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Identification and Structure of Four Yeast Genes (*SLY*) That Are Able To Suppress the Functional Loss of *YPT1*, a Member of the *RAS* Superfamily

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In *Saccharomyces cerevisiae*, the GTP-binding Ypt1 protein (Ypt1p) is essential for endoplasmic reticulum-to-Golgi protein transport. By exploiting a *GAL10-YPT1* fusion to regulate *YPT1* expression, three multicopy suppressors, *SLY2*, *SLY12*, and *SLY41*, and a single-copy suppressor, *SLY1-20*, that allowed *YPT1*-independent growth were isolated. Wild-type Sly1p is hydrophilic, is essential for cell viability, and differs from Sly1-20p by a single amino acid. *SLY2* and *SLY12* encode proteins with hydrophobic tails similar to synaptobrevins, integral membrane proteins of synaptic vesicles in higher eucaryotes. Sly41p is hydrophobic and exhibits sequence similarities with the chloroplast phosphate translocator. *SLY12* but not *SLY41* is an essential gene. The *SLY2* null mutant is cold and heat sensitive. The *SLY* gene products may comprise elements of the protein transport machinery.

A large and still increasing number of genes encoding small GTP-binding proteins have been identified in both lower and higher eucaryotes. They collectively constitute the *ras* superfamily, the best known members of which are the *ras* proto-oncogenes (for a review, see reference 6). *ras* and *ras*-related proteins are evolutionary highly conserved and share similar structural and biochemical properties (for reviews, see references 17 and 23). The conformational change occurring during the transition from the GDP- to the GTP-bound form is the characteristic feature that allows the regulation of these proteins and gives them their regulatory function (42, 50). When these proteins are complexed with GTP, they assume an active conformation that allows interaction with specific targets and associated activation of specific cellular reactions. The subsequent hydrolysis of protein-bound GTP, which is brought about by an intrinsic GTPase activity and accelerated by interacting GTPase-activating proteins (25, 33, 75, 77), ensures that the action of the regulator is temporally limited and allows the regeneration of an activation-competent complex.

A challenging problem is to characterize the normal cellular function of the many members of the *ras* superfamily of proteins. Examining the functions of these proteins in biological systems accessible to classical genetics may be the most feasible approach to determining their various roles. This applies to the unicellular yeasts, in which more than 10 small GTP-binding proteins, most of them with essential functions, have been identified. For example, defects in the *RAS2* gene of the budding yeast *Saccharomyces cerevisiae* interfere with normal cyclic AMP metabolism. In fact, evidence for the *RAS2* gene influencing the activity of adenylyl cyclase was first obtained by observing suppression of a *ras2* defect in *bcy1* mutants (73) lacking a functional regulatory subunit of cyclic AMP-dependent protein kinase (41). Further analysis of suppressors led to the identification of several genes acting downstream or upstream of *RAS2* (14, 15, 55, 69).

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Whereas yeast Ras proteins are signal amplifiers, several *ras*-related proteins are likely to have another mode of action. Through the analysis of conditional and null mutants of *SEC4* (26, 57), *YPT1* (4, 21, 60, 63), *SAR1* (47), and *ARF1* (65), at least four genes encoding GTP-binding proteins have been shown to be involved in protein transport from the endoplasmic reticulum (ER) to the plasma membrane. Mechanistically, these proteins are thought to cycle between a membrane-bound and a soluble form (43) and to direct the vesicular transport between different cellular compartments, i.e., from the ER to the Golgi complex, between separate Golgi cisternae, and from the Golgi apparatus to the plasma membrane (4, 11, 63, 78). At the restrictive temperature, *sec4* mutants are blocked late in the secretory pathway and accumulate small vesicles (49, 57). The *YPT1* gene product, which is structurally highly related to Sec4p (21), in contrast, participates in the transport process from the ER to the Golgi compartment and possibly also in intra-Golgi vesicle movements. Ypt1p-depleted cells as well as conditional *ypt1* mutants at the nonpermissive temperature are characterized by a massive accumulation of ER and partially glycosylated invertase (60, 63). The secretion defect has also been observed in cell-free transport systems from *ypt1* mutant cells or by blocking in vitro protein transport with anti-Ypt1p antibodies (3, 4). Evidence for a similar function of the mammalian ypt1 protein (27), which has also been named rab1 (74), comes from immunofluorescence and subcellular fractionation studies showing that ypt1p is primarily localized to Golgi membranes (51a, 63) and from the finding that the mouse ypt1p can functionally replace its yeast counterpart (28).

In a search for proteins that interact with Ypt1p or fulfill a related function, we made use of both genetic and molecular genetic methods whose combination makes the yeast system exceptionally attractive. The basic strategy was to switch off the expression of the essential *YPT1* gene under control of the repressible *GAL10* promoter (61) and to identify genes able to suppress the loss of *YPT1* function. In this way, a mutant gene, *SLY1-20* (for suppressor of loss of *YPT1* function), whose protein product carries a single amino acid

TABLE 1. *S. cerevisiae* strains used in this study

Strain	Genotype	Source or reference
GYW8-2A	*MATα leu2 his3 GAL10-YPT1-LEU2*	61
HLR3	*MATa leu2 his3 GAL10-YPT1-HIS3*	60
GFUII-2B	*MATα leu2 his3 GAL10-YPT1-HIS3*	This work
GFUII-1B	*MATα ura3 his3 GAL10-YPT1-HIS3*	This work
LSY20-1A	*MATa leu2 his3 GAL10-YPT1-LEU2 SLY1-20*	This work
DAH430H	*MATa/MATα YPT1/ypt1::HIS3 leu2/leu2 his3/his3*	60
DAH430	*MATa/MATα YPT1/YPT1 leu2/leu2 his3/his3*	60
INT1[a]	Same as GFUII-2B with plasmid YRp5L-SLY1/5 integrated at the *SLY1* locus	This work

[a] This transformant is phenotypically Glu⁻ [presumably *SLY1(wt)*-YRp5L-*SLY1(wt)*].

substitution compared with the wild-type Sly1p, and three multicopy suppressor genes, *SLY2*, *SLY12*, and *SLY41*, were isolated and characterized. Cells overexpressing wild-type *SLY2*, *SLY12*, and *SLY41*, but not *SLY1*, and cells producing the Sly1-20 mutant protein were viable even after disruption of the *YPT1* gene. The sequences of Sly2p, Sly12p, and Sly41p suggest that they are integral membrane proteins. Moreover, the protein products of the *SLY2* and *SLY12* multicopy suppressor genes have structural features in common with synaptobrevin, a synaptic vesicle membrane protein that is highly conserved in higher eucaryotes (67).

MATERIALS AND METHODS

Genetic techniques. *Escherichia coli* RR1 and JM101 were used for cloning experiments and the expression of *lacZ* fusion genes. *S. cerevisiae* strains used in this study are listed in Table 1. Crosses, sporulation of diploids, and dissection of tetrads were done as described by Sherman et al. (64). Rich medium (YEP) contained 1% yeast extract and 2% peptone (Oxoid), supplemented with 8% glucose (YEPD) for full repression of the *GAL10* promoter in front of the *YPT1* gene in strains HLR3, GFUII-1B, GFUII-2B, and GYW8-2A and in mutants and transformants derived from these strains. To induce the expression of the *YPT1* gene in these strains, YEP medium was supplemented with 2% galactose (YEPGal) instead of 8% glucose. Transformants and diploids were selected in minimal medium containing 0.67% yeast nitrogen base without amino acids (Difco) and either 2% glucose (SD) or 2% galactose (SGal). These media were supplemented to meet the auxotrophic requirements of particular strains (64). Yeast cells were made competent and transformed by the lithium acetate technique as described by Ito et al. (30).

Since growth of *GAL10-YPT1* strains stops after at least 12 h on glucose medium (61), transformants derived from these strains had to be replica plated to YEPD plates a second time after 24 h. This allowed the determination of Glu⁺ and Glu⁻ phenotypes after 2 days of incubation on YEPD plates.

For mutant isolation, strain HLR3 was grown in YEPGal medium and washed with 50 mM KPO_4 buffer, pH 6.5. The cells were treated with 1% ethyl methanesulfonate (EMS) for 1 h at 25°C in the same buffer. After washing with YEPD medium, cells were incubated in this medium at 25°C for mutation fixation. After 4 h of incubation, cells were spread on selective YEPD plates. The EMS treatment did not reduce the viability of the cells and had no strong effect on the number of colonies. All colonies, however, appeared at the same time and had about the same size; therefore, the colonies could be replica plated conveniently after 4 days of incubation at 25°C.

For genetic analysis, the *YPT1*-independent mutants identified by the *YPT1* deletion (*ypt1*⁻) were crossed to strain GYW8-2A, a strain carrying a *GAL10-YPT1* fusion (phenotype Glu⁻) and complementary amino acid requirements. All diploids obtained were Glu⁺. Since these diploids were homozygous for the *GAL10-YPT1* fusion, the Glu⁺ phenotype of the diploids suggests that the suppressor mutations are dominant. About half of the spores derived from these diploids were again Glu⁺. Although the spore viability was low in some cases, this result suggests that mutations in one particular gene are sufficient to make the cells *YPT1* independent. Since all suppressor mutations were dominant, allelism between the mutations in the different isolates had to be tested by crossing spores derived from different clones and subsequent tetrad analysis. This test for allelism was performed by using five isolates that showed good spore viability as well as four spores derived from a second screen for *YPT1*-independent mutants. None of the different combinations tested gave rise to Glu⁻ spores, suggesting that all clones were mutated in the same gene (*SLY1*).

Plasmids and nucleic acid techniques. DNA manipulations, such as *E. coli* transformation, restriction enzyme digestions, and ligations, were performed as described by Sambrook et al. (58). Preparation of yeast DNA and RNA and Northern (RNA) blotting were described previously (36, 37).

Plasmid YCp50 (52) was used as a low-copy-number yeast vector. The high-copy-number library was constructed by using the 2μm-based vector YEp13 (13). Other high-copy-number vectors used were YEp511, which was derived from YEp51 (12) by deleting the *GAL10* sequences from this vector, and YEp24 (16), which carries the *URA3* marker. These two plasmids were used for subcloning fragments derived from the original isolates YEp13-SLY2, YEp13-SLY12, YEp13-SLY41, and YCp50-SLY1/5(wt) to localize the complementing activity or to check whether the *SLY1(wt)* allele acts as a suppressor when overexpressed.

The *GAL10-YPT1* fusion gene together with the *HIS3* marker gene were deleted from mutants by transformation with a 5.4-kb *Hin*dIII fragment derived from plasmid pΔYL-2 (60). This plasmid contains parts of the *TUB2* and *ACT1* genes, which flank the *YPT1* gene on chromosome VI. The *Nar*I-*Bam*HI fragment containing the *YPT1* gene was replaced in this vector by a *Nar*I-*Bgl*II fragment containing the *LEU2* gene. The transformants were checked for the absence of any *YPT1* sequences by Southern blot analysis (data not shown).

To check the suppressor activity of the *SLY2* and *SLY12* genes on a low-copy-number plasmid, a 3.0-kb-long *Bam*HI fragment containing the *SLY2* gene and a 3.0-kb *Hin*dIII fragment carrying the *SLY12* gene were subcloned into YCp50 (52). The resulting plasmids were transformed into the *ura3* strain GFUII-1B. To achieve moderate overexpression of the *SLY41* gene, a 1.9-kb *Spe*I fragment containing the 2μm origin of replication was deleted from plasmid YEp24-SLY41, which carries the *SLY41* gene as a 3.0-kb *Cla*I fragment. The resulting plasmid was cut either with *Nco*I to direct integration into the *URA3* locus or with *Xba*I to direct integration into the *SLY41* locus. The linearized plasmids were mixed and transformed into GFUII-1B.

Transformants were selected on SGal plates, and colonies were replica plated onto YEPD plates.

Yeast DNA libraries were constructed by using DNA from a yeast strain with a deletion of the *YPT1* gene and carrying the dominant *SLY1-20* allele. This DNA was partially digested with the endonuclease *Sau*3A or *Taq*I. The endonuclease digestion was stopped by adding EDTA to the reaction mixture after different time intervals. The DNA was run on a preparative agarose gel, and the region containing DNA fragments longer than 4 kb was cut out of the gel. DNA was purified from the gel slice according to the method of Tautz and Renz (70) and ligated into the vectors YEp13 and YCp50. YEp13 and YCp50 DNA were cut with *Bam*HI or *Cla*I, respectively, and dephosphorylated to prevent religation. The *Bam*HI-*Sau*3A ligation and the *Cla*I-*Taq*I ligation yielded about 40,000 and 4,000 *E. coli* clones. About 90% of the plasmids contained an insert, as determined by checking for tetracycline-sensitive clones (the insert should disrupt the *tet* gene).

Three different clones containing the *SLY1* mutant gene, YCp50-SLY1/2, -SLY1/5, and -SLY1/6, were derived from the *Taq*I library. The *LEU2* marker gene was inserted into the unique *Sal*I site of the smallest of these isolates, YCp50-SLY1/5, as a 2.2-kb *Xho*I-*Sal*I fragment which was excised from YEp13 (13). The resulting centromere plasmid, YCp5LU-SLY1/5, was transformed into the *leu2/leu2* strain DAH430H to check the ability of this plasmid to complement the *YPT1* deletion. YCp5LU-SLY1/5 was cut with *Xho*I to delete the *URA3* gene and most of the *CEN4* sequences from this plasmid, thus creating YRp5L-SLY1/5. As an *ARS1* plasmid, this vector can either integrate into the genome or replicate autonomously. Hence, either fast- or slow-growing transformants were obtained when this plasmid was cut at the unique *Pvu*II site in the cloned *SLY1* sequence and used for yeast transformation. The *GAL10-YPT1* strain was transformed with this linearized plasmid, and transformants were selected on minimal plates lacking leucine and containing galactose as the sole carbon source. Both the fast- and the slow-growing clones were either Glu$^+$ or still Glu$^-$. We assume that in these Glu$^-$ clones the *SLY1(wt)* allele on the chromosome was used as template for the repair of the linearized plasmid DNA. DNA from a small Glu$^-$ colony was used for *E. coli* transformation to reisolate plasmid YRp5L-SLY1/5 containing the wild-type allele of the *SLY1* gene (YRp5L-SLY1/5^{wt}). A fast-growing stable Glu$^-$ transformant (INT1) was mated with a *SLY1-20* mutant (LSY20-1A) to prove the integration at the *SLY1* locus by tetrad analysis.

For DNA sequence analysis, DNA fragments from subclones with *ypt1*$^-$-complementing activity were inserted into the vector pSPT18 (Pharmacia). Supercoiled plasmid DNA was purified from agarose gels according to Tautz and Renz (70) and sequenced by the chain termination method (18, 59), using the SP6 and T7 primers and other synthetic primers synthesized according to sequences established. In each case, both strands were sequenced.

Southern transfers to Nytran-NY13N membrane (Schleicher & Schüll) were performed by using standard procedures. Hybridization was carried out at 65°C in 1 M NaCl–10% dextran sulfate–1% sodium dodecyl sulfate with 100 mg of calf thymus DNA per ml as the carrier. The same conditions were used when a yeast DNA library (vector YCp50) was screened for clones containing the *SLY1(wt)* gene with a 0.8-kb *Bgl*II fragment from the mutant sequence as a probe. One positive clone was obtained, which was named YCp50-SLY1(wt). The cloned sequence was not able to suppress the turning off of *YPT1* expression even when subcloned into the multicopy vector YEp511.

One-step gene disruption was carried out according to Rothstein (53). A 2.2-kb *Hpa*I fragment and a 3.0-kb *Bgl*II fragment containing the *LEU2* gene, a 2.2-kb *Eco*RV-*Hin*cII fragment containing the *HIS3* gene, and a 1.2-kb *Hin*dIII fragment containing the *URA3* gene were inserted into the coding regions of the *SLY1*, *SLY12*, *SLY2*, and *SLY41* genes, respectively. These fragments were derived from vectors YEp13 (13), pBR322-HIS3 (66), and YEp24 (16) and inserted into the unique restriction sites of the particular *SLY* genes present as pSPT18 subclones: the *Hpa*I site in *SLY1*, the *Bgl*II site in *SLY12*, the *Xba*I site in *SLY41*, and the *Nco*I site in *SLY2*. Then 57 or 96 nucleotides were removed from the coding region of the *SLY2* gene with BAL 31 nuclease. Where necessary, the ends of the DNA fragments and the vector DNA were made blunt by using the Klenow fragment of *E. coli* DNA polymerase I. Fragments containing the disrupted *SLY* genes were cut out of the plasmids by using endonucleases *Spe*I (*SLY1*), *Nco*I-*Hin*dIII (*SLY12*), *Hin*dIII-*Nhe*I (*SLY41*), and *Sma*I-*Sal*I (*SLY2*). These fragments were purified from agarose gels and used to transform strain DAH430 (*SLY1*, *-2*, and *-12*) or DUR3 (*SLY41*) (see Fig. 4, 10, 11, and 12). In the case of *SLY2*, some nucleotides were left which were derived from the pSPT18 polylinker next to the *Bam*HI sites at the boundaries of the yeast DNA insert. However, this small mismatch at the end of the fragments did not interfere with the gene disruption, because the effects of the *SLY2* disruption can be fully complemented by plasmids carrying as an insert a 1.5-kb *Bam*HI-*Eco*RV fragment that contains only *SLY2* sequences and no sequences from the neighboring reading frames. Moreover, Southern blot analysis showed that the *Bam*HI sites present at the end of the yeast DNA sequence are still present in the transformants (data not shown). Transformants were sporulated, and tetrad analysis was performed on YEPD plates. The integration of the DNA fragments at the different *SLY* loci was verified by Southern blot analysis as shown in Fig. 4, 10, 11, and 12.

Sequence comparison and secondary structure predictions. The search for proteins with sequence similarities to the products of the *SLY1*, *SLY2*, and *SLY12* genes by screening the MIPSX protein data base (Martinsrieder Institut für Proteinsequenzen; release 16 with 31435 sequences) did not detect any entry in this data base which yielded an optimized score of more than 82 with use of the FASTA algorithm (word size = 2) of Pearson and Lipman (51). Two proteins were identified which gave an optimized score of >100 compared with the primary sequence of the putative Sly41p. Two algorithms, RDF2 (51) and one algorithm written by Argos (2), were used to test the significance of these similarities. The score for the sequences aligned in Fig. 8B is 5.4 (region 1 and 3) or 6.5 (region 2) standard deviations above the mean score for the randomly permutated sequences.

Nucleotide sequence accession numbers. The sequences of the *SLY1*, *SLY2*, *SLY12*, and *SLY41* genes have been assigned the accession numbers X54323, X54236, X54237, and X54238, respectively, by the EMBL data base.

RESULTS

Isolation of *YPT1*-independent mutants. The *YPT1* gene in *S. cerevisiae* encodes a protein with an essential function (61, 62). *YPT1*-independent yeast mutants were isolated by using a *GAL10-YPT1* fusion. Placing the regulatable *GAL10* promoter in front of the *YPT1* coding region makes cells

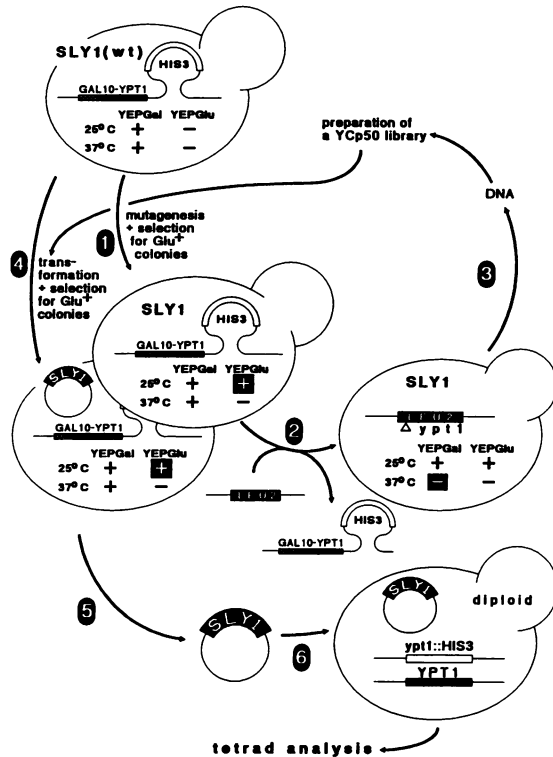

FIG. 1. Scheme for isolation and cloning of the suppressor *SLY1-20*. (1) Mutagenized HLR3 cells were spread on selective YEPD plates. Mutant colonies appearing after 4 days of incubation at 25°C were replica plated onto different media to identify clones with the indicated phenotypes. (2) *YPT1* was replaced by the *LEU2* gene in Ts⁻ clones (Glu⁺ at 25°C but not at 37°C). (3) A DNA library with fragments of a genetically analyzed *ypt1*⁻ strain, inserted into the single-copy vector YCp50, was (4) used to transform a *GAL10-YPT1* strain to identify Glu⁺ Ts⁻ colonies. (5) The recombinant plasmid was isolated and (6) was transformed into a DAH430H diploid.

galactose dependent and sensitive to the presence of glucose in the growth medium (61). With use of this selective system with EMS-mutagenized cells, however, mutations in the regulatory *GAL4* and *GAL80* gene products may also allow mutants to grow on glucose-containing medium. With this in mind, we isolated temperature-sensitive revertants (step 1 in Fig. 1), assuming that a *YPT1*-specific extragenic suppressor might be unable to rescue *YPT1*-deficient cells at all temperatures. In fact, high expression of the *GAL4* gene suppresses the shutdown of *GAL10-YPT1* expression at both 25 and 37°C (see below). Moreover, the efficient selection of conditional mutations in regulatory *GAL* genes requires primary mutations in the *GAL80* gene (40).

To ensure that the conditional phenotype of the revertants isolated was specific for *YPT1* deficiency, we further analyzed only clones that were Ts⁻ on glucose-containing but not on galactose-containing medium (Fig. 1). Most of the revertants isolated could also survive the deletion of the *YPT1* coding region, indicating that they were indeed *YPT1* independent. To delete the *YPT1* gene, the revertants were transformed with a linear DNA fragment containing the *LEU2* marker gene in place of the *YPT1* coding region (step 2 in Fig. 1). As the original strain HLR3 carries the *HIS3* marker gene near the 3′ end of the *GAL10-YPT1* fusion gene on chromosome VI (61), *ypt1*⁻ transformants became His⁻ and Leu⁺ after correct integration of the linear DNA fragment at the *YPT1* locus. In at least one of the His⁻/Leu⁺ transformants derived from each revertant, the absence of *YPT1* coding sequences was verified by Southern analysis (data not shown).

Five of the *YPT1*-independent mutants, crossed with a wild-type haploid, had good spore viability. The genetic analysis of these isolates showed that (i) mutations in one particular gene were sufficient to suppress the *YPT1* defect, (ii) the mutations were not linked to the *YPT1* gene, (iii) all mutations were either in the same gene or in closely linked genes, and (iv) all mutations behaved as dominant suppressors of the *YPT1* deficiency. Four mutants derived from a second screening for *YPT1*-independent growth were also found to be mutated in the same or a closely linked gene. This gene was named *SLY1* (for suppressor of loss of *YPT1* function). In accordance with standard *S. cerevisiae* nomenclature, dominant mutant alleles were designated *SLY1-20* for example, while we refer to the wild-type gene as *SLY1(wt)*.

Cloning of *SLY1-20*, a mutant gene acting as single-copy suppressor. The dominance of the *SLY1* suppressor mutations allowed to easily clone the gene. As outlined in Fig. 1, fragmented chromosomal DNA from a *SLY1-20* mutant lacking the *YPT1* gene was inserted into the yeast single-copy vector YCp50 (52), which carries the *URA3* marker, *ARS1* (yeast replication origin), and *CEN4* (yeast centromere) sequences (step 3 in Fig. 1). The plasmid collection was used to transform an *ura3* strain with the *GAL10-YPT1* fusion gene on chromosome VI. All Ura⁺ colonies selected on galactose medium were replica stamped onto glucose-containing plates (step 4 in Fig. 1). Transformants containing a plasmid with the dominant *SLY1-20* allele should be able to survive the shutdown of *YPT1* transcription in the presence of glucose. As expected, all of the isolated Glu⁺ colonies were temperature sensitive on glucose- but not on galactose-containing media. From such Glu⁺ transformants, three different plasmids with overlapping DNA inserts were isolated (step 5 in Fig. 1). The smallest of these plasmids (YCp50-SLY1/5) contained a 3.1-kb insert with a 1,998-bp-long open reading frame (Fig. 2). Deletions starting from either side of the insert and ranging into this open reading frame destroyed the suppressor activity. This result suggested that the 1,998-bp open reading frame encodes the *ypt1*⁻-complementing activity.

Evidence for the cloned sequence representing the *SLY1-20* mutant gene was obtained in two different ways. First, the cloned sequence could suppress not only the turning off of the *GAL10-YPT1* fusion but also the deletion of the *YPT1* gene. To prove this, the diploid strain DAH430H with one *YPT1* gene replaced by the *HIS3* marker gene (60) was transformed with plasmid YCp5LU-SLY1/5. This centromere plasmid was derived from the original *SLY1-20*-containing isolate YCp50-SLY1/5 by insertion of the *LEU2* gene as an additional marker. Leucine-prototrophic transformants were selected and sporulated. Eleven tetrads dissected gave rise to 22 large His⁻ colonies. The His⁻ phenotype indicated that they carried the *YPT1* allele. In addition to these wild-type spores, 12 somewhat smaller His⁺/Leu⁺ colonies grew out of the spores derived from plasmid-containing diploids. The His⁺/Leu⁺ phenotype suggested that they required for growth the suppressor gene present on the plasmid. The segregation pattern (12 His⁺ and 10 non-growing spores within 11 tetrads) is consistent with the

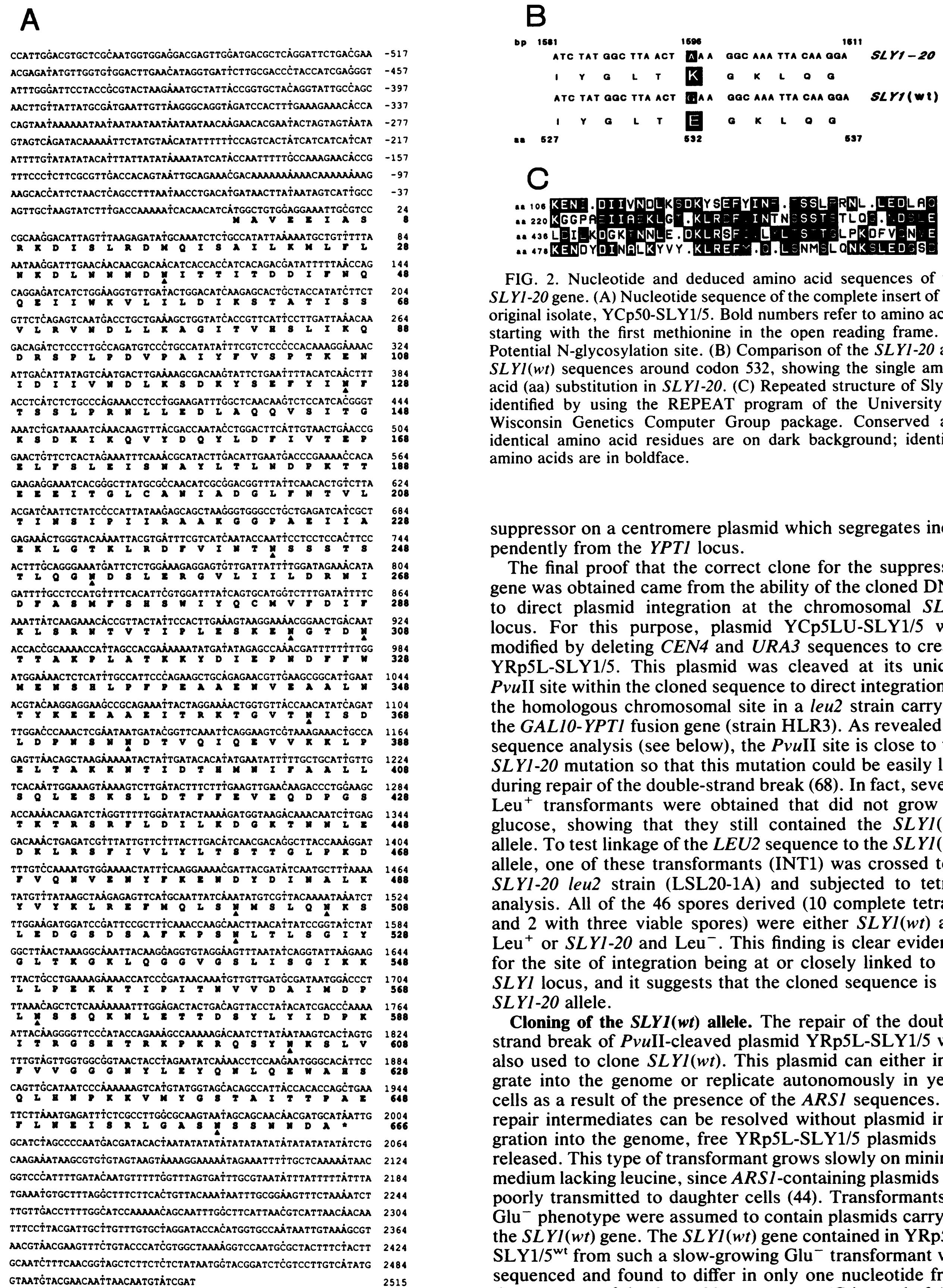

FIG. 2. Nucleotide and deduced amino acid sequences of the *SLY1-20* gene. (A) Nucleotide sequence of the complete insert of the original isolate, YCp50-SLY1/5. Bold numbers refer to amino acids starting with the first methionine in the open reading frame. ▲, Potential N-glycosylation site. (B) Comparison of the *SLY1-20* and *SLY1(wt)* sequences around codon 532, showing the single amino acid (aa) substitution in *SLY1-20*. (C) Repeated structure of Sly1p, identified by using the REPEAT program of the University of Wisconsin Genetics Computer Group package. Conserved and identical amino acid residues are on dark background; identical amino acids are in boldface.

suppressor on a centromere plasmid which segregates independently from the *YPT1* locus.

The final proof that the correct clone for the suppressor gene was obtained came from the ability of the cloned DNA to direct plasmid integration at the chromosomal *SLY1* locus. For this purpose, plasmid YCp5LU-SLY1/5 was modified by deleting *CEN4* and *URA3* sequences to create YRp5L-SLY1/5. This plasmid was cleaved at its unique *Pvu*II site within the cloned sequence to direct integration to the homologous chromosomal site in a *leu2* strain carrying the *GAL10-YPT1* fusion gene (strain HLR3). As revealed by sequence analysis (see below), the *Pvu*II site is close to the *SLY1-20* mutation so that this mutation could be easily lost during repair of the double-strand break (68). In fact, several Leu$^+$ transformants were obtained that did not grow on glucose, showing that they still contained the *SLY1(wt)* allele. To test linkage of the *LEU2* sequence to the *SLY1(wt)* allele, one of these transformants (INT1) was crossed to a *SLY1-20 leu2* strain (LSL20-1A) and subjected to tetrad analysis. All of the 46 spores derived (10 complete tetrads and 2 with three viable spores) were either *SLY1(wt)* and Leu$^+$ or *SLY1-20* and Leu$^-$. This finding is clear evidence for the site of integration being at or closely linked to the *SLY1* locus, and it suggests that the cloned sequence is the *SLY1-20* allele.

Cloning of the *SLY1(wt)* allele. The repair of the double-strand break of *Pvu*II-cleaved plasmid YRp5L-SLY1/5 was also used to clone *SLY1(wt)*. This plasmid can either integrate into the genome or replicate autonomously in yeast cells as a result of the presence of the *ARS1* sequences. As repair intermediates can be resolved without plasmid integration into the genome, free YRp5L-SLY1/5 plasmids are released. This type of transformant grows slowly on minimal medium lacking leucine, since *ARS1*-containing plasmids are poorly transmitted to daughter cells (44). Transformants of Glu$^-$ phenotype were assumed to contain plasmids carrying the *SLY1(wt)* gene. The *SLY1(wt)* gene contained in YRp5L-SLY1/5^{wt} from such a slow-growing Glu$^-$ transformant was sequenced and found to differ in only one nucleotide from the sequence of the *SLY1-20* mutant gene (G instead of A in

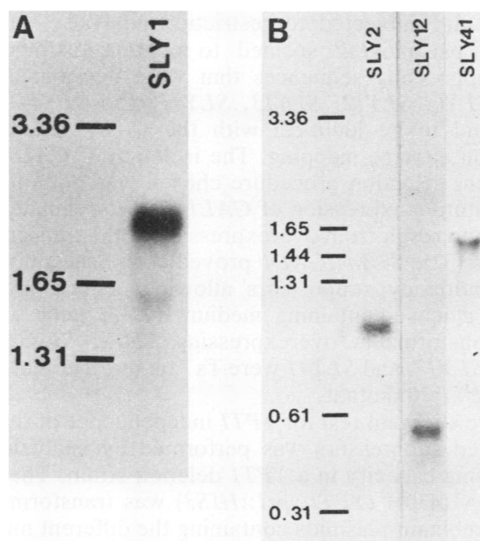

FIG. 3. Detection of specific transcripts of *SLY1*, *SLY2*, *SLY12*, and *SLY41*. Total cellular RNA was glyoxylated, 10 μg per lane was separated on 1.5% agarose gels, and mRNAs were hybridized with ^{32}P-labeled DNA fragments. To identify *SLY1* (A) and *SLY12* and *SLY41* (B) transcripts, the same DNA fragments were used that are indicated as probes for Southern blot analysis shown in Fig. 4, 11, and 12, respectively. The *SLY2*-specific probe was an *Aat*II fragment ranging from 186 bp 5′ of the translation start to 4 bp 3′ of the stop codon. Sizes of marker fragments (in kilobases) are shown on the left.

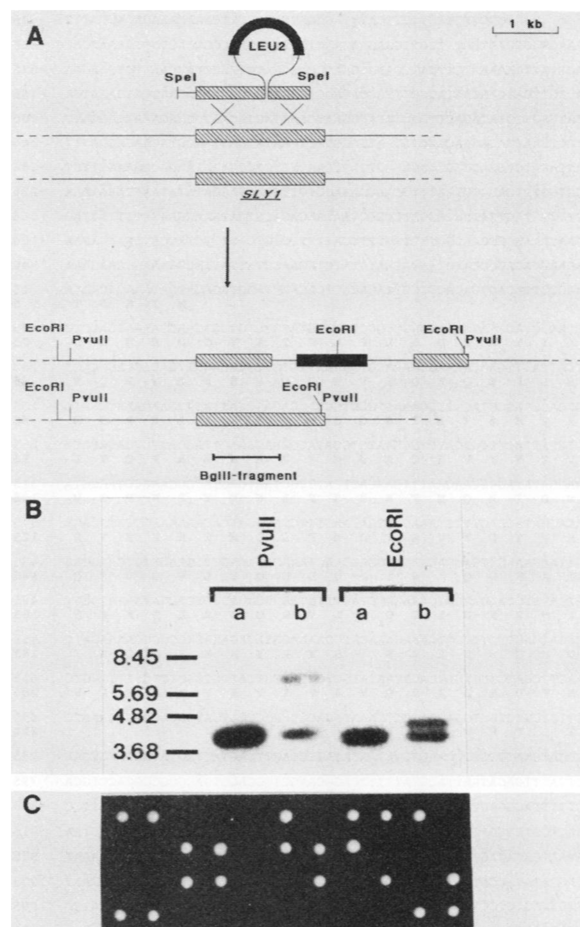

FIG. 4. Disruption of the *SLY1* gene. (A) The *SLY1* gene was interrupted by inserting a 2.2-kb *Hpa*I fragment carrying the *LEU2* gene as a selectable marker into the *Hpa*I site of the *SLY1* coding region. The linear 4.3-kb *Spe*I fragment carrying the disrupted *SLY1* gene was used to transform a diploid Leu$^-$ strain. Transformants with a wild-type *SLY1* gene on one chromosome and the disrupted *SLY1* gene on the other were identified by Southern blot analysis and used for sporulation and tetrad dissection. The 0.8-kb *Bgl*II fragment used for Southern blot hybridization. (B) Southern blot analysis of a diploid strain with one disrupted *SLY1* allele. Chromosomal DNAs from the untransformed Leu$^-$ diploid strain (lanes (lanes a) and from a Leu$^+$ transformant (lanes b) were digested with *Pvu*II or *Eco*RI and probed with the ^{32}P-labeled 0.8-kb *Bgl*II fragment. The appearance of a 7.2-kb *Pvu*II fragment and a 4.5-kb *Eco*RI fragment (lanes b) indicates correct integration and disruption of one *SLY1* allele. The sizes of marker fragments (in kilobases) are shown on the left. (C) Tetrad analysis of the *sly1*$^-$/*SLY1*$^+$ diploid strain analyzed in panel B, showing that only two (*SLY1*$^+$) spores form colonies.

the first position of codon 532 of the 1,998-bp open reading frame).

The wild-type *SLY1* gene was also isolated from a subgenomic library constructed with *Hin*dIII fragments (7 to 9 kb) of wild-type yeast DNA and vector YCp50. The gene was identified and cloned on a *Hin*dIII fragment of about 8 kb by hybridization to a ^{32}P-labeled probe of the *SLY1-20* mutant gene. The recombinant plasmid YCp50-SLY1(wt) contained the entire 1,998-bp open reading frame and flanking sequences. Sequence analysis showed the same single base difference within codon 532 (compared with *SLY1-20*) as *SLY1(wt)* derived from YRp5L-SLY1/5^{wt}.

It should be noted that *SLY1(wt)* overexpressed from a multicopy vector was unable to suppress the loss of *YPT1* function, whereas *SLY1-20* either on a plasmid or integrated into the genome could rescue cells lacking Ypt1p.

Sequences of *SLY1(wt)* and *SLY1-20* genes and their protein products. As mentioned above, dideoxy sequencing of the 3.1-kb DNA insert contained in YCp50-SLY1/5 revealed a long open reading frame of 1,998 bases (Fig. 2A). Deletions within the cloned sequence extending into this reading frame eliminated *ypt1*$^-$-complementing activity. Assuming that the first AUG of this reading frame is the translation initiation codon, the *SLY1-20* mutant gene would encode a protein of 666 amino acids. Sequence comparison of the wild-type and mutant genes disclosed only one nucleotide difference (G-to-A transition) which would result in a substitution of lysine for glutamic acid in position 532 of the Sly1-20 mutant protein (Fig. 2B).

Sly1p is a hydrophilic protein with a total of 25% charged amino acids that are evenly distributed over the entire length of the protein. There are no hydrophobic regions long enough to predict a membrane-spanning domain. The protein has 14 potential N-glycosylation sites (marked in Fig. 2A) and four internal repeats of about 40 amino acids (Fig. 2C). Neither these repeats nor any other region of Sly1p exhibits significant homology to proteins listed in available data banks.

***SLY1* encodes an essential function.** When a *Bgl*II fragment ranging from nucleotides 258 to 1294 of the *SLY1* protein-coding region was used as a hybridization probe in a RNA blot analysis, a unique 2.2-kb RNA was detected in growing yeast cells (Fig. 3A). To determine whether the gene coded for an essential function, the chromosomal *SLY1* allele was

```
GGATCCCCTTTACTTCTTCATTTAGCGATGATACCTTCAAACAAAGACACCATTT  -646
AAAGAGGCCAATGATTTGCTTGAAGAACAACTCTTTAAGATCGCTCACTGTCAAAATCAT  -586
ACGTATTGAAAAATGTTCATCCATCCGAATCCAGGGATTTTACCTACCAAATCTGCAGGA  -526
ACTGTTCATCAACAATACCCTTTGCGACACCACCCAACACCAAAAACAAGCGTCAAATGA  -466
TATGAGTTGTATAGAGTTCACTTCATGGAATGAACTACCACAATGCAAGAAATTGGGATT  -406
TGCTCAATTAGAGGACGACTCTAACTACGTTCTTAATATCAGTAACCTACAAGACCATTT  -346
ACCAAATCTGGACCTGCGGGAGAGTTTCCCAACTTTCTTCGATATAAGACAGAAGTTTGT  -286
CGTGGTTTGAGGTCTAATTTTCAACCAAACGTGTTGTACTAGCATATATAATTAAATAAA  -226
TTTCTCTAGTTATCACTTGATTTATCTAATAAGAGACGTCATAAACGAATTTTTTTTTAG  -166
CAATAAATCCTCAACGCTTTTTGTTTCATTTTTCGGTCTTTTTCTACCATAGAAGATAA  -106
ACAAGTAGAGCCCATGTAATATAATATATCTACAACCTATTATTACCCAAAACCAACAAA   -46
AACCCTGACAGTGACACCCCGTTACACACTCACAATTAAGTAGGGATGATAAAGTCAACA    15
                                               M  I  K  S  T     5
CTAATCTACAGAGAAGATGGGCTGCCTCTTTGTACGTCTGTGGACAACGAAAATGATCCC    75
 L  I  Y  R  E  D  G  L  P  L  C  T  S  V  D  N  E  N  D  P    25
TCATTATTTGAACAAAAGCAAAAGGTGAAAATCGTCGTTTCCAGATTGACACCACAGTCT   135
 S  L  F  E  Q  K  Q  K  V  K  I  V  V  S  R  L  T  P  Q  S    45
GCCACGGAGGCTACTTTGGAAAGTGGCTCCTTTGAGATCCATTATTTGAAGAAATCCATG   195
 A  T  E  A  T  L  E  S  G  S  F  E  I  H  Y  L  K  K  S  M    65
GTGTACTACTTCGTCATTTGCGAATCTGGATACCCAAGAAACTTAGCATTCTCATACCTT   255
 V  Y  Y  F  V  I  C  E  S  G  Y  P  R  N  L  A  F  S  Y  L    85
AACGATATAGCGCAAGAATTTGAACACTCATTTGCTAATGAGTACCCCAAGCCTACCGTA   315
 N  D  I  A  Q  E  F  E  H  S  F  A  N  E  Y  P  K  P  T  V   105
AGACCATACCAGTTTGTAAACTTTGATAACTTTCTACAGATGACAAAAAAGTCATACAGT   375
 R  P  Y  Q  F  V  N  F  D  N  F  L  Q  M  T  K  K  S  Y  S   125
GATAAGAAAGTTCAGGACAATTTGGACCAACTAAACCAAGAGCTTGTAGGTGTCAAGCAA   435
 D  K  K  V  Q  D  N  L  D  Q  L  N  Q  E  L  V  G  V  K  Q   145
ATCATGTCCAAGAACATCGAAGACCTACTTTACAGGGGAGATTCTCTCGATAAAATGAGT   495
 I  M  S  K  N  I  E  D  L  L  Y  R  G  D  S  L  D  K  M  S   165
GACATGAGTTCTTCTTTGAAAGAAACATCCAAAAGGTACAGAAAGTCCGCGCAAAAGATC   555
 D  M  S  S  S  L  K  E  T  S  K  R  Y  R  K  S  A  Q  K  I   185
AACTTCGATCTCTTGATCAGTCAATATGCTCCTATTGTCATTGTCGCTTTCTTTTTCGTC   615
 N  F  D  L  L  I  S  Q  Y  A  P  I  V  I  V  A  F  F  F  V   205
TTTCTCTTCTGGTGGATCTTCCTCAAATAGACGTCACCCCATCATCCCACATCACAAATC   675
 F  L  F  W  W  I  F  L  K  *                                  214
CCGATCAATTTGGTCCAAGTGTATAACATAGTCTAGTTCAGTATTAGTATAGTATGGCAA   735
TTTATTTGACATAATTACGTATTTTTCAGGCAAGCTGGCAGGTGACCCGCCGCACCTGCA   795
CTTTTTAGGGAGTCTTTTCAAGGATATCATCACTATATATCGAAGGAAAAGAGGCTTTCC   855
AACTCTTTTGGATGATCGAATTGCCATCACTACTCTATTAACTGTCTGTGTATTAACTAA   915
ATAATCACATAGTATTAAGTTATAATTCCTGGAAAAAAAAAGAAAGCGAAACGACAAAAT   975
TAGCATATACCATGTCTCAACTGCCAACAGATTTTGCTTCATTGATCAAGAGATTCCAAT  1035
TTGTTAGTGTCCTCGATTCCAACCCTCAAACCAAAGTTATGTCCTTATTGGGAACCATTG  1095
ACAACAAGGATGCTATTATCACGGCTGAAAAGACGCACTTTCTATTTGACGAAACCGTGA  1155
GAAGACCCTCTCAAGATGGCCGCTCCACTCCAGTTTTATATAATTGTGAAAACGAATATT  1215
CTTGCATCAACGGAATTCAGGAATTAAAGGAGATTACTTCCAATGATATTTACTACTGGG  1275
GTTTGTCAGTGATAAAGCAG                                          1295
```

FIG. 5. Nucleotide and deduced amino acid sequences of the *SLY2* gene.

disrupted by insertion of the *LEU2* marker gene into the *Hpa*I site at codon 390. A linear 4.3-kb *Spe*I fragment with part of the interrupted *SLY1* coding region was used to transform a homozygous Leu⁻ diploid strain and replace one chromosomal *SLY1* gene with the disrupted allele (Fig. 4A). Southern analysis of several Leu⁺ transformants confirmed the integration of the *LEU2* marker at the *SLY1* locus (Fig. 4B). Each of 10 tetrads derived from such a transformant gave rise to only two viable spores (Fig. 4C), all of them Leu⁻. Microscopic inspection of the other spores carrying the disrupted *SLY1* allele showed that they were unable to divide, indicating that an intact *SLY1* gene is essential for cell viability.

Isolation of multicopy suppressors of *YPT1* deletion. Multicopy suppressors of essential genes often exhibit a similar function or act in the same pathway as the gene they are able to suppress. In searching for suppressors of the loss of *YPT1* function, a genomic library with partially *Sau*3A-digested DNA of a *SLY1-20 ypt1*⁻ strain and the multicopy vector YEp13 (13) was constructed and used to transform a haploid strain whose chromosomal *YPT1* gene was under *GAL10* promoter control. Recombinant plasmids were retrieved from several transformants growing in glucose-containing medium and subjected to restriction analysis. Among 11 different plasmids, 7 seemed to contain distinguishable *ypt1*⁻-suppressing sequences that were designated *SLY2*, *SLY3*, *SLY6*, *SLY12*, *SLY13*, *SLY41*, and *SLY44*. *SLY44* was found to be identical with the *GAL4* gene (38) by restriction enzyme mapping. The isolation of *GAL4* by the suppressor selection procedure chosen was not surprising, as constitutive expression of *GAL1-GAL10*-regulated genes is known to result from overexpression of the transcriptional activator (32). *SLY44*/*GAL4* proved to be the only of the seven multicopy suppressors allowing cellular growth at 37°C in glucose-containing medium (*YPT1* gene switched off). Transformants overexpressing *SLY2*, *SLY3*, *SLY6*, *SLY12*, *SLY13*, and *SLY41* were Ts⁻ on this medium, similar to the *SLY1-20* mutant.

A more stringent test for *YPT1* independence of the newly discovered suppressors was performed by analyzing their suppressing capacity in a *YPT1* deletion strain. The diploid strain DAH430H (*YPT1*/*ypt1*::*HIS3*) was transformed with the recombinant plasmids containing the different multicopy suppressors, Leu⁺ transformants were sporulated, and tetrads were dissected. Since *YPT1* deletion is lethal, no His⁺ colonies were generated from spores of untransformed DAH430H. In contrast, a small number of slowly growing His⁺/Leu⁺ colonies (one to six per 10 tetrads) grew out of spores derived from *SLY2*-, *SLY12*-, and *SLY41*-transformed diploids on rich medium. Their His⁺/Leu⁺ phenotype indicated that they contained the *YPT1* deletion and were

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CCCCATGGTGTTTACTCTGTGATAGGAACCAAAGATCCCGTAATTATCGCTTTGTAATTG  -386
TATTTCCAGTTATTTCTGTACATTCTAGGTTACTGTTCAGGGATATGTTTCACTCTTATC  -326
GATGTGGCGTGCAAAAAATTTGAAGGGGCGACATATCAGGGAAAGTATTAGTGATTTTTT  -266
ATAATGTTGCACTATCTCAGATGAAATTTATGCATGCTAATTGGCGGATACTACTCTACC  -206
GTTGTTGCAAGCTACGTAGGAACGTTCACGGCTCTTTCTTCCATTAATTTTAAATTGCGA  -146
AAGTATCCAGTAGTTGTTAAAAATGGAGTTTTTTCAACTCGCGCCTTTTGCCAAGGAATG   -86
GTAGAAAACAAACAGTAGTTCACTTGAGACTGTGAATGTGTTAGAAAATCGTGATTGAAG   -26
AGCCTCACAACACTAGGCTACACAGATGAGTTCAAGGTATGAGAAATTATTAGACTATTC    35
                          M  S  S  R                           4
TAAAGTTAGAGTAGCGCTTTTTGTTAAACATCTACCATAACACCTCGATATTGGGAGTTA    95
TTGACCACGAGTGAACTTCGTTTACTAACTATAGCGTGTGTATATAGATTTGCAGGGGGA   155
                                              F  A  G  G       8
AACGCTTATCAACGTGATACTGGTAGAACACAGTTATTCGGACCGGCTGATGGATCAAAT   215
 N  A  Y  Q  R  D  T  G  R  T  Q  L  F  G  P  A  D  G  S  N    28
AGTCTCGATGACAATGTATCATCAGCGCTAGGGAGCACAGATAAATTAGACTACTCCCAA   275
 S  L  D  D  N  V  S  S  A  L  G  S  T  D  K  L  D  Y  S  Q    48
AGTACTTTGGCATCTCTTGAATCTCAAAGTGAGGAACAGATGGGAGCTATGGGTCAGAGA   335
 S  T  L  A  S  L  E  S  Q  S  E  E  Q  M  G  A  M  G  Q  R    68
ATAAAAGCACTCAAGTCATTATCGTTGAAGATGGGTGATGAGATTAGAGGCAGCAATCAA   395
 I  K  A  L  K  S  L  S  L  K  M  G  D  E  I  R  G  S  N  Q    88
ACTATTGACCAGCTTGGTGATACTTTCCATAACACTTCTGTAAAACTCAAAAGGACTTTT   455
 T  I  D  Q  L  G  D  T  F  H  N  T  S  V  K  L  K  R  T  F   108
GGAAACATGATGGAGATGGCCAGAAGATCTGGGATCAGTATAAAAACATGGTTAATAATA   515
 G  N  M  M  E  M  A  R  R  S  G  I  S  I  K  T  W  L  I  I   128
TTTTTTATGGTAGGCGTGCTATTTTTTTGGGTATGGATTACATAAATATTTGCATATTGT   575
 F  F  M  V  G  V  L  F  F  W  V  W  I  T                     142
ACATTAAACCTAAGTACAAAATAAGAGCGACGTTTGTTAGATGATTGTAGATATTGATAC   635
GTGAAATAAAGGATAGTTATGATAGCATCATAAGATATCAGCATTCTTTTATTTTTATAA   695
CTACTTAAGCGTTTAATCTTGATTTTTTTTTTTCTCCATATTTCCTAGGATACCGACAGC   755
GTTCCTTTTCGCCCTATAGTGTTTCCTTACTTTGTATCTTCTGTAAATAGATGGACCTTT   815
GAGTATTGCGATAAACAAAACAATAACAATTAAGTAACCGGGCATGCGTTCCCGTGGATT   875
ATGTCTAGGAAACTGAGGACATTATGGAATGGAGAATTTATCAATCTCCCCTTCAACTTA   935
GAGCTGTATAGAGCTTTTGATGGAATATTTAAGGACACGAATGCTTCTCTCAGCAATTGA   995
GGGTTGTTAGAAGTCAAGGAGTTGCCAAAATTATCTTTATTGTAGTAGTAATTATTTGAT  1055
TTATCAATGATAATCACATCACCAATTTTATATTCTCCAGTAATACCATATTTTCTAAAA  1115
AAATTCTTCTCCTTTGAAATATCTAAATATCCTAATAACAATTTTTTTTGATCCATATGG  1175
GGAATCTCTTCACGCATTTTATCAACAATTTTGTTAGCCGGAGCCTTTTTTTCCTTTATT  1235
TTCTTGATCCCGTCTGCTTTCCTTGCCCTTCTCTCATTAATTTCTTCGAAGTTATTTTCC  1295
ATCCGAATATGTTCATACTCCCAACTTGCGAGGAGCAAGTTTTTAAT               1342
```

FIG. 6. Nucleotide and deduced amino acid sequences of the *SLY12* gene. The splicing consensus sequences of the intron are underlined.

A

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Sly2p                  MIKSTLIYREDGLPLCT_SVDNEND
Sly12p MSSRFAGGNAYQRDTGRTQLFGPADGSNSLDDNVS

Sly2p  PSLFE_QKQKVKIVVSRLTPQSATEATLESGSFEI
Sly12p SALGSTDKLD_________YSQS_TLASLESQSEE_

Sly2p  HYLKKSMVYYFVICESGYPRNLAFSYLNDIAQEFE
Sly12p -----------------------------------

Sly2p  HSFANEYPKPTVRPYQFVNFDNFLQMTKKSYSDKK
Sly12p -----------------------------------

Sly2p  VQDNLDQLNQELVGVKQIMSKNIEDLLYRGDSLDK
Sly12p ___QMGAMGQRIKALKSLSLKMGDEI__RG_SNQT

Sly2p  MSDMSSSLKETS_KRYRKSAQKINFD__LLISQYA
Sly12p IDQLGDTFHNTSVKLKRTFGNMMEMARRSGISIKT

Sly2p  PIVIVAFFFVFLFWWIFLK
Sly12p WLIIFFMVGV_LFFWVWIT
```

B

```
aa125 S D K K V Q D N L D Q L N Q E L V G V . K Q I M S
aa 61 . . . . . . . . Q M G A M G Q R I K A L . K S L S L
aa 43 A Q K K L Q . Q T Q A K V D E V V G I M R V N V E
aa 28 S N R R L Q . Q T Q A Q V D E V V D I M R V N V D

      K N I E . D . . L L Y R G D S L D K M S D M S S S
      K M G D . E . . . . I R G . S N Q T I D Q L G D T
      K V L E R D Q K L S E L G E R A D Q L E Q G A S Q
      K V L E R D Q K L S E L D D R A D A L Q A G A S Q

      L K E T S . . K R Y R K S A Q K I N F D . . L L I S
      F H N T S . V K L K R T F G N M M E M A R R S G I S
      F . E Q Q A G K L K R K Q W W A N M K . . . . . . .
      F . E T S A A K L K R K Y W W K N L K . . . . . . .

      Q Y A P I V I V A F F F V F L F W W I F L K •    Sly2p
      I K T W L I I F F M V G V . L F F W V W I T •    Sly12p
      M M I I L G V I A V V L L I I V L V S V W P S . . SB, Dro.
      M M I I L G V I C A I I L I I I I V . Y F S S •  SB, bov.
```

C

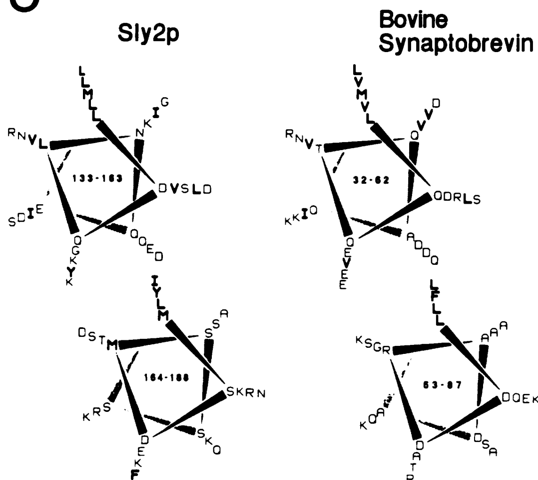

FIG. 7. Sequence comparison of yeast Sly2p and Sly12p and synaptobrevins from higher eucaryotes. (A) Comparison of amino acid sequences of Sly2p and Sly12p. Identities (bold) and favored substitutions are highlighted by shaded boxes. The C-terminal hydrophobic regions are boxed. The regular spacing of tyrosine and phenylalanine residues in the central region of Sly2p are indicated by large bold letters. (B) Alignment of c-terminal regions of Sly2p and Sly12p with the corresponding regions of synaptobrevins from *Drosophila* head (Dro.) and bovine brain (bov.) (66). Only those amino acid (aa) residues are highlighted that are conserved or identical (bold) between at least one Sly protein and one of the synaptobrevins. The hydrophobic C-terminal regions are boxed. (C) Helical wheel analysis of portions of Sly2p and bovine synaptobrevin. In each case, two consecutive segments are displayed (see numbers within the schematic α helix). The idealized α helix is drawn according to the conventions given by Landschulz et al. (35).

dependent on the recombinant plasmids carrying the *LEU2* marker gene. The small number of His$^+$/Leu$^+$ colonies might be explained by an unequal transmission of plasmids to different spores during meiosis if one assumes that a certain number of vectors containing the suppressor genes is required to allow growth of the *ypt1*$^-$ spores.

To confirm that *SLY2*, *SLY12*, and *SLY41* are multicopy suppressors, the cloned sequences harboring the suppressing activity were either cloned into the single-copy vector YCp50 (*SLY2* and *SLY12*) to transform a *GAL10-YPT1* haploid strain or duplicated in the genome (*SLY41*) through integration of a recombinant vector lacking the 2μm origin of replication (for details, see Materials and Methods). None of the Leu$^+$ transformants survived the shutdown of *YPT1* transcription on glucose medium. This result proved that the three genes are indeed multicopy suppressors. *SLY3*, *SLY6*, and *SLY13*, which were unable to suppress the deletion of *YPT1*, were not analyzed further.

***SLY2* and *SLY12* encode related proteins similar in structure to synaptobrevin.** Deletion analysis of the cloned DNA fragments contained in YEp13-SLY2, YEp13-SLY12, and YEp13-SLY41 established that the suppressor activities were confined to rather short regions, a 1.5-kb *Bam*HI-*Eco*RV fragment (*SLY2*), a 1.8-kb *Nco*I-*Hin*dIII fragment (*SLY12*), and a 1.6-kb *Hin*dIII-*Sau*3A fragment (*SLY41*). These or longer fragments were subjected to sequence analysis. In the case of *SLY2*, the longest open reading frame indicated a protein of 214 amino acids (Fig. 5). This predicted reading frame correlates well with the 0.9-kb *SLY2* mRNA (Fig. 3B) if one allows for about 200 nucleotides of untranslated sequence.

The nucleotide sequence of *SLY12* (Fig. 6) revealed the presence of a 131-bp intron interrupting the fourth codon. The intron was predicted from the highly conserved yeast splice site sequences, GTATGT at the 5′ and TAG at the 3′ splice junction, and the branchpoint TACTAAC sequence near the 3′ splice site (22, 36, 37). The predicted 5′ splice site sequence of the *SLY12* intron, GTATGA, deviates by one nucleotide from the more typical GTATGT sequence, but there is at least one other yeast gene, *S10-2* (39), that has the same GTATGA donor sequence. As no other typical 5′ splice site sequence was found up to the *Nco*I restriction site (position −442; Fig. 6) and introns typically interrupt *S. cerevisiae* protein-coding regions within the first few codons (22), the predicted intron size and location must be correct. According to the DNA sequence, the *SLY12* gene encodes a protein of 142 amino acids. This size is compatible with the length of the *SLY12* mRNA, which by RNA blot analysis was shown to be 0.6 kb long (Fig. 3B).

An alignment of Sly2p and Sly12p sequences is shown in Fig. 7A. At their C termini, both proteins contain a highly hydrophobic stretch of more than 20 amino acids rich in aromatic amino acids, which is followed by only one polar or charged residue. Sly12p shares other sequence similarities with Sly2p besides the hydrophobic C terminus. Between

A

```
GCTTACGATATTTAGAGAGTAAGTGGATTGACGATAAAAGTGTTTAATAAGGAGCCATTC  -34
GTTTTTATAAAGGAGAAACAATACCGGCCTAACATGATTCAAACGCAAAGTACAGCGATC   27
                                  M  I  Q  T  Q  S  T  A  I     9
AAACGACGTAATTCTGTTCATAAAAACCTCTTTGATCCATCACTGTATCAAATACCAGAA   87
 K  R  R  N  S  V  H  K  N  L  F  D  P  S  L  Y  Q  I  P  E    29
CCACCACGGGGGTTCCAACACCAAAAAAAAGAATACAGTAAGGAGACATTCAGTAATCAA  147
 P  P  R  G  F  Q  H  Q  K  K  E  Y  S  K  E  T  F  S  N  Q    49
GTCTTTGGATATGATATTACTAGCCTTAAAAAAAGATTCACGCAACTATTTCCTAGTAAT  207
 V  F  G  Y  D  I  T  S  L  K  K  R  F  T  Q  L  F  P  S  N    69
ATACAAGGGTACTTACCTGAAGTTGACCTAAGAATAACCATTATTTGTTCTATATGGTAC  267
 I  Q  G  Y  L  P  E  V  D  L  R  I  T  I  I  C  S  I  W  Y    89
GTCACGTCATCTATTTCAAGTAACCTATCTAAAGCCATTTTAAGAACTTTCAACCATCCC  327
 V  T  S  S  I  S  S  N  L  S  K  A  I  L  R  T  F  N  H  P   109
ATAGCTCTCACAGAATTGCAATTTCTTGTTAGTGCTGTTCTATGTGTTGGATTTGCTTCC  387
 I  A  L  T  E  L  Q  F  L  V  S  A  V  L  C  V  G  F  A  S   129
ATCGTAAATTTATTCCGACTACCTCGTTTGAAGCATACGAAGTTTTCAAAGGCACTCAAT  447
 I  V  N  L  F  R  L  P  R  L  K  H  T  K  F  S  K  A  L  N   149
AGTTTCCCTGACGGTATCCTTCCTGAATATCTCGATGGCAATTTCAGGAGTTCTATCCTG  507
 S  F  P  D  G  I  L  P  E  Y  L  D  G  N  F  R  S  S  I  L   169
CACAAGTTTTTAGTTCCCTCAAAGCTGGTTTTGATGACCACTTTCCCTATGGGAATATTC  567
 H  K  F  L  V  P  S  K  L  V  L  M  T  T  F  P  M  G  I  F   189
CAATTTATTGGTCATATCACATCGCACAAGGCGGTATCTATGATACCAGTATCACTAGTG  627
 Q  F  I  G  H  I  T  S  H  K  A  V  S  M  I  P  V  S  L  V   209
CATTCCGTGAAGGCATTATCCCCAATAATAACAGTTGGCTACTATAAATTTTTCGAACAT  687
 H  S  V  K  A  L  S  P  I  I  T  V  G  Y  Y  K  F  F  E  H   229
CGTTATTACAATTCCATGACTTATTATACCTTGTTACTTTTAATTTTTGGCGTTATGACT  747
 R  Y  Y  N  S  M  T  Y  Y  T  L  L  L  L  I  F  G  V  M  T   249
ACTTGCTGGTCAACACATGGCAGTAAGAGGGCTTCAGATAACAAAAGCGGATCTTCATTA  807
 T  C  W  S  T  H  G  S  K  R  A  S  D  N  K  S  G  S  S  L   269
ATCGGGTTGCTTTTTGCCTTTATTTCCATGATAATATTTGTAGCACAGAATATTTTTGCA  867
 I  G  L  L  F  A  F  I  S  M  I  I  F  V  A  Q  N  I  F  A   289
AAGAATATTTTAACCATCAGAAGGAAGGTAGGAATACTGCCGTCTTCTTCTACGGATGAC  927
 K  N  I  L  T  I  R  R  K  V  G  I  L  P  S  S  S  T  D  D   309
GTCACGTCGAAGGAAGGGCAACCGAGTCTAGACAAAACAAGATTTTCTCCATTGCAAGTG  987
 V  T  S  K  E  G  Q  P  S  L  D  K  T  R  F  S  P  L  Q  V   329
GATAAGATTACCATATTGTTCTACTCGTCCTGCATTGGGTTTTCTTTAACCCTATTACCT 1047
 D  K  I  T  I  L  F  Y  S  S  C  I  G  F  S  L  T  L  L  P   349
TTTTTAACCGGCGAATTAATGCATGGCGGTAGCGTTATCAACGATTTAACGCTAGAAACA 1107
 F  L  T  G  E  L  M  H  G  G  S  V  I  N  D  L  T  L  E  T   369
GTAGCCCTTGTAGCGATTCATGGAATAGCCCATTTTTTCCAAGCAATGCTTGCTTTCCAG 1167
 V  A  L  V  A  I  H  G  I  A  H  F  F  Q  A  M  L  A  F  Q   389
TTGATCGGTTTACTATCTTCCATTAATTATTCGGTAGCAAACATCATGAAGAGGATTGTT 1227
 L  I  G  L  L  S  S  I  N  Y  S  V  A  N  I  M  K  R  I  V   409
GTTATATCCGTGGCACTCTTTTGGGAAACAAAGTTAAATTTTTTTCAGGTGTTTGGTGTT 1287
 V  I  S  V  A  L  F  W  E  T  K  L  N  F  F  Q  V  F  G  V   429
ATCTTGACAATTGCCGGATTGTACGGTTATGACAAATGGGGGCTTTCCAAAAAAGATGGA 1347
 I  L  T  I  A  G  L  Y  G  Y  D  K  W  G  L  S  K  K  D  G   449
CGTCAGGCATAATATGAAGTAATACTATTGAGTACAAACCATCATTCAAGGTAGGACACT 1407
 R  Q  A  *                                                   452
TAGTACTATATCAAATATATAATCAAAAAAAAAACCCTCGAAGGTCTTTTTTATCTTATT 1467
TACTGCTACTACCTCTGTTGCTAGCATTACTTCAAGATCC                     1507
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B

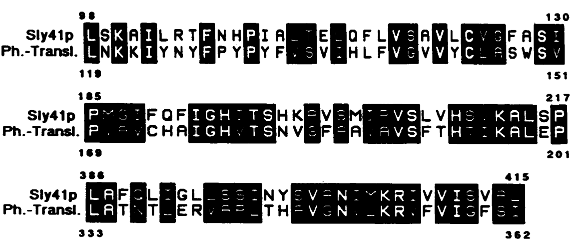

FIG. 8. Nucleotide sequence of the *SLY41* gene and comparison of Sly41p with the phosphate translocator of spinach chloroplasts. (A) Nucleotide sequence of the 1.6-kb *Hin*dIII-*Sau*3A fragment that carries the suppressor function. (B) Comparison of three regions of Sly41p and the phosphate translocator from spinach chloroplasts (20) that display the highest degree of homology (regions 1, 2, and 3 indicated in Fig. 9).

two of these regions, there is a 74-residue-long gap at position 62 of Sly12p. The domain, present only in Sly2p, is very unusual in having either tyrosyl or phenylalanyl residues in every eighth position.

Notably, the primary structures of Sly2p and Sly12p resemble that of VAMP-1 from *Torpedo* electric lobe (76) and synaptobrevin from bovine brain and *Drosophila* head (67). These proteins are known to be integral membrane proteins found in synaptic vesicles. The hydrophobic carboxy-terminal domain serves as a membrane anchor, with the rest of the protein exposed to the cytoplasm (67, 76). The highest degree of conservation between these proteins from different species is found in a region of about 65 amino acids which precedes the membrane-spanning domain (67). This region is flanked by a group of basic amino acids, and it can be readily aligned with the corresponding regions from Sly2p and Sly12p. If these 52 amino acid residues are aligned as shown in Fig. 7B, 30.8 or 32.6% identity, respectively, is observed between Sly2p and synaptobrevin from either bovine brain or *Drosophila* cells. The alignment of 47 amino acids of the same region of Sly12p and the two synaptobrevins shows 21.3% identical residues. These domains may have maintained the ability to form similar secondary structures. Secondary structure predictions (24) strongly suggest that large parts of these domains exist in an α-helical conformation. Helical wheel analysis (Fig. 7C) of these domains reveals a nonrandom distribution of hydrophobic as well as charged and polar amino acids around the putative helices.

***SLY41* encodes a protein related to the phosphate translocator from chloroplasts.** Sequence analysis of the 1.6-kb *Hin*dIII-*Sau*3A fragment of YEp13-SLY41 containing the suppressor of *YPT1* deletion disclosed a long open reading frame for a very hydrophobic protein of 452 amino acids (Fig. 8A). The predicted size of this protein is consistent with the 1.6-kb transcript identified on Northern blots with a ^{32}P-labeled fragment of the deduced *SLY41* coding region (Fig. 3B).

Four different regions of Sly41p give hydrophobicity values of more than 1.6 as determined according to Kyte and Doolittle (34), using a window of 19 amino acids. Thus, Sly41p may contain at least four transmembrane domains. If a protein forms or is part of a channel embedded in the membrane, it may also contain amphiphilic helices. A structure prediction analysis which uses an algorithm for plotting the hydrophobicity along the side of putative α helices (31) predicts that Sly41p may indeed form nine membrane-spanning α helices, two of them hydrophobic and seven amphiphilic (data not shown). Corroborating these assumptions, the channel-forming phosphate translocator of spinach chloroplasts (20) was the only protein detected by a computer-assisted alignment that exhibits significant similarity with the primary sequence of Sly41p. Two different algorithms, RDF2 (51) and one written by Argos (2), found three regions of high similarity (standard deviations of up to 6); these regions are indicated in the hydropathy profiles presented in Fig. 9. A comparison of these sequences is shown in Fig. 8B. Most remarkably, Lys-353 and Arg-354, which are thought to be involved in substrate binding by the phosphate translocator (79), correspond well with Lys-406 and Arg-407 in Sly41p (Fig. 8B). These basic amino acids are located at equivalent positions within similar amphipathic helices at a distance of 45 to 50 amino acids from the C terminus.

***SLY12* but not *SLY2* or *SLY41* is an essential gene.** Since the *SLY* genes were isolated by complementation of a defective *YPT1* gene, their protein products might function in the same

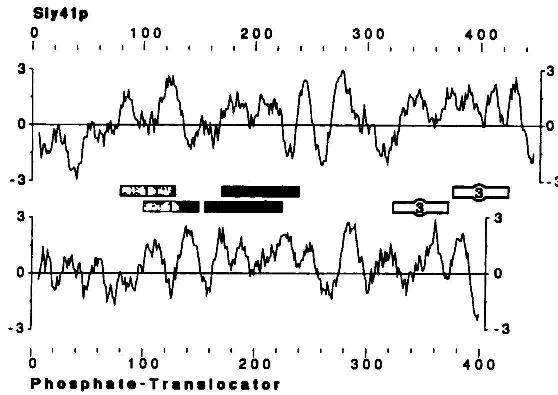

FIG. 9. Hydropathy profile of Sly41p and the phosphate translocator from spinach chloroplasts. Hydrophobicity of Sly41p and the phosphate translocator was determined according to Kyte and Doolittle (34) with a window of 11. The relative positions of the three regions displaying significant homologies, as determined according to Argos (2), are indicated.

pathway as the GTP-binding protein. In a first attempt to prove this, mutants were constructed with disruptions in the multicopy suppressors. Different marker genes, *HIS3*, *LEU2*, and *URA3*, were inserted into the coding regions of *SLY2*, *SLY12*, and *SLY41*, respectively (Fig. 10 to 12). Linear DNA fragments containing the disrupted genes were used to transform diploids homozygous for either *his3*, *leu2*, or *ura3* defects. In each case, two representative transformants were shown by Southern blot analysis to have one disrupted and one wild-type allele and were subjected to sporulation. Tetrad analysis showed that insertion of the *URA3* gene into the middle of the *SLY41* coding region (codon 208) had no effect on spore viability. Of 32 tetrads dissected, 28 gave rise to four spores that formed colonies of equal size. The severe truncation of Sly41p was without any effect on cell viability, which suggests, but does not necessarily prove, that this protein has no essential function.

Different results were obtained when *SLY2* or *SLY12* was inactivated. No viable spores carrying the *LEU2* marker inserted into codon 117 of the *SLY12* gene were obtained. None of 37 tetrads dissected produced more than two viable spores, and all of the 53 viable spores were Leu$^-$; i.e., they contained the wild-type *SLY12* allele. Microscopic inspection of the inviable spores showed that about 25% of them had germinated but stopped dividing after not more than two cell divisions. Thus, the *SLY12* gene may encode a function that is essential for yeast vegetative growth. For the disruption of *SLY2*, 57 and 96 bp around the *NcoI* site at codon 64 were deleted by BAL 31 digestion before insertion of the *HIS3* marker into the protein-coding region. As the 5′ deletion endpoints differed by one base pair, truncated proteins could be formed with the N-terminal 55 amino acids of Sly2p fused to either 17 or 9 amino acids encoded by the inserted DNA. In both cases, about 80% of the tetrads derived from different transformants having one wild-type and one disrupted *SLY2* allele produced four viable spores. After 2 days at 25°C, there was a clear 2:2 segregation in the size of the colonies. The smaller colonies were always His$^+$ and therefore carried the *SLY2* disruption. They were also strictly cold sensitive (Cs$^-$), and about half of them were thermosensitive (Ts$^-$), as judged by their inability to grow on plates at 15 and 37°C, respectively. The heterogeneity with respect to the Ts$^-$ phenotype of *sly2*$^-$ cells might be

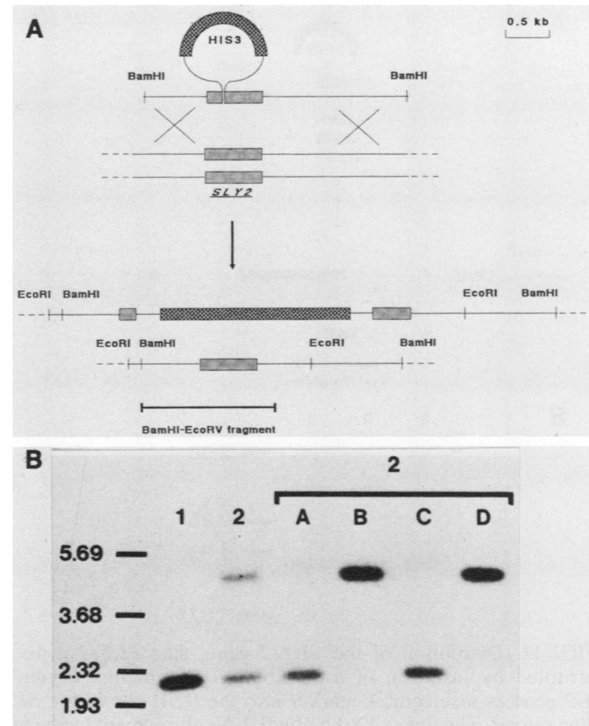

FIG. 10. Disruption of the *SLY2* gene. The *HIS3* gene as a selectable marker was inserted into the *SLY2* coding region to disrupt the *SLY2* gene as described in Materials and Methods. A linear 5.2-kb *SmaI-SalI* fragment carrying the disrupted *SLY2* gene was used to transform a diploid *his3/his3* strain (DAH430). (A) The 1.5-kb *BamHI-EcoRV* fragment used for Southern analysis. Relevant restriction sites are indicated. (B) Southern blot analysis to demonstrate the correct integration of the disrupted gene into one chromosome. Chromosomal DNAs from the untransformed His$^-$ diploid strain (lane 1), from the His$^+$ transformant (lane 2), and from the four spores derived from the His$^+$ transformant (lanes 2A to 2D) were digested with *EcoRI* and probed with the ^{32}P-labeled 1.5-kb *BamHI-EcoRV* fragment. The hybridizing 4.3-kb fragment in lanes 2, 2B, and 2D indicates the correct integration and disruption of one *SLY2* allele. The sizes of marker fragments (in kilobases) are shown on the left.

explained by the fact that the diploid strain used for transformation was not absolutely isogenic and contained a modifier gene determining this phenotype of some *sly2::HIS3* haploids. To prove this, a Cs$^-$/Ts$^-$ spore (*sly2::HIS3*) was crossed to a wild-type spore (*SLY2*) having a Ts$^-$ background, as inferred from the Ts$^+$ phenotype of the two Cs$^-$ spores within the same tetrad. The wild-type strain used in this cross was derived from a tetrad containing two Cs$^-$ spores that were not Ts$^-$. Assuming that the modifier gene segregates in a 2:2 fashion, this strain should carry the allele causing heat sensitivity of *SLY2*-disrupted cells. The resulting diploid gave rise to *sly2::HIS3* spores that, without exception, were Cs$^-$ and Ts$^-$.

DISCUSSION

We have isolated four genes able to suppress the deletion of *YPT1*, an essential yeast gene encoding a small GTP-binding protein. Three of the genes identified are multicopy suppressors (*SLY2*, *SLY12*, and *SLY41*), while the *SLY1-20* mutant allele exerts its suppressing activity when present in

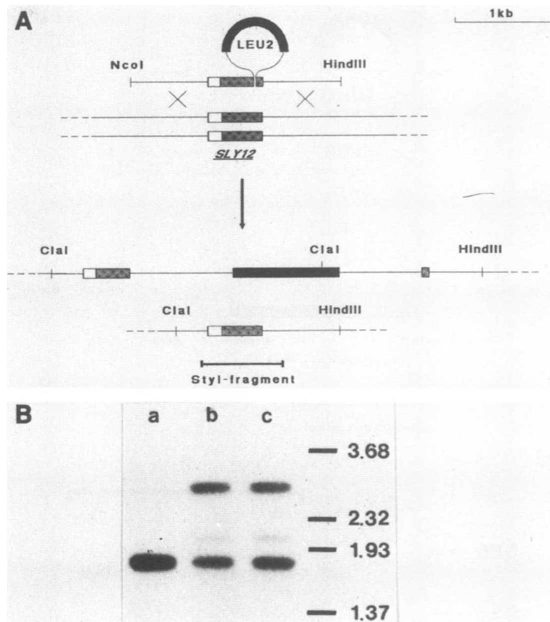

FIG. 11. Disruption of the *SLY12* gene. The *SLY12* gene was interrupted by insertion of a 3.0-kb *Bgl*II fragment carrying the *LEU2* gene as a selectable marker into the *Bgl*II site of the *SLY12* coding region. The linear 4.8-kb *Hin*dIII-*Nco*I fragment carrying the disrupted *SLY12* gene was used to transform a diploid Leu$^-$ strain. Transformants with a wild-type *SLY12* gene on one chromosome and a disrupted *SLY12* gene on the other were identified by Southern blot analysis. (A) Relevant restriction sites and the 0.83-kb *Sty*I fragment used as hybridization probe for Southern blot analysis. (B) Southern blot analysis. Chromosomal DNAs from the untransformed Leu$^-$ diploid strain (lane a) and from two Leu$^+$ transformants (lanes b and c) were digested with *Cla*I and *Hin*dIII and probed with the ^{32}P-labeled 0.83-kb *Sty*I fragment. The appearance of the additional hybridizing 2.8-kb fragment indicates correct integration and disruption of one *SLY12* allele. The sizes of marker fragments (in kilobases) are shown on the right.

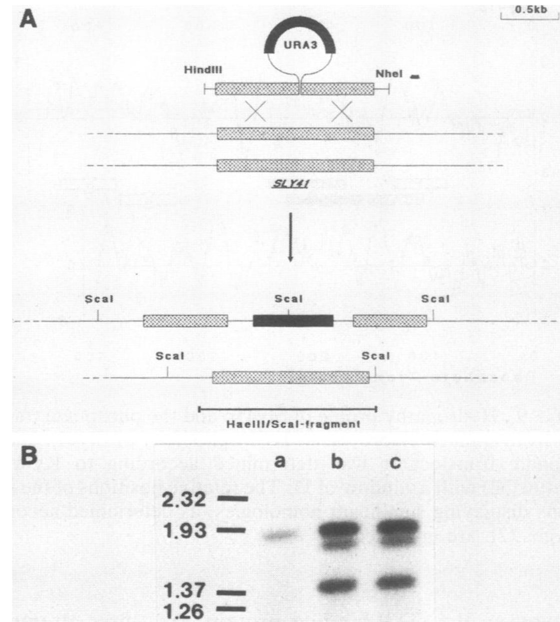

FIG. 12. Disruption of the *SLY41* gene. The *SLY41* gene was interrupted by insertion of a 1.2-kb *Hin*dIII fragment carrying the *URA3* gene as a selectable marker into the *Xba*I site of the *SLY41* coding region. The linear 2.7-kb *Hin*dIII-*Nhe*I fragment was used to transform a diploid Ura$^-$ strain. (A) Relevant restriction sites and the 1.4-kb *Hae*III-*Sca*I fragment used for Southern blots. (B) Southern blot analysis. Chromosomal DNAs from the untransformed Ura$^-$ diploid strain (lane a) and from two Ura$^+$ transformants (lanes b and c) were digested with *Sca*I. The appearance of the two additional hybridizing 1.6- and 1.3-kb fragments indicates correct integration and disruption of one *SLY41* allele. The sizes of marker fragments (in kilobases) are shown on the left.

only one copy. From a published restriction map of a cloned DNA fragment carrying the *BET1* gene, whose protein product acts in the ER-to-Golgi transport (48), it seems possible that *BET1* and *SLY12* are identical genes.

The selection of genes suppressing the deletion instead of point mutations of an essential gene has certain advantages. It precludes the isolation of intragenic suppressors and suppressors that simply act by altering the expression of the mutant gene or the processing, stability, or translational efficiency of its transcription product. Both the suppression of a deletion and the suppression by overproduction are indicative of a suppressor gene that either replaces the deleted gene in the same pathway or else induces a pathway that bypasses the need for the deleted gene. Other investigators have shown that in yeast cells, the suppression of a deletion (15) as well as multicopy suppression (for review, see reference 7) can be due to genes that are functionally related to the defective gene. Interestingly, four yeast genes encoding small GTP-binding proteins, *SEC4* (57), *SAR1* (47), *RSR1* (7), and *CDC42* (7), have been identified as multicopy suppressors whose protein products are structurally unrelated to those of the genes they suppress. Similarly, functional relatedness but totally different primary structures are now observed in the case of Ypt1p and the *SLY* gene products that can compensate for the complete loss of the GTP-binding protein. In particular, sequence motifs typical for GTP-binding proteins are absent from the different Sly proteins. Sly2p, Sly12p, and Sly41p may be integral membrane proteins of either ER, Golgi, or vesicular structures, cellular compartments involved in the functioning of Ypt1p (4, 60, 63). Evidence for membrane localization has been obtained for Sly2p (49a).

How can one rationalize that overexpression of a gene complements the deletion of an essential gene encoding a protein with totally unrelated primary structure? This kind of genetic complementation is often found in signal transmission pathways, in which a defect of an essential protein can be suppressed by overexpression of a downstream element. For example, a functional adenylyl cyclase is dispensable in yeast cells overexpressing different cyclic AMP-dependent protein kinases (72). In contrast, proteins like Ypt1p and Sec4p are thought to act in unidirectional vesicle transport (26, 63), in which the transport cycle is regulated by the switch from a GDP- to a GTP-bound form. As was first pointed out by Bourne (11), the role of these proteins might be analogous to that played by the elongation factor EF-Tu, a GTP-binding protein that increases translational accuracy (71). According to this hypothesis, Ypt1p in its GTP-bound state would bind to a protein on the vesicle surface and, simultaneously, to a docking protein on the target membrane. GTP hydrolysis and release of the GDP-bound protein would follow after vesicle docking to the correct target membrane.

Speculating along this line, one might envisage that specific vesicle surface or target membrane proteins used for vesicle docking are usually limiting, so that vesicle attachment to the acceptor membrane would be a rather inefficient process in the absence of the GTP-binding protein. Under conditions of an excess of either of these membrane components, the efficiency of vesicle docking might be increased such that the rate of protein transport would be sufficient to allow cell viability in the absence of Ypt1p. This may be the mechanism by which the multicopy suppressors *SLY2* and *SLY12* act. It is possible that the *SLY2* and *SLY12* genes code for vesicle-specific surface or docking proteins, since they contain domains with a degree of hydrophobicity that is sufficient to span a membrane. As noted above, Sly2p and Sly12p may be able to assume a secondary structure similar to that of the VAMP-1/synaptobrevin proteins from higher eucaryotes in a region preceding the C-terminal hydrophobic transmembrane region. If, indeed, parts of these domains exist in an α-helical conformation, hydrophobic residues will always be limited to a very thin ridge on one face of the helix (Fig. 7C). This structure would enable these proteins to form dimers composed of identical or different subunits (35). The α-helical regions may form long and flexible coiled-coil structures which expose the N-terminal domain of the protein (possibly a true receptor domain) in some distance from the surface of the membrane.

The single-copy suppressor *SLY1-20* may encode a protein acting downstream of Ypt1p. In contrast to the multicopy suppressors, the *SLY1* gene must carry a specific point mutation in order to complement the loss of Ypt1p. Overexpression of the wild-type *SLY1* allele has no equivalent effect. Therefore, the protein encoded by the *SLY1-20* mutant gene may exist in a specific conformation which the wild-type protein can adopt only after a conformational change is induced, perhaps in a complex with Ypt1p. A more trivial explanation would be the activation by *SLY1-20* of a parallel pathway substituting for the Ypt1p-requiring one. There is evidence, however, that Sly1p itself is required for the ER-to-Golgi transport (49a). Sly1p does not exhibit a significant hydrophobic character. This is not unusual, since most of the cloned and sequenced *SEC* genes in yeast cells, *SEC2* (45), *SEC7* (1), *SEC14* (5), *SEC15* (56), *SEC18* (19), *SEC23* (29), and *SEC53* (8), code for cytosolic factors that most likely are only transiently associated with membranes through the interaction with other proteins. Only three of the characterized *SEC* genes, *SEC11* (10), *SEC12* (46), and *SEC59* (9), are known to code for integral membrane proteins. Ypt1p and Sec4p, on the other hand, are bound to membranes through their C-terminally located cysteine residues, which in the case of Ypt1p have been shown to be modified by lipidation (43, 78).

The mechanism by which the *SLY41* gene product may act is certainly different from that of the other suppressors. The sequence similarity of Sly41p with the phosphate translocator from spinach or pea chloroplasts (20, 79) might suggest that *SLY41* codes for a channel protein which could translocate ions or metabolites across intracellular membranes. It is known that particular *ypt1* defects can be suppressed either by adding Ca^{2+} to the growth medium (60) or by defects in the Ca^{2+} ATPase (Pmr1p) which may sequester cytosolic Ca^{2+} into a storage compartment or into a compartment of the secretory pathway (54). This suppression seems to be rather nonspecific, since cell-free transport showed that Ca^{2+} addition could not restore protein transport in extracts from *ypt1* mutants (3) and that the Ypt1p-requiring step precedes the calcium-dependent one (4). Nevertheless, a defect in the transport of Ca^{2+} into specific membrane compartments or an increase in the phosphate release from such compartments seems to have comparable effects on *ypt1* defects. In contrast to *pmr1* mutations, however, which can suppress only the cold-sensitive *ypt1-1* mutant (54), Sly41p overproduction can suppress the deletion of *YPT1*. Moreover, the suppression by Sly41p overproduction is specific for *ypt1* defects, while the other $ypt1^-$ suppressors can also improve the growth of certain *sec* mutants at their restrictive temperatures (49a). Unfortunately, the $sly41^-$ cells are viable and show no particular phenotype when tested under different growth conditions. Therefore, the analysis of mutants may fail to determine the normal function of this gene.

SLY1, *SLY12*, and *SLY2* either are essential for cell viability or confer conditional lethality. This characteristic makes it possible not only to analyze the mode of their $ypt1^-$-complementing activity but also to determine the normal cellular functions of these genes.

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