Genetic Perturbation of Glycolysis Results in Inhibition of de Novo Inositol Biosynthesis*

Yihui Shi1,‡, Deirdre L. Vaden1†, Shulin Ju3, Daobin Ding4, James H. Geiger3 and Miriam L. Greenberg1‡

From the 1Department of Biological Sciences, Wayne State University, Detroit, Michigan 48202 and 3Department of Chemistry, Michigan State University, East Lansing, Michigan 48824

In a genetic screen for Saccharomyces cerevisiae mutants hypersensitive to the inositol-depleting drugs lithium and valproate, a loss of function allele of TPI1 was identified. The TPI1 gene encodes triose phosphate isomerase, which catalyzes the interconversion of dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate. A single mutation (N65K) in tpi1 completely abolished Tpi1p enzyme activity and led to a 30-fold increase in the intracellular DHAP concentration. The tpi1 mutant was unable to grow in the absence of inositol and exhibited the “inositol-less death” phenotype. Similarly, the pgk1 mutant, which accumulates DHAP as a result of defective conversion of 3-phosphoglycerol phosphate to 3-phosphoglycerate, exhibited inositol auxotrophy. DHAP as well as glyceraldehyde 3-phosphate and oxaloacetate inhibited activity of both yeast and human myo-inositol-3-phosphate synthase, the rate-limiting enzyme in de novo inositol biosynthesis. Implications for the pathology associated with TPI deficiency and responsiveness to inositol-depleting anti-bipolar drugs are discussed. This study is the first to establish a connection between perturbation of glycolysis and inhibition of de novo inositol biosynthesis.

Bipolar disorder, also called manic-depressive illness, is a severe psychiatric illness with a prevalence of about 1.5% (1). Lithium and valproate (VPA)3 are two FDA-approved drugs for the treatment of bipolar disorder. Neither drug is completely effective, but the development of new therapies is hindered by the fact that the mechanisms underlying the therapeutic effects of lithium and VPA are not known. The inositol depletion hypothesis has been proposed to explain the therapeutic effects of lithium in the treatment of bipolar disorder (2). This hypothesis is based on the evidence that inositol monophosphatase is inhibited by therapeutic concentrations of lithium, which can, thus, disrupt the phosphoinositide cycle. More recently, VPA has also been linked to inositol depletion. VPA was found to decrease the concentration of myo-inositol in rat brain after chronic administration (3). Both lithium and VPA cause a decrease in intracellular inositol in yeast (4). Moreover, a recent study showed that lithium, VPA, and carbamazepine, another mood-stabilizing drug, decreased growth cone collapse and increased growth cone area in sensory neurons in culture. These effects were abolished by the addition of inositol (5). These studies suggested that inositol metabolism may be associated with the mechanism of action of lithium and VPA. Surprisingly, very little is known about the molecular control of inositol de novo biosynthesis in human cells.

The de novo biosynthesis of inositol has been extensively characterized in the yeast Saccharomyces cerevisiae. Isolation of spontaneous mutants unable to grow in the absence of inositol in S. cerevisiae was first carried out three decades ago (6). Inositol auxotroph mutants undergo inositol-less death, the abrupt decrease in viable cells when deprived of inositol (7). In the rate-limiting step of inositol synthesis, glucose 6-phosphate (G6P) is converted to myo-inositol 3-phosphate (MIP), catalyzed by the INO1-encoded MIP synthase (8). MIP is then dephosphorylated to myo-inositol by inositol monophosphatase (8). MIP synthase has been purified from a number of species (9–14). Myo-2-inosose 1-phosphate, an intermediate in the reaction, is a strong competitive inhibitor of yeast MIP synthase (15). Further investigation also demonstrated that myo-2-inosose 1-phosphate analogues, including 2-deoxy-myoinositol 1-phosphate, 1-deoxy-1-(phosphonomethyl)-myo-2-inosose, and dihydroxyacetone 1-phosphate (DHAP), are MIP synthase inhibitors (16). The extensive genetic and biochemical characterization of inositol metabolism in yeast make it an excellent model for investigating the mechanisms of action of inositol-depleting drugs. Furthermore, MIP synthase is highly conserved from yeast to humans, as expression of human MIP synthase complements the inositol auxotrophy of yeast ino1 null mutants (17). Consistent with the hypothesis that VPA depletes inositol by indirect inhibition of MIP synthase, human MIP synthase activity is decreased in vivo in the presence of VPA (17).

To determine how lithium and VPA affect the inositol metabolic pathway, we used a genetic approach to identify mutants hypersensitive to lithium and VPA in the absence of inositol. We identified a yeast mutant defective in TPI1, which encodes triose phosphate isomerase. This enzyme catalyzes the interconversion of DHAP and glyceraldehyde 3-phosphate (G3P). In humans, TPI deficiency is a rare multisystem disorder characterized by autosomal recessive inheritance (18). It is associated with chronic hemolytic anemia, recurrent infections, cardiomyopathy, progressive neurologic dysfunction, and death in childhood (18). The crystal structures of Tpi1p from different species indicate that the enzyme is a dimer of two identical subunits (19–22). The subunit interface is of particular interest because it is critical for dimer stability (23, 24). In humans, 80% of TPI-deficient patients carry an inherited substitution of aspartate for glutamate at residue 104 (E104D). This mutation is located at the subunit interface of the Tpi1p dimer, which leads to loss of enzyme activity due to instability of the mutant dimer (25).

In this report we show that a single mutation (N65K) in the dimer interface region of Tpi1p completely abolished enzyme activity and led to a 30-fold increase in the intracellular DHAP level. Interestingly, tpi1 exhibited the inositol auxotrophy phenotype. Consistent with inositol auxotrophy, MIP synthase was inhibited by DHAP, and other metabolites of carbohydrate metabolism also inhibited the enzyme. This is the
first report indicating that the perturbation of glycolysis inhibits the inositol biosynthetic pathway.

**EXPERIMENTAL PROCEDURES**

**Materials**—All chemicals used were reagent grade or better. Glucose extract, and peptone were bought from Difco. Amino acids, NAD, NADH, myo-inositol, G6P, imidazole, lysyosyme, VPA, ampicillin, ethylmethane sulfonate, sodium thiocussate, triethanolamine, DHAP, and G3P were from Sigma. [14C]G6P, [14C]inositol, and [32P]UTP were from PerkinElmer Life Sciences. Protease inhibitor tablets and glycerol-3-phosphate dehydrogenase were from Roche Applied Science. The pGEM-T EASY vector, Wizard Miniprep DNA purification kit, amplified in Prep yeast plasmid miniprep kit, and retransformed into Invitrogen Research. The pGEM-T EASY vector, Wizard Miniprep DNA purification kit, and retransformed into Invitrogen Laboratory. The pGEM-T EASY vector, Wizard Miniprep DNA purification kit, and retransformed into Invitrogen Laboratory. The pGEM-T EASY vector, Wizard Miniprep DNA purification kit, and retransformed into Invitrogen Laboratory.

**Strains**—The yeast strains used in this study are shown in **TABLE ONE**.

**Genome Media**—YPD (complex medium) contained glucose (2% w/v), Bacto-peptone (2% w/v), yeast extract (1% w/v), and agar (2% w/v) for solid medium. Complete synthetic medium contained glucose (2% w/v), ammonium sulfate (2 g/liter), and the amino acids and nucleotides arginine (20 mg/liter), adenine (20 mg/liter), histidine (10 mg/liter), leucine (60 mg/liter), lysine (20 mg/liter), methionine (20 mg/liter), threonine (0.3 g/liter), tryptophan (20 g/liter), and uracil (40 mg/liter). The salts added are essentially the components of Difco vitamin-free yeast base and the full vitamin supplement. Minimal synthetic sporulation medium contained 0.1% yeast extract, 0.1% dextrose, 1% potassium acetate, and 2% agarose and was supplemented with methionine, histidine, and uracil.

**Chemical Mutagenesis**—Ethylmethane sulfonate mutagenesis was carried out according to the method of Lindegren et al. (26). Cells were mutagenized to 50–90% kill.

**Screen for Lithium- and VPA-hypersensitive (lvs) Mutants**—Mutagenized cells were plated on YPD plates and replica-plated to complete synthetic inositol-free medium with or without 60 mM LiCl or 6 mM VPA. lvs mutants were identified, patched to YPD plates, and replica-plated to medium containing or lacking lithium, VPA, or myo-inositol (75 μM). Mutants in which hypersensitivity to lithium and VPA was reversed in the presence of inositol were chosen for further analysis.

**Identification of Mutant Genes**—Mutants containing mutations in a single nuclear gene were transformed by electroporation with a cDNA library (YES), and transformants were selected by uracil prototrophy. The transformants were replica-plated onto plates containing lithium or VPA to select for increased drug resistance. To confirm that colonies acquired resistance from the plasmid, plasmid DNA was extracted from yeast using a Zymoprep yeast plasmid miniprep kit, amplified in Escherichia coli, extracted using a Wizard Miniprep DNA purification kit, and retransformed into the mutant.

**DNA Sequencing**—The plasmid inserts of the complementary clones were sequenced using ABI fluorescence technology with primers that flank the insertion site. The nucleotide sequences obtained from the forward and reverse primers were used to identify the cloned genes. Primers were designed that flanked the insertion site (the BamHI site, nucleotide 378) of the genomic fragment in the YCp50 vector. The forward primer begins at nucleotide 329, 5'-TTGAGGCCCCTACATTGATCCACAGG-3', and the reverse primer begins at nucleotide 399, 5'-ATGGTCCGAGGAGGATG-3'. The primer designed for the YES vector flanked the XhoI site, the forward primer beginning at nucleotide 7767, 5'-GAATTACCGAGGGAGGGG-3', and reverse primer beginning at nucleotide 95, 5'-ATGTTGTTGAATTTGATGGG-3'. Cycle sequencing was performed with ABI PRISM Dye terminators. The sequences obtained were analyzed by a data base search using Blast in the Saccharomyces Genomic Database to search for sequences showing homology to the sequenced clones.

**Yeast Strains used in this study**

| Strains          | Characteristics or genotype | Source/comments                  |
|------------------|----------------------------|----------------------------------|
| SMY15            | D273-10B derivative, met2, urs3-52, MATa | M. Greenberg laboratory         |
| SMY10            | Isogenic to SMY15 except lys2, met2, MATa | M. Greenberg laboratory         |
| tpi1             | Isogenic to SMY15 except N65K       | This study; original mutant out-crossed to SMY10 |
| BY4741           | his3Δ1, leu2Δ0, met15Δ0, urs3Δ0, MATa | Invitrogen                      |
| BY4741 ino1Δ     | his3Δ1, leu2Δ0, met15Δ0, urs3Δ0, ino1Δ::kanMX, MATa | Invitrogen                      |
| N501-1B          | leu2, mal, mel, gal2, MATa          | ATCC                            |
| N548-8A, pgk1    | pgk1, leu2, mal, me, gal2, MATa     | ATCC                            |

Inositol (75 μM) was plated to medium containing or lacking lithium, VPA, or myo-inositol, and [32P]UTP were from PerkinElmer Life Sciences. Protease inhibitor tablets and glycerol-3-phosphate dehydrogenase were from Roche Applied Science. The pGEM-T EASY vector, Wizard Miniprep DNA purification kit, and retransformed into Invitrogen Research. The pGEM-T EASY vector, Wizard Miniprep DNA purification kit, and retransformed into Invitrogen Research.

**Identification of Mutant Genes**—Mutants containing mutations in a single nuclear gene were transformed by electroporation with a genomic library in the YCp50 plasmid (pBR2236) containing ~10–15 kilobases of yeast genomic DNA or a cDNA library (YES), and transformants were selected by uracil prototrophy. The transformants were replica-plated onto plates containing lithium or VPA to select for increased drug resistance. To confirm that colonies acquired resistance from the plasmid, plasmid DNA was extracted from yeast using a Zymoprep yeast plasmid miniprep kit, amplified in *Escherichia coli*, extracted using a Wizard Miniprep DNA purification kit, and retransformed into the mutant.

**DNA Sequencing**—The plasmid inserts of the complementary clones were sequenced using ABI fluorescence technology with primers that flank the insertion site. The nucleotide sequences obtained from the forward and reverse primers were used to identify the cloned genes. Primers were designed that flanked the insertion site (the BamHI site, nucleotide 378) of the genomic fragment in the YCp50 vector. The forward primer begins at nucleotide 329, 5'-TTGAGGCCCCTACATTGATCCACAGG-3', and the reverse primer begins at nucleotide 399, 5'-ATGGTCCGAGGAGGATG-3'. Cycle sequencing was performed with ABI PRISM Dye terminators. The sequences obtained were analyzed by a data base search using Blast in the Saccharomyces Genomic Database to search for sequences showing homology to the sequenced clones.

The **Yeast Strains used in this study** are shown in **TABLE ONE**.
normalize for loading variation. RNA probes for Northern analysis were synthesized using the Promega Riboprobe system from plasmids linearized with restriction enzymes as follows (listed as plasmid, restriction enzyme, and RNA polymerase): pGEM-426, Ndel, T7 (TP11); pH310, HindIII, T7 (INO1); pAB309, EcoRI, SP6 (TCM1). The results of Northern blot hybridization were quantitated by phosphorimaging.

**Protein Assay**—Protein concentration was determined by the method of Bradford (28) using bovine serum albumin as the standard.

**Triose Phosphate Isomerase Assay**—The specific activity of triose phosphate isomerase was determined in cell-free extracts as described by Maitra and Lobo (29).

**Determination of Intracellular DHAP Concentration**—Determination of intracellular DHAP concentration was performed in cell-free extracts as described by Compagno et al. (30). Briefly, cells were rapidly collected by filtration through a 0.8-μm filter and resuspended in pre-cooled 9% HClO4. The acidic cell suspension was frozen in liquid nitrogen and thawed on ice three times. Neutralization of the cell-free extract was carried out with 2M KHCO3 at pH 7. The DHAP assay was performed in 0.1 M triethanolamine buffer, pH 7.4, 0.3 mM NADH, and 1 unit/ml glycerol-3-phosphate dehydrogenase. To calculate the intracellular DHAP concentration, a yeast cytosolic volume of 1.67 μl per mg of dry yeast biomass was assumed (31).

**RESULTS**

Identification of **tpi1**—To identify common targets of lithium and VPA, we implemented a genetic screen to isolate mutants hypersensitive to both drugs in the absence of inositol, in which hypersensitivity was rescued by exogenous inositol. Ethylmethane sulfonate mutagenesis was carried out according to the method of Lindegren et al. (26). Mutagenized wild type cells (35,000) were screened, and 26 mutants, termed **lvs** mutants (lithium and valproate sensitive), were isolated. All mutants were recessive, and 12 complementation groups were identified, including 6 in which mutants displayed a temperature-sensitive (ts) growth phenotype. One of the ts mutants, **lvs5**, was characterized further. Phenotypic analysis revealed that **lvs5** is auxotrophic for inositol and temperature-sensitive (Fig. 1A). The mutant was unable to grow at 30°C on synthetic media lacking inositol, but 5 μM inositol supported growth. The mutant was hypersensitive to lithium or VPA in the presence of 5 μM inositol. Supplementation with 75 μM inositol could partially restore growth of the mutant in the presence of lithium and VPA (Fig. 1B) but did not restore growth of the mutant at 39°C (Fig. 1A).

Further analysis indicated that the mutant exhibited inositol-less death, the precipitous decrease in viability when cells are starved for inositol (7). As seen in Fig. 2, **lvs5** cells shifted from 1+ to 1− media lost viability similar to **ino1Δ** cells, in which MIP synthase is deleted.

**Purification of Recombinant MIP Synthase**—Yeast (32) and human (17) MIP synthases were isolated and purified as previously described.

**MIP Synthase Assay**—Two well established methods were used to measure the MIP synthase activity; the paper chromatographic method of Chen and Charalampous (33) and the colorimetric method of Barnett et al. (34). The effect of high concentrations of DHAP on yeast MIP synthase was assayed by the chromatographic method of Chen and Charalampous (33), because contaminating phosphate in high concentrations of DHAP interfered with the latter procedure. Activity was measured in the presence of 100 mM Tris acetate, pH 7.0, 20 mM NH4Cl, 0.8 mM NAD⁺, 2 mM dithiothreitol, 5 mM G6P, and 0.2 mg of purified yeast MIP synthase protein. Specific activities, Michaelis constants (Km), and inhibition constants (Ki) for DHAP, G3P, and oxaloacetate (OAA) on yeast and human MIP synthase were determined by the colorimetric method of Barnett et al. (34) with minor modifications. Reaction tubes contained 100 mM Tris acetate, 20 mM NH4Cl, 0.8 mM NAD⁺, 2 mM dithiothreitol, 0.2 mg of purified protein buffered to pH 7.0 for yeast MIP synthase and pH 8.0 for human MIP synthase. Michaelis constants (Km) for G6P and inhibition constants (Ki) for DHAP, G3P, and OAA were determined by employing Lineweaver-Burk analysis of enzyme velocity in the reaction buffer with varying concentrations of G6P (0.5, 0.75, 1.0, 1.5, and 2.0 mM), DHAP (0, 100, 250, and 500 μM), G3P (0, 100, 250, and 500 μM), and OAA (0, 100, 250, and 500 μM).
Regulation of Inositol Biosynthesis by Glycolytic Intermediates

FIGURE 3. TPI1 complements the inositol auxotrophic, temperature-sensitive, and lvs phenotypes of lvs5. Isogenic lvs5 and wild type (WT) (SMY15) cells transformed with YCp50 (vec) or with YCp50 carrying the TPI1 gene (TPI1) were precluded in synthetic uracil media plus 75 μM inositol at 30 °C for about 24 h. Cells were washed twice, quantified, serially diluted, and spotted on uracil plates with or without 75 μM inositol, 60 mM lithium, or 6 mM VPA as indicated. Cells were incubated at the indicated temperature for 4 days.

TABLE TWO

Triose phosphate isomerase activity and intracellular DHAP concentration in tpi1

| Strains       | Triose phosphate isomerase activity | Intracellular DHAP |
|---------------|------------------------------------|--------------------|
|               | units/mg of protein                 | m M                |
| Wild type     | 11.3 ± 1.6                         | 0.76 ± 0.05        |
| tpi1          | ND                                 | 23.67 ± 4.04       |
| tpi1 + TPI1   | 9.5 ± 1.2                          | 0.77 ± 0.04        |
| tpi1 + YCp50  | ND                                 | 25.7 ± 3.51        |
| WT + YCp50    | 9.8 ± 1.4                          | 0.75 ± 0.04        |

To clone the gene mutated in lvs5, the lvs5 mutant was transformed with genomic and cDNA libraries, and transformants were screened for the ability to grow at 30 °C in the absence of inositol, in the presence of 60 mM lithium or 6 mM VPA, and at the non-permissive temperature of 39 °C on synthetic uracil drop out media. A genomic library plasmid bearing a 7.4-kilobase DNA sequence containing the TPI1 locus and a cDNA library plasmid containing the TPI1 gene complemented the mutant phenotypes, including drug sensitivity, inositol dependence, and temperature-sensitive growth. The TPI1 gene was subsequently cloned into the YCp50 plasmid and transformed into the lvs5 mutant cell. Expression of the TPI1 gene restored growth in the absence of inositol, in the presence of lithium and VPA, and at 39 °C (Figs. 3 and 4). Sequencing analysis revealed a single C to A mutation in the TPI1 locus of lvs5, resulting in a missense codon leading to a single amino acid change, Asn to Lys, at residue 65 (N65K). The Asn-65 residue is at the dimer interface and is conserved in all species for which the gene has been sequenced (NCBI GenBank™) (Fig. 4). The lvs5 mutant will be referred to as tpi1.

Loss of Tpi1p Enzyme Activity and Accumulation of DHAP in tpi1—The specific activity of triose phosphate isomerase and intracellular DHAP levels were measured in cell-free extracts of tpi1 and isogenic wild type (SMY15) cells. As seen in TABLE TWO, triose phosphate isomerase activity is not detectable in tpi1 mutant cells, which accumulate about 30-fold more DHAP than wild type cells. This is consistent with levels found in a previously identified tpi1 mutant (30). The presence of a plasmid-born TPI1 gene in tpi1 restored wild type levels of enzyme activity and intracellular DHAP. The loss of triose phosphate isomerase activity in the tpi1 mutant is not due to decreased expression of TPI1, the levels of which were similar in tpi1 and wild type cells (Fig. 5).

High Levels of DHAP Inhibit MIP Synthase Activity—To determine whether inositol auxotrophy could be explained by inhibition of MIP synthase by DHAP, we assayed the activity of purified yeast MIP synthase in the presence of DHAP. As seen in Fig. 6, DHAP inhibits MIP synthase activity. At 22 mM DHAP, the concentration observed in the tpi1 mutant, yeast MIP synthase activity was completely abolished. This result suggested that inositol auxotrophy of tpi1 is due to inhibition of MIP synthase by increased DHAP levels and predicted that other glycolysis mutants that accumulate DHAP are also inositol auxotrophs. The glycolysis mutant pgk1 is defective in the conversion of 3-phosphoglycerolylphosphate to 3-phosphoglycerate, and accumulation of DHAP has been demonstrated in this mutant (35). Consistent with this prediction, pgk1 was also unable to grow in the absence of inositol (Fig. 7A) and exhibited inositol-less death when deprived of inositol (Fig. 7B). Inositol auxotrophy of tpi1 could not be explained by the inability to derepress INO1. As seen in Fig. 8A, INO1 is derepressible in the tpi1 mutant when deprived of inositol. INO1 abundance was quantified by Northern analysis of RNA extracted from cells after the shift from I+ to I− media. INO1 expression was similar in tpi1 and wild type cells (Fig. 8A). Furthermore, when assayed in the absence of DHAP, MIP synthase was
active in tpi1 cell extracts (Fig. 8B). These results indicate that INO1 is expressed normally in tpi1 and translated into an active protein.

We wished to determine whether other intermediates of carbohydrate metabolism similar to DHAP inhibit MIP synthase at elevated concentrations. This might indicate tight control of MIP synthase during perturbation of carbohydrate metabolism. The glycolytic pathway intermediate, G3P, and the TCA cycle intermediate, OAA, inhibited MIP synthase activity as well (TABLE THREE). The Michaelis constant ($K_m$) for G6P, the substrate of yeast MIP synthase, was 250 $\mu$M. As seen in TABLE THREE, DHAP, G3P, and OAA were all competitive inhibitors of yeast MIP synthase with respective $K_i$ values of 156, 127, and 109 $\mu$M. Our result is consistent with the previous finding that DHAP is a competitive inhibitor of yeast MIP synthase. For human MIP synthase, we observed similar results. The $K_m$ for G6P was 185 $\mu$M. The $K_i$ values for DHAP, G3P, and OAA were 115, 102, and 88 $\mu$M, respectively. In patients with triose phosphate deficiency, DHAP levels as high as 3.772 mmol/g Hb (36) or 904.9 nmol/ml of RBC (37) have been reported. These levels can be extrapolated to 1–3 mM, which is in the range that can inhibit human MIP synthase activity in vitro.

### DISCUSSION

The demonstration of inositol auxotrophy in the tpi1 mutant intriguingly links perturbation of glycolysis to the inositol biosynthetic pathway. Intracellular DHAP increased about 30-fold in tpi1, consistent with DHAP levels reported for a previously isolated tpi1 mutant (30). DHAP is a competitive inhibitor of MIP synthase, probably due to its structural similarity to the ketone and phosphate moieties of myo-2-inosose 1-phosphate, an intermediate in the MIP synthase reaction. In the tpi1 mutant the INO1 gene is derepressed, and MIP synthase protein is expressed when inositol is limiting, as shown in Fig. 6. Therefore, inhibition of MIP synthase by DHAP is the most likely explanation for inositol auxotrophy of tpi1.
with this, the glycolysis mutant pgk1, which also accumulates DHAP, was also auxotrophic for inositol. Purified yeast and human MIP synthases were inhibited by DHAP as well as by other metabolites of carbohydrate metabolism, including G3P and OAA. These results suggest that genetic perturbation of glycolysis leads to inhibition of inositol de novo biosynthesis by direct inhibition of MIP synthase.

This report demonstrates a direct link between glycolysis and inositol biosynthesis and differs mechanistically and qualitatively from previous studies linking regulation of carbohydrate utilization to ino1 expression. Expression of INO1 is affected by the glucose response pathway, an energy-saving mechanism that turns off expression of genes required for utilization of carbon sources other than glucose when glucose is present, and turns them on when glucose is limiting (38, 39). In yeast, the glucose response pathway is mediated by the Snf1p-Snf4p kinase complex and the Reg1p-Glc7p phosphatase complex, which regulate Mig1p, a repressor of genes required for utilization of non-glucose carbon sources (39). When glucose is limiting, the Snf1p-Snf4p kinase inactivates Mig1p. When glucose is abundant, Mig1p is activated by Reg1p-Glc7p phosphatase. Interestingly, the glucose repression pathway affects inositol synthesis at the level of INO1 expression and is inositol auxotrophs (40, 41), whereas the reg1 mutant exhibits increased expression of INO1 and has an inositol excretion phenotype (41, 42). Thus, expression of INO1 is altered when the response to glucose is genetically perturbed.

The results reported here have implications for the pathology associated with TPI deficiency, one of several well studied glycolytic enzyme deficiencies (18, 25, 43). In contrast to other deficiencies, TPI-deficient patients have a progressive, severe neurologic disorder (44). The neurologic defects may be, in part, a result of inositol deficiency. The brain possesses ~6 mM inositol, and alterations in brain inositol concentrations have been reported in a number of pathologic conditions, including bipolar disorder, Down syndrome, and diabetic peripheral neuropathy (45). A recent study indicated that MIP synthase activity is inhibited by VPA in crude homogenates of human prefrontal cortex in patients with TPI deficiency, DHAP levels of 1–3 mM have been reported in a number of pathological conditions, including G3P and OAA. These results suggest that genetic perturbation of glycolysis leads to inhibition of inositol de novo biosynthesis by direct inhibition of MIP synthase.

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