**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<sup>−</sup>) 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<sup>−</sup> phenotype. Another mutant was complemented by a single open reading frame (ORF) termed GIT1 (glycerophosphoinositol). 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)C] and polyphosphoinositides (PIP, PI 3-P, PIP2), 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<sub>2</sub> (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 (glycerophosphoinositol), which appears to encode a transporter for GroPIns. To our knowledge, this is the first
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 (Glt') that is displayed in some mutants with an Ino– phenotype (i.e., sp7 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 glycerophosphomyo-[2-3H]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) and subjected to mild alkaline methanolation as described previously (Lindegren and Steiner 1968). Strain his3 leu2 ura3 ade2 MATa spt7 (Rutgers University, New Brunswick, NJ). Following termination of the assays by the addition of 10 ml of glycerophosphoinositol:water (32:4:5:1) and the lipids visualized with I2 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 N2. The PI was resuspended in methanol:toluene (1:1) and subjected to mild alkaline methanalysis as described previously (Lester et al. 1990). The resulting GroPIns was desalted over a column of Bio-Rex 70 (50–100 mesh, sodium form), eluting with H2O.

Strains and culture conditions: Strains (Table 1) were grown aerobically at 30°C with shaking. Turbidity was monitored by measurement at A650 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 (tp1 ur3 la2 his3 MATa) and the ino1 (tp3) strain (tp1 ur3 la2 his3 ino1::HIS3 MATa) were provided by P. McGraw (University of Maryland, College Park, MD). Strain T303 (his3 la2 ur3 ade2 MATa) was provided by G. Carman (Rutgers University, New Brunswick, NJ). Strain sp7 (spt7::LEU2 his4 la2 ur3 MATa) and the YCP50-based plasmid containing the SPT7 gene were provided by A. Nord (University of Pittsburgh, PA).

Mutagenesis and genetic manipulations: The ino1 (tp3) strain was subjected to ethylmethane sulfate (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°C for 3 days. Colonies were replica printed onto synthetic media (i) lacking inositol (1'), (ii) containing 75 μM inositol (1'), and (iii) lacking inositol but containing 25 μM GroPIns (GroPIns'). After 3–4 days incubation at 30°C, colonies able to grow on 1' plates but unable to grow on 1 plates and GroPIns' plates were selected. Two mutants isolated in this manner were backcrossed to an inositol auxotroph of the opposite mating type (tp16), and strains sp7 ino1 (tp18) and sp7 ino1 (tp42) 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 Bretinbach 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 BamHI site of the tetracycline resistance gene (tet') of the vector, an 18-base primer derived from a section of the tet' gene (~40 bases from the BamHI 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 BamHI to ClaI fragment, containing a single ORF and 406 bp upstream of ATG, was subcloned into the vector pRS314 (Gietz and Schieber 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 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' 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 1 litre synthetic media to log-phase (A650 of 0.3 to 0.8). The cells were harvested, washed with fresh media, and resuspended to an A650 of 5 in fresh media. Assays were started by adding 50 μl of glycerophosphoinositol[2-3H]inositol at the indicated concentration to 200 μl of cell suspension. Assays were carried out for 5 min at 30°C. 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[2-3H]inositol and a 40-fold excess (400 μM) of the indicated nonradioabeled 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 [3H]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 [3H]inositol, and [3H]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 Ecolube 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 strains complement each other and are, thus, most likely reisolated in our screen. 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 (Schoeder and Brentenbach 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 (JP1M1) and git2 ino1 (JP2M2) 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 (JP1M1) and git2 ino1 (JP2M2) 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
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 EcoRI-ClaI fragment encompassing the SPT7 gene (Gansheroff et al. 1995) was able to complement the git2 mutation. (2) A characterized sp7 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 sp7 ino1 and sp7 ino1 (JP61) segregants derived from this cross were unable to grow on GroPIns. (3) Diploids produced by crossing an sp7 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 sp7 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 a single ORF (previously identified as SPT7) included within that fragment was focused upon, as it appeared 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 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.

**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), •; git1 ino1 (JPM1), □; git 2 ino1 (JPM2), ○.

**Figure 2.**—Complementation of git1 mutant with pJP100. Wt, ino1 (JP3), git1 ino1 (JPM1), and git1 ino1 (JPM1) containing pJP100 (git1 ino1 + 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.
Glycerophosphoinositol Transport

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 Ino1 (i.e., not homozygous for ino1).

**GroPi ns transport activity**: At concentrations of 10 μM and below, GroPi ns 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 GroPi ns transport activity at high GroPi ns 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 GroPi ns transport (Patton et al. 1995). In fact, wild-type cells grown in the presence of 75 μM inositol and assayed with [3H]GroPi ns exhibit only 6% of the GroPi ns 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 GroPi ns transport (Table 2). Transport assays were performed with 10 μM [3H]GroPi ns and a 40-fold excess (400 μM) of nonradiolabeled

![Graph](image)

**Figure 3.**—GroPi ns 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 GroPi ns. Wild type, ; git1::HIS3 (JP89), .

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**Table 1**

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<th>Strain</th>
<th>Genotype</th>
<th>Source or reference</th>
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<td>P. McGraw</td>
</tr>
<tr>
<td>ino1 (JP3)</td>
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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 \( \mu \text{m} \) inositol and 400 \( \mu \text{m} \) of the unlabelled 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 triplicate determinations. For the other additions, data represent the mean ± standard deviation of triplicate determinations.

**Table 2**

<table>
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<td>Inositol</td>
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<td>GroPC</td>
<td>76.5 ± 5.5</td>
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<tr>
<td>GroPE</td>
<td>58.5 ± 3.8</td>
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<td>Glycerol-3-phosphate</td>
<td>23.3 ± 0.5</td>
</tr>
<tr>
<td>GroPS</td>
<td>14.9 ± 3.0</td>
</tr>
<tr>
<td>GroPIns</td>
<td>11.6 ± 1.5</td>
</tr>
</tbody>
</table>

Transport activity was assayed in the presence of 10 \( \mu \text{m} \) glycerophospho-[\( ^{3} \text{H} \)]inositol and 400 \( \mu \text{m} \) 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.

**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 kDa. A KYTE and Doolittle (1982) hydrophathy 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 Smit h 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 \( \mu \text{m} \) 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 no effect upon GroPIns transport.

![Figure 4](image-url)

**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 \( \mu \text{m} \) inositol and 3 \( \mu \text{Ci/ml} \) [\( ^{3} \text{H} \)]inositol. At the indicated times, the culture medium was analyzed for inositol (A) and GroPIns (B). Wild type, ■; git1::HIS3 (JP89), □.
no effect upon GroPln 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 GroPln 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 GroPlns, are negatively charged glycerophosphate esters. Whether GroPS and glycerol-3-phosphate are actually transported by Git1p remains to be established.

At GroPlns concentrations below 10 μm, the git1::HIS3 (JP89) mutant cannot transport GroPlns, but above this concentration transport is observed (Figure 3). We attribute the residual transport seen in the glycerophosphodiesters, this is the first report of such mutations, to transport of GroPIns held by Git1p remains to be established. derepression of IPC synthase activity when inositol is added to the media (Figure 4). While inositol availability is dramatically affected by the concentration of inositol in the media (Figure 4), extracellular inositol availability is required for the production of extracellular GroPlns, when extracellular inositol becomes limiting (approximately 1 μm), GroPlns is transported back into the cell as one of the extracellular GroPlns 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 G1T2 to be allelic to SPT7. Mutations in SPT7 were originally isolated as suppressors of Ty and a 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 GroPlns 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 GroPlns 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 glycerophosphodiester, this is the first report of such a transporter being identified in a eukaryotic cell. In E. coli, glycerophosphodiester 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 GroPlns 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 GroPlns 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 identified by Wodicka et al. to be regulated by nutrient availability. The mRNA of OP13, which is the structural gene for phospholipid-N-methyltransferase, which is coregulated with INO1, was found to be 18-fold more abundant in minimal medium than in 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 UASNO (CATGTG), which is found in the INO1 and OP13 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 GroPlns 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 continuously released from CDP-ethanolamine derived PE and recycled back into PE (Shiao and Vance 1995). In yeast, the production and reutilization of GroPlns 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 GroPlns 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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LITERATURE CITED


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