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GIT1, a Gene Encoding a Novel Transporter for Glycerophosphoinositol in *Saccharomyces cerevisiae*

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ABSTRACT

Phosphatidylinositol catabolism in *Saccharomyces cerevisiae* cells cultured in media containing inositol results in the release of glycerophosphoinositol (GroPIns) into the medium. As the extracellular concentration of inositol decreases with growth, the released GroPIns is transported back into the cell. Exploiting the ability of the inositol auxotroph, *ino1*, to use exogenous GroPIns as an inositol source, we have isolated mutants (Git^-) defective in the uptake and metabolism of GroPIns. One mutant was found to be affected in the gene encoding the transcription factor, *SPT7*. Mutants of the positive regulatory gene *INO2*, but not of its partner, *INO4*, also have the Git^- phenotype. Another mutant was complemented by a single open reading frame (ORF) termed *GIT1* (**g**lycerophospho**i**nosi**t**ol). This ORF consists of 1556 bp predicted to encode a polypeptide of 518 amino acids and 57.3 kD. The predicted Git1p has similarity to a variety of *S. cerevisiae* transporters, including a phosphate transporter (Pho84p), and both inositol transporters (Itr1p and Itr2p). Furthermore, Git1p contains a sugar transport motif and 12 potential membrane-spanning domains. Transport assays performed on a *git1* mutant together with the above evidence indicate that the *GIT1* gene encodes a permease involved in the uptake of GroPIns.

PHOSPHATIDYLINOSITOL (PI) is an essential membrane component of the yeast *Saccharomyces cerevisiae.* While PI acts as a precursor to several other phospholipids, including sphingolipids [IPC, MIPC, $M(IP)_2C$] and polyphosphoinositides (PIP, PI 3-P, PIP_2), it can also be deacylated to form extracellular glycerophosphoinositol (GroPIns). The production of extracellular GroPIns is a major catabolic pathway in *S. cerevisiae*, accounting for ~50% of the phosphorus and inositol lost from PI during growth in rich medium (Angus and Lester 1972). The production of extracellular GroPIns is regulated by both glucose and inositol (Angus and Lester 1975; Patton *et al.* 1995) and is postulated to be the result of the activity of cell surface phospholipases localized in the plasma membrane and/or periplasmic space (Angus and Lester 1975). *S. cerevisiae* not only releases GroPIns into the medium but is also capable of transporting GroPIns back into the cell in an energy-requiring process regulated by the amount of inositol in the medium (Patton *et al.* 1995).

Phosphoinositide deacylation, resulting in the production of lysophosphoinositols and GroPIns, has important biological consequences in mammalian cells. For example, lysophosphatidylinositol has been shown to have mitogenic activity in k-*ras*-transformed epithelial cells (Falasca and Corda 1994). Furthermore, the level of GroPIns increases during hemopoietic cell differentiation (Mountford *et al.* 1994). Similarly, during transformation by *ras* and other oncogenes, the amounts of GroPIns and polyphosphoinositide deacylation products increase (Valitutti *et al.* 1991; Corda and Falasca 1996). GroPIns-4-P has been reported to be an inhibitor of adenylate cyclase in thyroid cells (Iacovelli *et al.* 1993), and its formation has been shown to be hormone-induced (Falasca *et al.* 1997). In *S. cerevisiae*, the addition of glucose to glucose-starved cells stimulates the production of extracellular GroPIns, GroPIns-4-P, and GroPIns-4,5-P_2 (Hawkins *et al.* 1993). This suggests that the nutrient signal of glucose refeeding activates a phospholipase(s) and/or lysophospholipase that act to deacylate the respective phosphoinositides. Thus, *S. cerevisiae* would appear to be a good system in which to study phosphoinositide deacylation and subsequent metabolic events.

S. cerevisiae requires a source of inositol to synthesize the inositol-containing phospholipids. The required inositol can be obtained from endogenous biosynthesis or by transporting exogenous inositol into the cell. Inositol-1-phosphate synthase carries out the rate-limiting step in inositol biosynthesis and is encoded by the *INO1* gene (Carman and Henry 1989). By exploiting the ability of the inositol auxotroph, *ino1*, to use exogenous GroPIns as an inositol source, we have isolated mutants defective in the uptake and subsequent metabolism of GroPIns. One such mutant is defective in a single gene of previously unassigned function termed *GIT1* (**g**lycerophospho**i**nosi**t**ol), which appears to encode a transporter for GroPIns. To our knowledge, this is the first

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report of such a transporter in a eukaryotic cell. Furthermore, our analysis of the ability of yeast cells to take up GroPIns as a source of inositol provides a novel phenotype (Git$^-$) that is displayed in some mutants with an Ino$^-$ phenotype (*i.e.*, *spt7* and *ino2* mutants) and not in others (*i.e.*, *ino1* and *ino4* mutants).

MATERIALS AND METHODS

Materials: Materials and sources are as follows: crude soybean PI, GroPIns (Sigma Chemical Co., St. Louis); silica gel plates, GF/C filters (Whatman); Bio-Rex 70 (Bio-Rad, Richmond, CA); yeast media components (Difco, Detroit); oligonucleotides (GIBCO, Grand Island, NY); and glycerophospho-*myo*-[2-^{3}H]inositol (American Radiolabeled Chemicals Inc., St. Louis).

Isolation of GroPIns from crude soybean PI: Soybean PI (10 mg/plate) suspended in chloroform:methanol (1:1) was spotted onto 1 mm silica gel preparative plates (Whatman). The chromatograms were run in chloroform:methanol:acetic acid:water (32:4:5:1) and the lipids visualized with I_2 vapor. The band running at the same Rf as standard PI (Sigma Chemical Co.) was marked and scraped into a test tube following evaporation of the iodine. The PI was eluted from the silica gel with chloroform:methanol:water (16:16:5), the tube was centrifuged to sediment the silica gel, and the solvent containing PI was dried under N_2. The PI was resuspended in methanol:toluene (1:1) and subjected to mild alkaline methanolysis as described previously (Lester and Steiner 1968). The resulting GroPIns was desalted over a column of Bio-Rex 70 (50–100 mesh, sodium form), eluting with H_2O.

Strains and culture conditions: Strains (Table 1) were grown aerobically at 30° with shaking. Turbidity was monitored by measurement at A_{650} nm on a Beckman (Foster City, CA) DU 64 spectrophotometer. Synthetic complete media were prepared as described previously (Patton *et al.* 1995). Some media were supplemented with the indicated amounts of *myo*-inositol or GroPIns. YEPD media consisted of 20 g glucose, 10 g yeast extract, and 20 g bacto peptone per liter. The wild-type strain (*trp1 ura3 leu2 his3 MAT*α) and the *ino1* (JP3) strain (*trp1 ura3 leu2, his3, ino1::HIS3 MAT*α) were provided by P. McGraw (University of Maryland, College Park, MD). Strain T303 (*his3 leu2 ura3 ade2 MAT***a**) was provided by G. Carmen (Rutgers University, New Brunswick, NJ). Strain *spt7* (*spt7::LEU2 his4 leu2 ura3 MAT*α) and the YCp50-based plasmid containing the *SPT7* gene were provided by K. Arndt (University of Pittsburgh, PA).

Mutagenesis and genetic manipulations: The *ino1* (JP3) strain was subjected to ethylmethane sulfonate (EMS) mutagenesis as described by Lindegren *et al.* (1965) with a survival rate of 40%. Cells were spread onto YEPD plates and incubated at 30° for 3 days. Colonies were replica printed onto synthetic media (i) lacking inositol (I$^-$), (ii) containing 75 μm inositol (I$^+$), and (iii) lacking inositol but containing 25 μm GroPIns (GroPIns$^+$). After 3–4 days incubation at 30°, colonies able to grow on I$^+$ plates but unable to grow on I$^-$ plates and GroPIns$^+$ plates were selected. Two mutants isolated in this manner were backcrossed to an inositol auxotroph of the opposite mating type (JP16), and strains *git1 ino1* (JPM1) and *git2 ino1* (JPM2) were isolated from the resulting diploids by tetrad dissection.

Mating, sporulation, and tetrad dissection were performed using standard methodologies (Rose *et al.* 1990). Sporulation medium was supplemented with 100 μm inositol, since diploid strains that are inositol auxotrophs require exogenous inositol for sporulation (Schroeder and Breitenbach 1981).

Bacterial and yeast transformations: Bacterial strains were transformed with plasmid DNA using calcium chloride (Rose *et al.* 1990), and yeast strains were transformed using lithium acetate (Rose *et al.* 1990).

Isolation of the *GIT1* gene: The *GIT1* gene was cloned by complementation of the GroPIns auxotrophy of the *git1 ino1* (JPM1) mutant. DNA from the yeast genomic library YCp50-*LEU2* (obtained from P. Hieter) was transformed into the *git1 ino1* strain. Approximately 40,000 transformants were screened for leucine and GroPIns prototrophy and for inositol auxotrophy. Two colonies were isolated that contained a single complementing plasmid. The plasmid was recovered from yeast and amplified in *Escherichia coli.* Since the YCp50 library was constructed by inserting yeast DNA in the *Bam*HI site of the tetracycline resistance gene (tet^r) of the vector, an 18-base primer derived from a section of the tet^r gene ~40 bases from the *Bam*HI site was used for sequencing (courtesy of Mark Hiller, Carnegie Mellon University, Pittsburgh, PA). Using the Saccharomyces Genome Database (SGD), the open reading frames (ORFs) contained on the complementing plasmid were analyzed. A 2-kb *Bam*HI to *Cla*I fragment, containing a single ORF and 406 bp upstream of ATG, was subcloned into the vector pRS314 (Sikorski and Hieter 1989). The resulting plasmid, pJP100, was shown to contain the complementing activity. This ORF is YCR098C in the SGD, and the GenBank accession number is X59720. The identity of the gene contained in pJP100 was verified by sequencing into the gene on both ends using the universal priming sites (T7, T3) contained in the pRS314 plasmid.

Isolation of the *GIT2/SPT7* containing plasmid: The *GIT2* gene was cloned by complementation of the GroPIns auxotrophy of the *git2 ino1* (JPM2) mutant. DNA from the yeast genomic library YCp50-*LEU2* was transformed into the *git2 ino1* (JPM2) strain. The transformation efficiency of this strain was quite low and, out of those transformants, a relatively high percentage contained the complementing plasmid. Approximately 200 transformants were screened for leucine and GroPIns prototrophy and for inositol auxotrophy. Two colonies were isolated that contained a single complementing plasmid. The plasmid was recovered from yeast and amplified in *E. coli.* Sequencing was performed using a primer derived from the tet^r gene, as described for the sequencing of the *GIT1* gene. With the aid of SGD, the ORFs contained on the complementing plasmid were determined.

DNA sequencing: DNA was sequenced with an ABI PRISM 377 automatic DNA sequencer (University of Pittsburgh Research Support Facilities).

Construction of *GIT1* disruption alleles: A *git1::HIS3* disruption allele in which 1 kb of the *GIT1* ORF was replaced by *HIS3* was constructed using a PCR-targeting approach (Lorenz *et al.* 1995). Two bifunctional oligos were designed (GIBCO) that consisted of 45 nt at their 5′ ends homologous to the target sequences. The 3′ ends contained 20 nt homologous to the flanking regions of the marker gene. The marker gene was amplified from vector pRS303 using these primers. The resulting PCR product was used for transformation using a one-step gene disruption procedure. Integration at the correct locus would result in a disrupted *git1* locus in which nucleotides 288–1226 were removed and replaced with a 1.3-kb fragment of pRS303 bearing the *HIS3* gene. Histidine prototrophs were screened by PCR to verify integration at the correct locus.

Transport assays: Cell cultures were grown in I$^-$ synthetic media to log-phase (A_{650} of 0.3 to 0.8). The cells were harvested, washed with fresh media, and resuspended to an A_{650} of 5 in fresh media. Assays were started by adding 50 μl of glycerophospho[2-^{3}H]inositol at the indicated concentration to 200 μl of cell suspension. Assays were carried out for 5 min at 30°. Following termination of the assays by the addition of 10 ml

ice-cold water, cells were collected by filtration through glass fiber filters. The filters were washed with 20 ml water and subjected to liquid scintillation counting. For the experiments of Table 2, assays were performed with 10 μm glycerophospho[2-^{3}H]inositol and a 40-fold excess (400 μm) of the indicated nonradiolabeled compounds. The experiments involving GroPIns transport (Figure 2), and GroPIns production and reutilization (Figure 3) were performed three times with essentially identical results. Because these processes displayed some variation with slight changes in growth rate and culture density, statistical analysis was not performed on the independent experiments; only a single representative experiment is presented.

Determination of GroPIns and inositol in the media: Cells were inoculated in synthetic medium containing 10 μm inositol and 3 μCi/ml [^{3}H]inositol. At various time points, 1-ml aliquots of culture were removed and centrifuged to pellet the cells. The resulting supernatants were analyzed for inositol and GroPIns using anion exchange chromatography, as described previously (Patton *et al.* 1995). In short, the supernatants were passed through Dowex-1 resins, the resins were washed with water to remove all free [^{3}H]inositol, and [^{3}H]GroPIns was eluted from the resin with 5 mm sodium tetraborate, 60 mm ammonium formate. Liquid scintillation counting was performed in a Beckman (Fullerton, CA) LS5801 liquid scintillation counter using Ecolume liquid scintillation cocktail (ICN Biomedicals).

RESULTS

Identification of mutants defective in GroPIns utilization: The inositol auxotroph, *ino1* (JP3), is capable of using either inositol or GroPIns as an inositol source (Git$^+$ phenotype). Following EMS mutagenesis, approximately 8,000 colonies were screened for growth on media containing inositol and on media containing GroPIns. Previously, we reported that GroPIns can enter the cell via both inositol transporter-dependent and -independent pathways (Patton *et al.* 1995). Strains bearing a deletion in the minor inositol transporter gene, *ITR2*, do not display aberrant growth on inositol or GroPIns in an *ino1* background, so we did not expect to reisolate *itr2* mutants in this screen. However, during the screening, three mutants exhibiting poor growth in the presence of inositol were eliminated to avoid the reanalysis of *itr1* mutants defective in the major inositol transporter (Nikawa *et al.* 1991). Focusing only on mutants able to grow in the presence of inositol, but unable to grow when GroPIns was supplied as the sole source of inositol (Git$^-$ phenotype), seven mutants were identified for further study. The growth characteristics of two such mutants, *git1 ino1* (JPM1) and *git2 ino1* (JPM2), wild type, and the parental *ino1* (JP3) strain are shown in Figure 1.

Genetic analysis: To determine whether the mutations conferring the Git$^-$ growth phenotype were dominant or recessive with respect to wild type, the mutants were crossed to an *ino1* (Git$^+$) strain of the opposite mating type (JP16) and were examined for growth on GroPIns. All such diploids grew as well on GroPIns as the parent strain, *ino1* (JP3), demonstrating that the Git$^-$ growth phenotype was recessive; one mutant was dropped from further genetic analysis because of its inability to mate. To determine whether the mutant phenotype was conferred by a single gene, the diploids heterozygous for the mutation conferring the Git$^-$ phenotype but homozygous for *ino1* were sporulated, and the resulting tetrads analyzed for growth on GroPIns. One candidate was eliminated because of failure of its progeny to show 2:2 segregation of the Git$^-$ phenotype. Three candidates were eliminated from further genetic analysis because of the inability of the corresponding diploids to produce tetrads containing four viable spores. In progeny derived from two diploids simultaneously homozygous for *ino1* and heterozygous for *git1* or *git2*, the Git$^-$ mutant growth phenotype segregated 2:2 as expected for a phenotype conferred by a mutation in a single gene, although the sporulation efficiency was low. It is known that diploid strains homozygous for the *ino1* mutation cannot sporulate unless inositol is supplemented (Schroeder and Breitenbach 1981). However, the addition of 100 μm exogenous inositol did not completely cure the sporulation defect in our Git$^+$ Ino$^-$ (*i.e.*, *ino1* homozygous) strains. Nevertheless, out of the five full tetrads that we were able to obtain from the *git1 ino1* × *GIT1 ino1* (JP16) diploid and eight tetrads derived from the *git2 ino1* × *GIT2 ino1* (JP16) diploid, all segregated 2:2 with regard to growth on GroPIns. The *git1 ino1* (JPM1) and *git2 ino1* (JPM2) segregants obtained from those crosses were crossed to each other. In each case, the resulting diploids were able to grow on GroPIns, indicating that the mutations harbored in the *git1 ino1* (JPM1) and *git2 ino1* (JPM2) strains complement each other and are, thus, most likely to reside at different genetic loci.

GroPIns transport activity is down-regulated by the availability of inositol in the media and up-regulated by the absence of inositol (Patton *et al.* 1995). The phospholipid biosynthetic genes are known to be regulated in a similar fashion by the negative regulatory protein encoded by the *OPI1* gene and by the positive transcription factors encoded by the *INO2* and *INO4* genes (Carman and Henry 1989). Thus, we tested the positive regulatory mutants *ino2* (SH303) and *ino4* (SH307) for their ability to grow on GroPIns. The *ino4* (SH307) mutant was able to grow on GroPIns while the *ino2* (SH303) mutant was not (data not shown), suggesting that growth on GroPIns requires the *INO2* gene product but not the *INO4* gene product. To determine if an *ino2* mutant was reisolated in our screen, an *ino2* (SH303) inositol auxotroph was crossed to the Git$^-$ mutants and the resulting diploids were tested for their ability to grow on GroPIns. In each case, the diploids were able to grow on GroPIns, indicating that the mutations harbored in these Git$^-$ strains were not likely to be *ino2* alleles.

***GIT2* is allelic to *SPT7*:** The *git2* mutation was complemented using a YCp50-based genomic library. Partial

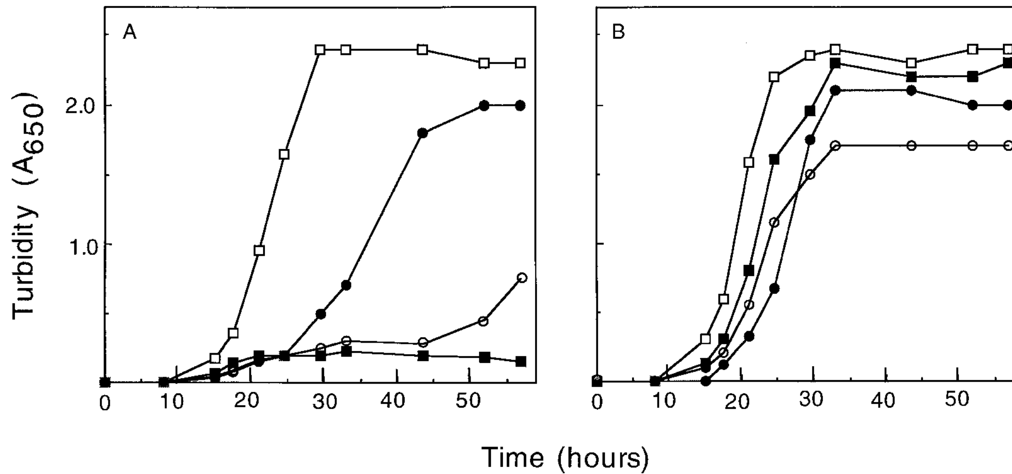

Figure 1.—Growth using (A) GroPIns or (B) inositol as an inositol source. Strains were inoculated at A_{650} = 0.005 into synthetic media containing either 50 μm GroPIns or 50 μm inositol. At the indicated times, A_{650} was measured. Wild type, □; *ino1* (JP3), ●; *git1ino1* (JPM1), ■; *git 2 ino1* (JPM2), ○.

sequencing of the complementing plasmid followed by data analysis utilizing the SGD localized the complementing activity to a 7-kb fragment on chromosome *II*. A single ORF (previously identified as *SPT7*) included within that fragment was focused upon, as it appeared most likely to contain the complementing activity. Based upon the following facts (data not shown), *GIT2* was determined to be identical to *SPT7*: (1) A YCp50 plasmid containing a 7-kb *Eco*RI-*Cla*I fragment encompassing the *SPT7* gene (Gansheroff *et al.* 1995) was able to complement the *git2* mutation. (2) A characterized *spt7* deletion mutant, which is an inositol auxotroph (Gansheroff *et al.* 1995), was crossed with an *ino1* strain (JP16) and sporulated, and random spore analysis was performed since the sporulation efficiency was quite poor. Both *spt7 INO1* and *spt7 ino1* (JP61) segregants derived from this cross were unable to grow on GroPIns. (3) Diploids produced by crossing an *spt7 ino1* strain (JP61) with two different *git2 ino1* segregants [JP2-9(5C) and JP2-9(9A)] from a cross of the original *git2 ino1* mutant to a *GIT1 ino1* (JP16) strain, were unable to grow on GroPIns and failed to sporulate. Since diploids homozygous for an *spt7* mutation have also been reported to be sporulation defective (Gansheroff *et al.* 1995), further genetic analysis on these diploids was not attempted. The plasmid bearing the *SPT7* gene was not able to complement the Git$^-$ phenotype of any of the six other mutants isolated in this study.

Cloning and disruption of *GIT1*: The *git1* mutation was complemented using a YCp50-based genomic library. Partial sequencing of the complementing plasmid allowed the *GIT1* gene to be localized to the right arm of chromosome *III*. A single ORF (YCR098C), labeled as "probable metabolite transport protein" in the SGD, was subcloned into vector pRS314 to produce pJP100 and was transformed into the *git1 ino1* mutant. This subclone contained the complementing activity (Figure 2). However, pJP100 was not able to complement the Git$^-$ phenotype of any of the six other mutants isolated in this study. The GCG BestFit program (University of Wisconsin, Madison, WI), revealed significant sequence homology between the predicted amino acid sequence of the *GIT1* ORF and the amino acid sequences of the *S. cerevisiae* transport proteins Pho84p, Itr1p, and Itr2p. Also, an analysis of the *GIT1* promoter region revealed the presence of a core basic helix-loop-helix binding motif, CACGTG (Crowther *et al.* 1996), and three Stress Response Element consensus sequences, AGGG or CCCT (Ruis and Schuller 1995), which could potentially be involved in transcriptional regulation.

A disruption allele of the cloned gene was created by replacing a 938-bp region in the middle of the ORF by a 1.3-kb DNA fragment bearing the *HIS3* gene; this fragment was used to replace the chromosomal copy of *GIT1* in a wild-type strain. The gene disrupted strain, *git1::HIS3* (JP89), no longer containing an *ino1* allele and having neither an Ino$^-$ nor a Git$^-$ phenotype, was crossed to a wild-type strain of the opposite mating type (T303) and the resulting diploid was sporulated. Since the *git1* mutation confers no growth phenotype in a wild-type genetic background (*i.e.*, in the absence of an *ino1* mutation), His$^+$ prototrophy was used to follow the

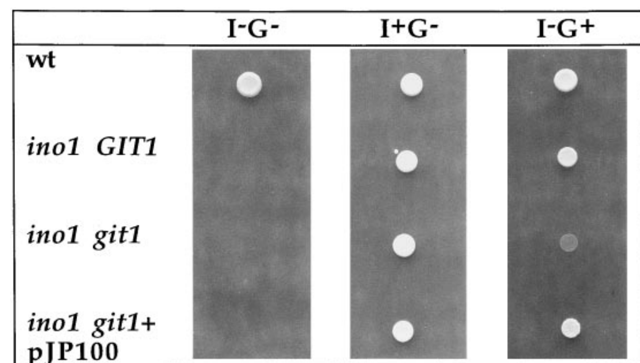

Figure 2.—Complementation of *git1* mutant with pJP100. Wt, *ino1* (JP3), *git1ino1* (JPM1), and *git1ino1* (JPM1) containing pJP100 (*git1ino1* + pJP100) were spotted onto plates lacking an inositol source (I$^-$G$^-$), containing 75 μm inositol but lacking GroPIns (I$^+$G$^-$), and lacking inositol but containing 75 μm GroPIns (I$^-$G$^+$). Plates were allowed to incubate at 30° for 4 days.

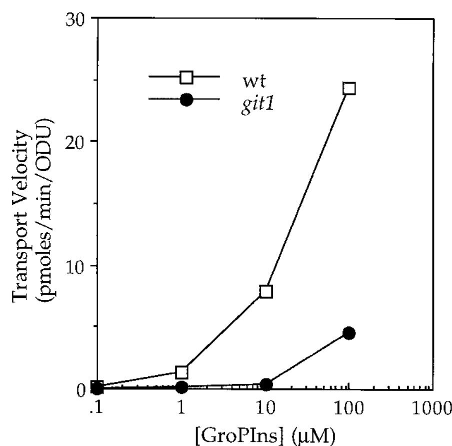

Figure 3.—GroPIns transport is greatly decreased in a *git1::HIS3* (JP89) deletion mutant. Transport assays were performed as described in materials and methods with the indicated concentrations of GroPIns. Wild type, □; *git1::HIS3* (JP89), ●.

gene disruption, and the *git1* phenotype was confirmed by assay of GroPIns transport activity. The 15 tetrads derived from this cross segregated 2:2 with regard to His$^+$ prototrophy. All four spores in each of three complete tetrads were assayed for GroPIns transport activity. The lack of GroPIns transport activity cosegregated with His$^+$ prototrophy, and all His$^-$ spores showed wild-type transport activity (data not shown). Furthermore, when the *git1 ino1* (JPM1) mutant was crossed to a strain of the *git1::HIS3 ino1* genotype [derived from sporulating a *git1::HIS3* (JP89) × *ino1* (JP16) diploid], the diploid was unable to grow on GroPIns, confirming that the constructed deletion mutation was most likely allelic to the original *git1* mutation.

Phenotypic analysis of *git1::HIS3* mutant: The doubling times and ultimate culture densities of the *git1::HIS3 INO1* (JP89) strain were nearly identical to wild type when it was grown in rich medium (YEPD) as well as in defined I$^+$, I$^-$, and GroPIns$^+$ media. The portion of chromosome *III* containing the ORF YCR098C had been analyzed as part of the genome sequencing project, and, consistent with the above observations, it was reported that disruption of this ORF results in no obvious phenotype as compared to wild type when tested for growth on lactose, lactate, or glycerol media. It also reportedly showed no osmotic or detergent sensitivity. Sporulation of the heterozygous and homozygous *git1* diploid strains was also reportedly normal (Sor *et al.* 1992), and we likewise observed no defect in the sporulation of *git1/git1* diploids that are Ino$^+$ (*i.e.*, not homozygous for *ino1*).

GroPIns transport activity: At concentrations of 10 μm and below, GroPIns transport ability was virtually abolished in the *git1::HIS3* (JP89) strain (Figure 3). At concentrations above 10 μm, the *git1::HIS3* (JP89) strain displays some transport capability but much less than the wild-type strain. This observed GroPIns transport activity at high GroPIns concentrations is most likely the result of transport occurring through another, lower affinity permease, such as an inositol transporter (Patton *et al.* 1995).

The experiments shown in Figure 3 were performed on cultures grown in the absence of inositol, since inositol was shown to repress GroPIns transport (Patton *et al.* 1995). In fact, wild-type cells grown in the presence of 75 μm inositol and assayed with 10 μm [^{3}H]GroPIns exhibit only 6% of the GroPIns transport activity observed for cells grown in the absence of inositol.

***GIT1* substrate specificity:** The substrate specificity of the Git1p was assessed by examining the ability of various compounds to inhibit GroPIns transport (Table 2). Transport assays were performed with 10 μm [^{3}H]GroPIns and a 40-fold excess (400 μm) of nonradiolabeled

TABLE 1

Strains

Strain	Genotype	Source or reference
Wild type	*trp1 ura3 leu2 his3 MATα*	P. McGraw
ino1 (JP3)	*trp1 ura3 leu2 his3 ino1::HIS3 MATα*	P. McGraw
JP16	*leu2 lys2 his3 ino1::HIS3 MAT***a**	This study
git1 ino1 (JPM1)	*trp1 ura3 leu2 his3 git1 ino1::HIS3 MATα*	This study
git2 ino1 (JPM2)	*leu2 trp1 his3 git1 ino1::HIS3 MAT***a**	This study
git1::HIS3 (JP89)	*trp1 ura3 his3 git1::HIS3 MATα*	This study
T303	*his3 leu2 ura3 ade2 MAT***a**	G. Carman
JP16(1C)	*lys2 git1 ino1::HIS3 his3 MAT***a**	This study
JP57	Diploid from *git1::HIS* × JP16(1C) *MAT***a**/α	This study
spt7	*spt7::LEU2 his4 leu2 ura3 MATα*	K. Arndt
JP61	*spt7::LEU2 ino1::HIS3 his3 ura3 MAT***a**	This study
JP62	Diploid from JP16 × 2-9(5C) *MAT***a**/α	This study
JP2-9(9A)	*git2 ino1::HIS3 leu2 his3 MATα*	This study
JP2-9(5C)	*git2 ino1::HIS3 leu2 his3 trp1 MATα*	This study
SH303	*ino2::TRP1 his3 leu2 ura3 trp1 MAT***a**	S. Henry
SH307	*ino4::LEU2 his3 leu2 ura3 trp1 MATα*	S. Henry

TABLE 2

Effect of related compounds on GroPIns transport

Addition	Activity (%)
None	100
Inositol	99.5
Glycerol	91.3 ± 9.8
GroPC	76.5 ± 5.5
GroPE	58.5 ± 3.8
Glycerol-3-phosphate	23.3 ± 0.5
GroPS	14.9 ± 3.0
GroPIns	11.6 ± 1.5

Transport activity was assayed in the presence of 10 μm glycerophospho[2-3H]inositol and 400 μm of the unlabeled indicated compounds. Values are the percentage of activity remaining as compared to the activity obtained when no additions were made (None). For the inositol addition, value represents the mean of duplicate determinations. For the other additions, data represent the mean ± standard deviation of triplicate determinations.

compounds. Glycerol and inositol had virtually no effect upon GroPIns transport. GroPC and GroPE inhibited transport to some extent, and glycerol-3-phosphate and GroPS caused a marked inhibition of GroPIns transport (Table 2).

Production of extracellular GroPIns and its reutilization through *GIT1*: A dynamic relationship exists between extracellular inositol concentration and the production and subsequent reutilization of extracellular GroPIns. Wild-type and *git1::HIS3* (JP89) cells were inoculated in synthetic media containing 10 μm [3H]inositol, and the concentrations of extracellular inositol and GroPIns were analyzed as the cultures progressed (Figure 4). Both strains displayed an initial production of GroPIns. However, when the level of inositol reached approximately 1 μm ($A_{650} \approx 1$), the wild-type strain began to transport GroPIns back into the cell, as evidenced by the decrease in extracellular GroPIns levels. In contrast, the GroPIns concentration in the *git1::HIS3* (JP89) culture began to level off at 1 μm inositol, but there was no subsequent decrease corresponding to GroPIns reuptake.

DISCUSSION

The *GIT1* gene (YCR098C) resides on the right arm of chromosome *III.* It consists of 1556 bp encoding a 518-amino acid protein with a predicted molecular mass of 57.3 kD. A Kyte and Doolittle (1982) hydropathy profile of the deduced amino acid sequence (Sor *et al.* 1992) indicates the presence of 12 potential membrane-spanning regions and hydrophilic N and C termini. A sugar transport motif [SDRIGR(K/R)(4-5)G] at amino acid 329 was also identified (Sor *et al.* 1992). The *GIT1* gene was originally sequenced as part of the *S. cerevisiae* genome sequencing project (this ORF was first labeled as YCR137 but is now YCR098C), but no function was assigned to it other than homology to carbohydrate transport proteins (Sor *et al.* 1992). Within the *S. cerevisiae* genome, *GIT1* bears similarity to a number of other metabolite transport proteins including the inorganic phosphate transporter, *PHO84,* and the inositol transporters, *ITR1* and *ITR2.* Using the algorithm of Smith and Waterman (1981) with gap weight 3.0 and gap weight length 0.1, *GIT1* is predicted to have 49.6% similarity and 26.2% identity with *PHO84,* 51.1% similarity and 23.7% identity with *ITR1,* and 50.8% similarity and 26.2% identity with *ITR2.*

The *git1::HIS3* mutation eliminates measurable GroPIns transport activity as defined as transport below 10 μm GroPIns (Figure 3). Thus, we infer that this specific GroPIns transport activity is carried out by the *GIT1* gene product, Git1p. We have shown previously that GroPIns can be transported into the cell intact, without first being hydrolyzed to inositol extracellularly (Patton *et al.* 1995). Consistent with this finding is the fact that at a concentration 40 times that of radiolabeled GroPIns, unlabeled inositol and glycerol had virtually

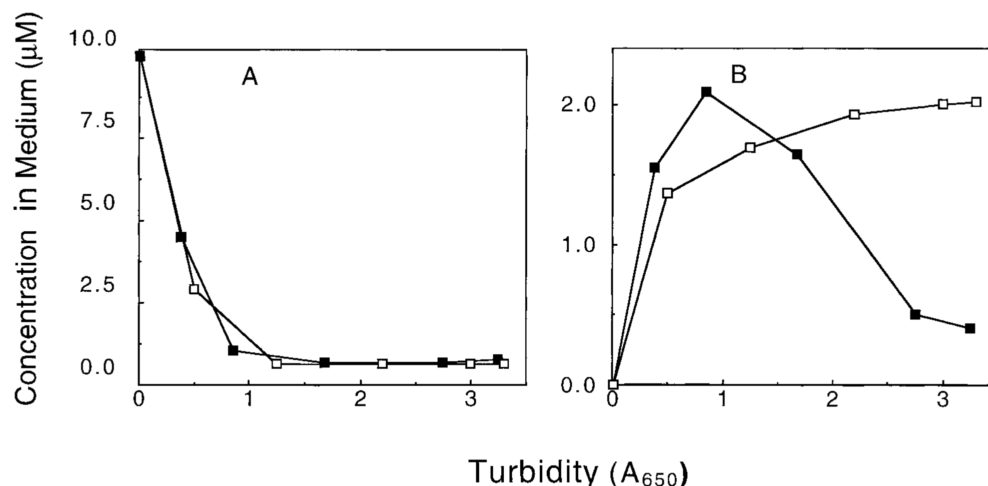

Figure 4.—A *git1* deletion mutant (JP89) cannot reutilize GroPIns when the medium becomes depleted of inositol. Cells were inoculated in synthetic medium containing 10 μm inositol and 3 μCi/ml [3H]inositol. At the indicated times, the culture medium was analyzed for inositol (A) and GroPIns (B). Wild type, ■; *git1::HIS3* (JP89), □.

no effect upon GroPIns transport (Table 2). Thus, inositol and glycerol appear to have little or no affinity for Git1p while GroPC and GroPE may have limited affinity because they do cause some inhibition of GroPIns transport. However, both glycerol-3-phosphate and GroPS caused marked inhibition, suggesting that they have significant affinity for Git1p. It is interesting to note that GroPS and glycerol-3-phosphate, like GroPIns, are negatively charged glycerophosphate esters. Whether GroPS and glycerol-3-phosphate are actually transported by Git1p remains to be established.

At GroPIns concentrations below 10 μm, the *git1::HIS3* (JP89) mutant cannot transport GroPIns, but above this concentration transport is observed (Figure 3). We attribute the residual transport seen in the *git1::HIS3* (JP89) deletion mutant and in the wild-type strain at higher concentrations to the action of other nonspecific transporter(s), most likely the major inositol transporter (Patton *et al.* 1995). In synthetic medium containing nonlimiting amounts of inositol, the extracellular GroPIns concentration reaches 1–2 μm per A_{650} of culture during the course of growth (Figure 4; Angus and Lester 1972). Thus, under normal laboratory conditions the concentration of extracellular GroPIns in yeast cultures does not go above 10 μm, making Git1p the primary GroPIns transporter under normal physiological conditions. This pathway, by which extracellular GroPIns produced via PI degradation is subsequently taken up and reutilized by the cell, is dramatically affected by the concentration of inositol in the media (Figure 4). While inositol availability is required for the production of extracellular GroPIns, when extracellular inositol becomes limiting (approximately 1 μm), GroPIns is transported back into the cell through the action of the Git1p permease. The starting inositol concentration for the experiment of Figure 4 (10 μm) is approximately the inositol concentration found in standard synthetic media when using yeast nitrogen base (Difco) with or without amino acids. Thus, under normal laboratory growth conditions, there exists a dynamic interplay between extracellular inositol and extracellular GroPIns levels. In the *git1::HIS3* (JP89) strain, this recycling pathway does not occur.

The genetic screen described here also resulted in the identification of *SPT7*, a global transcription factor (Gansheroff *et al.* 1995). We have shown *GIT2* to be allelic to *SPT7*. Mutations in *SPT7* were originally isolated as suppressors of Ty and ∂ insertion mutations in the 5′ region of the *HIS4* and *LYS2* genes (Gansheroff *et al.* 1995). Although it is tempting to speculate that *SPT7* is involved in the transcriptional regulation of *GIT1*, that has yet to be demonstrated. The *INO2* gene, which encodes a positive transcriptional regulator of *INO1* and other phospholipid biosynthetic genes, is also required for the utilization of GroPIns as an inositol source. Surprisingly, mutations at the *INO2* locus were not identified in our screen, suggesting that the screening may not have identified all loci capable of conferring the Git^- phenotype when mutated. While growth on GroPIns requires the *INO2* gene product, it does not require the *INO4* gene product. Other phenotypic differences between *ino2* and *ino4* mutants have been observed. For example, the *ino4* mutant expresses a small amount of *INO1* transcript under limiting inositol conditions, while the *ino2* mutant does not (Graves 1996). In contrast, the expression of a fusion gene driven by the *INO4* promoter (Ashburner and Lopes 1995) and derepression of IPC synthase activity when inositol is supplemented in the growth media require a functional *INO4* but not *INO2* gene product (Ko *et al.* 1994).

Although *E. coli* is known to have transporters for glycerophosphodiesters, this is the first report of such a transporter being identified in a eukaryotic cell. In *E. coli*, glycerophosphodiesters and glycerol-3-phosphate can be transported either via the *pho* regulon-dependent Ugp system (Schweizer *et al.* 1982; Xavier *et al.* 1995) or the *glp* regulon-dependent GlpT system (Larson *et al.* 1982; Xavier *et al.* 1995). The Ugp system is induced by phosphate starvation, while the GlpT system is induced in response to the presence of glycerol and glycerol-3-phosphate in the medium. Similarly, the transport of GroPIns via Git1p in *S. cerevisiae* is dependent upon the nutritional environment of the cell.

As shown previously (Patton *et al.* 1995), inositol causes repression of the ascribed Git1p transport activity (*i.e.*, specific transport of GroPIns at concentrations below 10 μm). Data reported in a recent publication by Wodicka *et al.* (1997), who performed genome-wide expression monitoring in *S. cerevisiae*, lend credence to the idea that *GIT1* transcription is regulated by nutritional factors. These authors identified *GIT1* as one of ~250 genes showing significant regulation in response to nutrient availability. *GIT1* mRNA was reported to be 31-fold more abundant in minimal medium as compared to rich medium; only four other genes displayed a greater fold difference under those conditions (Wodicka *et al.* 1997). Consistent with previously published data, other phospholipid biosynthetic genes were also reported by Wodicka *et al.* to be regulated by nutrient availability. The mRNA of *OPI3*, which is the structural gene for phospholipid-N-methyltransferase, which is coregulated with *INO1*, was found to be 18-fold more abundant in minimal as compared to rich medium, and *INO1* mRNA was found to be highly abundant in minimal medium and undetectable in rich medium (Wodicka *et al.* 1997). Our inspection of the promoter region (defined as 1000-bp upstream of the start codon) of *GIT1* failed to detect a copy of the repeated element UAS_{INO} (CATGTG), which is found in the *INO1* and *OPI3* promoters, but we did find a bHLH consensus sequence (CACGTG) located at nucleotides 518–523 upstream of ATG to which *INO2* and *INO4* (bHLH proteins) might bind to activate transcription (Bachhawat *et al.* 1995).

S. cerevisiae adjusts its metabolism to use the available sources of carbon, phosphate, sulfate, and nitrogen, among other things (Jones *et al.* 1992). We now report that yeast cells can use the GroPIns that they have released into the medium as a result of PI turnover during normal growth, as an alternate source of inositol during times of inositol depletion. Phospholipid metabolites recycle during normal growth in yeast and in other organisms. For example, we have recently shown that when PC is acted upon by a phospholipase D in wild-type *S. cerevisiae*, the released choline is immediately reincorporated into PC (Patton-Vogt *et al.* 1997). Similarly, in Chinese hamster ovary cells it appears that ethanolamine is continually released from CDP-ethanolamine derived PE and recycled back into PE (Shiao and Vance 1995). In yeast, the production and reutilization of GroPIns following the deacylation of PI, most likely mediated by a phospholipase B or a phospholipase A and a lysophospholipase, is a major metabolic route (Angus and Lester 1972, 1975; Patton *et al.* 1995). Furthermore, we have shown that GroPIns production and reutilization is regulated by the nutrient status of the cell. The exact mechanism of this regulation and its significance for cellular function will be the subject of future studies. Clearly, this pathway of PI turnover must be taken into account in future studies of PI metabolism and signal transduction in yeast. In mammalian cells, the deacylation of phosphoinositides and the production of glycerophosphoinositols has already been shown to have important biological consequences (Valitutti *et al.* 1991; Iacovelli *et al.* 1993; Falasca and Corda 1994; Mountford *et al.* 1994; Corda and Falasca 1996; Falasca *et al.* 1997).

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