In *Saccharomyces cerevisiae* deletion of phosphoglucose isomerase can be suppressed by increased activities of enzymes of the hexose monophosphate pathway

J. Richard Dickinson,¹ Michael A. Sobanski¹ and Michael J. E. Hewlins²

Author for correspondence: J. Richard Dickinson. Tel: +44 1222 874000 ext. 5762. Fax: +44 1222 874305.

¹ School of Pure and Applied Biology, University of Wales College of Cardiff, PO Box 915, Cardiff CF1 3TL, UK

² Department of Chemistry, University of Wales College of Cardiff, PO Box 912, Cardiff CF1 3TB, UK

***Saccharomyces cerevisiae* mutants defective in the structural gene *PGI1* lack phosphoglucose isomerase and hence cannot grow on glucose. Spontaneous mutants were isolated by selecting for the regained ability to grow on YEPD (yeast extract/peptone/glucose). Three complementation groups called *spg29–31* (suppressor of *pgi1Δ*) were identified. The metabolism of [2-¹³C]glucose was studied by ¹³C NMR spectroscopy. This led to the conclusion that in a *spg29* mutant suppression of the glycolytic defect was achieved by increased carbon flux through the hexose monophosphate pathway. The specific activities of enzymes of the hexose monophosphate pathway (except glucose-6-phosphate dehydrogenase) and NAD- and NADP-dependent glutamate dehydrogenase were increased in the bypass mutant.**

Keywords: *Saccharomyces cerevisiae*, *pgi1Δ* suppressor mutations, hexose monophosphate pathway, ¹³C NMR

INTRODUCTION

Phosphoglucose isomerase is the enzyme which catalyses the interconversion of glucose 6-phosphate and fructose 6-phosphate. Thus, it is both the second step of the glycolytic sequence and the last step of gluconeogenesis. Mutants of *Saccharomyces cerevisiae* defective in the structural gene (*PGI1*) for phosphoglucose isomerase have been described by many authors (Maitra, 1971; Herrera & Pascual, 1978; Clifton *et al.*, 1978; Ciriacy & Breitenbach, 1979; Aguilera, 1986). All such *pgi1* mutants cannot grow on glucose, the assumption being that in *S. cerevisiae* flux through the hexose monophosphate pathway is inadequate to support growth. It is reckoned that the hexose monophosphate pathway is only capable of 8% of the carbon flux that occurs during growth on glucose (Bruinenberg *et al.*, 1986). In this respect *S. cerevisiae* is different from both *Escherichia coli* and *Kluyveromyces lactis*, in which mutants lacking phosphoglucose isomerase can still grow on glucose (Vinopal *et al.*, 1975; Goffrini *et al.*, 1991).

One way of trying to understand all aspects of the phenotype of *pgi1* mutants has been the isolation of extragenic suppressor mutations which confer upon *pgi1* mutants the ability to grow on glucose. Aguilera (1987) described *spg1* (suppressor of *pgi1Δ*) mutations which

restored growth on glucose and resulted in obligately high levels of mitochondrial respiration and no ethanol formation. Gamo *et al.* (1993) described *rgl1* and *rgl2* (resistance to glucose) mutations from which it was also presumed that glucose may be channelled through the hexose monophosphate pathway to respiration. Recently, Boles *et al.* (1993) showed that overexpression of *GDH2*, which encodes the NAD-dependent glutamate dehydrogenase, can suppress the growth defect on glucose caused by *pgi1Δ* mutations. The explanation for this is that overexpression of NAD-dependent glutamate dehydrogenase causes a cycle of metabolic interconversion between 2-oxoglutarate and glutamate in which the anabolic reaction results in the conversion of 2-oxoglutarate to glutamate with concomitant conversion of NADPH to NADP, whilst the catabolic reaction results in the formation of 2-oxoglutarate from glutamate with conversion of NAD to NADH. Hence, the rapid depletion of NADP which would otherwise result from overuse of glucose-6-phosphate dehydrogenase (if flux via the hexose monophosphate pathway was increased in *pgi1Δ* bypass mutants) is avoided and the extra NADH which is formed can be oxidized by the electron transport chain. This also explains the high obligatory respiration observed in *pgi1* bypass mutants.

Boles *et al.* (1993) also showed that deletion of the *ZWF1*

gene (which encodes glucose-6-phosphate dehydrogenase) blocks suppression of a *pgi1Δ* mutation. This is also highly indicative of the importance of the hexose monophosphate pathway, but it must be stressed that, to date, none of the studies have proved that the suppression of a *pgi1* mutation actually involves a metabolic bypass in which glucose is catabolized by means of increased flux via the hexose monophosphate pathway; i.e. the flow of carbon from substrate to product(s) has not been demonstrated. One could postulate three possible metabolic routes. Firstly, increased flux via the hexose monophosphate pathway. Secondly, use of the glycolytic sequence involving an isoenzyme of phosphoglucose isomerase encoded by a gene which is normally cryptic. This is not without precedent in *S. cerevisiae*: for example *ADH4* (encoding an isoenzyme of ethanol dehydrogenase) was discovered in *adh1 adh2 adh3* triple mutants (Paquin & Williamson, 1986). Thirdly, the use of a hitherto-unknown pathway of glucose catabolism. This concept has also been suggested previously to explain the bypass of *pfk1 pfk2* double mutants which lack phosphofructokinase (Breitenbach-Schmitt *et al.*, 1984). ^{13}C nuclear magnetic resonance (NMR) spectroscopy is a very good technique for metabolic studies (London, 1988; Davies & Brindle, 1992); hence it was decided to use ^{13}C -labelled glucose to analyse the patterns of metabolism by ^{13}C NMR in suppressed *pgi1Δ* mutants. Providing yeast with $[2-^{13}\text{C}]\text{glucose}$ allows one to discriminate between the known pathways as described below.

Use of the hexose monophosphate pathway will give rise to fructose 1,6-bisphosphate with label at C-1 and C-3. Subsequent cleavage by aldolase would yield dihydroxyacetone phosphate labelled at C-1 and C-3, which would be metabolized by the conventional glycolytic sequence to produce pyruvate labelled at C-3 and C-1, respectively. This labelled pyruvate has three possible fates, the first being conversion to ethanol (labelled at C-2) and $^{13}\text{CO}_2$ via pyruvate decarboxylase and ethanol dehydrogenase (Fig. 1). Since it is common for ethanolic fermentation to be accompanied by the formation of some glycerol (to preserve the NAD/NADH balance) one would also expect to see glycerol labelled at C-1 and C-3 which was derived from the C-1,3-labelled dihydroxyacetone phosphate (Fig. 1). A second possible fate of the pyruvate would be via pyruvate dehydrogenase, which would result in loss of $^{13}\text{CO}_2$ and formation of $[2-^{13}\text{C}]\text{acetate}$. Subsequent metabolism of the $[2-^{13}\text{C}]\text{acetate}$ via the tricarboxylic acid and/or glyoxylate cycles would give predictable labelling patterns as described previously (Dickinson *et al.*, 1983; Dickinson & Hewlins, 1991) when the fate of $[2-^{13}\text{C}]\text{acetate}$ was analysed by ^{13}C NMR. The third possible route for metabolism of some pyruvate would be via pyruvate carboxylase to 1,3-labelled oxaloacetate. The ^{13}C at C-1 of oxaloacetate would be lost as $^{13}\text{CO}_2$ at the isocitrate dehydrogenase step of the tricarboxylic acid cycle and the remaining label would result in glutamate labelled only at C-2 – an isotopomer that would be easy to identify, but has never been seen in yeast. Metabolism of isocitrate via the glyoxylate cycle would give readily identifiable intermediates.

The expression of a previously cryptic gene resulting in a new phosphoglucose isomerase activity would give catabolism of $[2-^{13}\text{C}]\text{glucose}$ via a completely reinstated glycolytic sequence. This would be expected to produce $[2-^{13}\text{C}]\text{pyruvate}$ and hence ethanol labelled at C-1 along with some $[2-^{13}\text{C}]\text{glycerol}$ (Fig. 1). Oxidative decarboxylation of this $[2-^{13}\text{C}]\text{pyruvate}$ via pyruvate dehydrogenase would give $[1-^{13}\text{C}]\text{acetate}$, which could enter the tricarboxylic acid cycle to yield glutamate labelled at C-1 on the first turn of the cycle and thence at C-5 on the second turn. The labelling of glutamate at C-1 and C-5 would yield a very characteristic spectrum. Metabolism of the $[1-^{13}\text{C}]\text{acetate}$ to $[6-^{13}\text{C}]\text{isocitrate}$ would give rise via the glyoxylate cycle to glutamate labelled only at C-1 on each successive turn of that cycle. The conversion of $[2-^{13}\text{C}]\text{pyruvate}$ to $[2-^{13}\text{C}]\text{oxaloacetate}$ by pyruvate carboxylase would give rise to glutamate labelled at C-3 on the first turn of the tricarboxylic acid cycle, 2,3- on the second turn and 1,2,3- on the third and subsequent turns of that cycle. This would also be a unique labelling pattern, which, even if it was combined with the formation of 1-, and 1,5-labelled glutamate, would still be very different from patterns which have been recorded previously. Finally, conversion of any $[2-^{13}\text{C}]\text{oxaloacetate}$ to $[3-^{13}\text{C}]\text{isocitrate}$ and subsequent intervention of the glyoxylate cycle would yield glutamate labelled only at C-2 and C-3. Hence, the catabolism of $[2-^{13}\text{C}]\text{glucose}$ via all known metabolic routes (Fig. 1) produces predictable labelling patterns in intermediates whose constituent carbon atoms have well-defined resonances in the NMR spectrum. With this knowledge we embarked upon an NMR analysis of the metabolism of glucose in a *pgi1Δ* mutant carrying a *spg29* suppressor mutation which allows growth on glucose.

METHODS

Media, strains and genetic methods. Cultural conditions and complex growth media have been described previously (Dickinson, 1991). Complex media contained, per litre: yeast extract (10 g), bacteriological peptone (20 g), adenine (0.1 g), uracil (0.1 g) and the specified carbon source (YEPD, 20 g D-glucose; YEPF, 20 g D-fructose). Minimal medium with fructose (2%, w/v) as major carbon source was supplemented with 0.1% (w/v) glucose. Standard genetic techniques were used for mating, sporulation and dissection (Sherman, 1975; Mortimer & Hawthorne, 1975). *S. cerevisiae* strain AAG2 (*MATα ura3 his4 can pgi1Δ25::LEU2*) was obtained from A. Aguilera (University of Seville, Spain). Strain S9 (*MATα ura3 his4 can pgi1Δ25::LEU2 spg29-1*) was derived by crossing E9 (obtained as a spontaneous revertant of AAG2 as described below) to strain 329 (*MATα ade8*) and repeated backcrossing of appropriate *MATα* segregants to AAG2. Strain OH1 (*MATα ura3 his4 can*) is a wild-type (i.e. *PGI1*) haploid derived from a cross between 329 and AAG2. Strains 26.1A (*MATα ade1 ade2 his7 pgi1-1*) and 26.6B (*MATα ura1 pgi1-1*) were constructed from mutant 9520b/TIC (*MATα pgi1-1*) (Maitra, 1971), which was provided by P. K. Maitra (Tata Institute of Fundamental Research, Bombay, India).

Isolation of mutants carrying suppressors of *pgi1Δ* mutations. A 100 ml batch of strain AAG2 was grown overnight in YEPF to OD₆₀₀ 1.00. The cells were then harvested by

centrifugation and resuspended in 100 ml YEPD. The culture was divided into 10 separate aliquots, each of which was incubated in a 100 ml conical flask at 30 °C for 6 d. After this time 0.1 ml samples were removed from each small culture and plated directly onto YEPD. The YEPD plates were inspected after 3, 5 and 7 d. Colonies which had appeared at each time and were large enough to handle were transferred to YEPF, incubated for 4–5 d and then replica-plated to YEPF and YEPD. Clones which were able to grow well on YEPD when re-tested were retained for further analysis.

NMR analyses. For *in vivo* NMR analysis cells of S9 were grown in YEPD to OD₆₀₀ 4.0 (late exponential phase for this strain), harvested by centrifugation, resuspended in 2 ml 50 mM potassium phosphate buffer pH 6.0 containing 30% (v/v) ²H₂O and transferred to a 10 mm NMR tube with a gassing system which provides both aeration and mixing (described by Lloyd *et al.*, 1993). Then 70 mg [2-¹³C]glucose (99 atom % enrichment) was added and ¹³C data were collected in blocks of 500 accumulations (each of 8K data points over 22000 Hz) with an acquisition time of 0.18 s and a 1 s delay between pulses using a Bruker WM360 spectrometer operating at 90.5 MHz. High-power ¹H-decoupling was used during acquisition, with low-power decoupling during delays: preliminary experiments had determined the correct settings to effect proton decoupling with sufficient dielectric heating to maintain a temperature of 30 °C ± 1 °C.

Metabolic analyses were also done on cells which had been grown to OD₆₀₀ 4.0 in YEPD, harvested by centrifugation and resuspended in YEP containing [2-¹³C]glucose. After 150 min incubation at 30 °C cells were harvested and perchloric acid extracts prepared for ¹³C NMR analysis as described previously (Dickinson & Hewlins, 1988, 1991). The spectrum was recorded for the solution in a 5 mm NMR tube, using 32K data points over 22000 Hz, with broad-band ¹H-decoupling, and also by the DEPT method to determine the number of protons attached to each carbon signal. A two-dimensional ¹³C-¹H chemical shift correlation spectrum was carried out using the XHCORRD routine in the standard Bruker NMR software. All chemical shifts are reported in p.p.m. relative to the signals (¹H and ¹³C as appropriate) from sodium 3-(trimethylsilyl)propane-1-sulphonate (assigned $\delta = 0$ for both nuclei) measured in ²H₂O solution as the external standard.

Enzyme assays. Cells were harvested by centrifugation, resuspended in 50 mM potassium phosphate buffer pH 7.4 containing 2 mM EDTA and 2 mM 2-mercaptoethanol and disrupted using a Braun homogenizer as described by Dickinson & Williams (1986). Aliquots of this homogenate were used immediately as the source of enzyme. Phosphoglucose isomerase (EC 5.3.1.9), glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and gluconate-6-phosphate dehydrogenase (EC 1.1.1.43) were assayed as described by Maitra & Lobo (1971). Ribulose-5-phosphate 3-epimerase (EC 5.1.3.1) was assayed according to Williamson & Wood (1966). Transaldolase (EC 2.2.1.2) was assayed by the method of Tchola & Horecker (1966). NAD- and NADP-dependent glutamate dehydrogenases (EC 1.4.1.2 and 1.4.1.4) were both assayed in 0.1 M imidazole buffer pH 7.9 in a total volume of 1.8 ml. NADP-dependent glutamate dehydrogenase was assayed by following NADPH disappearance when 2-oxoglutarate (22.8 μ mol) was converted to glutamate in the presence of ammonium acetate (614 μ mol) using 0.03 μ mol NADPH. NAD-dependent glutamate dehydrogenase was monitored by following the increase in absorbance at 340 nm due to NADH formation when glutamate (25 μ mol) was converted to 2-oxoglutarate using 0.375 μ mol NAD.

RESULTS

Isolation and genetic characterization of mutants carrying suppressors of *pgi1*Δ mutations

The spontaneous mutants which arose on YEPD after 3 d were called 'early mutants' and numbered E1–E33. The 'mid mutants' collected after 5 d were numbered M1–M14 and the 'late mutants' were numbered L1–L29. There was no correlation with the original time of appearance of an individual mutant and its rate of proliferation when examined on YEPD plates or in YEPD liquid medium: i.e. there was a range of generation times amongst all three sets of mutants. However, all of the 'mid mutants' subsequently died over the next 4 weeks (irrespective of whether they were stored on YEPD or YEPF) before they had been characterized genetically. Nevertheless, the frequency of suppressor mutations would seem to be 10⁻⁷–10⁻⁸. Sixteen of the remaining mutants which grew most rapidly on YEPD were mated on YEPF to strain 26.1A. The diploids which formed were selected on fructose minimal medium supplemented with 0.1% glucose. These diploids were then replicated onto YEPD: none of them grew on YEPD, showing that the suppressor mutations were all recessive. A series of outcrosses to suitable wild-type haploids allowed a subsequent complementation analysis, which indicated the existence of at least three distinct *spg* mutations. It seemed prudent to retain the terminology of Aguilera (1987) for such mutants rather than add a further acronym to an already crowded literature of phenotypes. However, to avoid the possibility of a future overlap of mutants from our different laboratories it was decided to call these three mutations *spg29* to *spg31*. It was not possible to

Fig. 2. Typical growth curves for S9 (*pgi1*Δ *spg29*-1; ○) and wild-type haploid OH1 (*PGI1*; ●) in YEPD liquid medium. Both strains were inoculated into fresh pre-warmed medium from starter cultures grown to exponential phase in YEPD.

Fig. 3. Time course of utilization of [2-¹³C]glucose by strain S9. *In vivo* time-elapsed proton decoupled NMR spectra are shown. α , C-2 of α -D-glucose; β , C-2 of β -D-glucose; T, C-2 of trehalose; G, C-1,3 glycerol.

Fig. 4. ¹³C NMR spectrum of a perchloric acid extract of strain S9 made after growth in YEP-[2-¹³C]glucose. E, C-2 of ethanol; 4, C-4 of glutamate; 3, C-3 of glutamate; 2, C-2 of glutamate; N, C-4 of glutamine; other resonances marked are as in Fig. 3.

definitely assign all of the mutants, mainly due to their different rates of proliferation on YEpd prior to mating. The *spg29* mutation was represented by three alleles all resulting in very similar phenotypes, the most prominent of which was the most rapid growth (generation time of 335 min in exponential phase) in glucose (Fig. 2).

¹³C NMR analysis of metabolism in a *pgi1Δ spg29* mutant

In vivo NMR analysis of strain S9 showed that the major metabolic product derived from [2-¹³C]glucose was glycerol labelled at C-1 and C-3 (Fig. 3). This signal corresponding to glycerol (65.3 p.p.m.) increased with

time as did the resonance corresponding to C-2 of the storage compound trehalose (75.7 p.p.m.). Three other resonances can be seen in Fig. 3 (78.6 p.p.m., 72.4 p.p.m. and 63.5 p.p.m.); they are explained below. The *in vivo* experiment was not run for longer because although it could be seen (from comparison of the peak heights of the signals due to C-2 of glucose) that very little glucose had been metabolized, the experiment had served its purpose, i.e. identification of the metabolic pathway by which the glucose was catabolized. Detailed isotopomer analysis is always better done on perchloric acid extracts because field inhomogeneity within the sample of *in vivo* experiments leads to signal broadness.

One possible criticism of the *in vivo* NMR study above is that the cells are in a non-growing situation because no nitrogen source was provided, although other workers actually strive to devise conditions of rigorous non-growth for metabolic analysis (e.g. Benevolensky *et al.*, 1994). However, in this case the criticism would seem to be irrelevant because analysis of an extract made from cells grown in YEP-[2-¹³C]glucose gave a similar result (Fig. 4). In addition, the expanded spectrum allows the identification of resonances at 18.9 p.p.m. (ethanol C-2), 29.4 p.p.m. (glutamate C-3), 35.6 p.p.m. (glutamate C-4) and 57.3 p.p.m. (glutamate C-2). Although small, the signal due to ethanol C-2 is highly significant because it confirms that ¹³C in this molecule has been derived via the hexose monophosphate pathway (as explained in the Introduction). The fact that the intensity of the signal for glutamate C-4 is significantly greater than that for C-3, which in turn is much greater than the intensity of the glutamate C-2 resonance, is also important because these data indicate that glutamate is being labelled as predicted for metabolism of [2-¹³C]glucose around the hexose monophosphate pathway to yield pyruvate labelled at C-3 and C-1. This is decarboxylated to [2-¹³C]acetate, which then enters the tricarboxylic acid cycle. Indeed, it is just possible to observe the appearance of label in the C-4 position of glutamate first, followed by label at C-3 and C-2, in the time-elapsed spectra in Fig. 3. The resonance at 34.6 p.p.m. is due to C-4 of glutamine formed directly from glutamate. The multiplets at 98.6 p.p.m. and 94.8 p.p.m. are respectively C-1 of β -D-glucose and α -D-glucose which are visible mainly due to natural abundance of ¹³C at this position. The singlet (centre line) resonance in each case corresponds to glucose labelled only at C-1; the doublets are due to interaction with the heavily labelled C-2. Similarly, the resonance at 63.5 p.p.m. seen here and noted in Fig. 3 along with the resonances at 78.6 p.p.m. and 72.4 p.p.m. are due to glucose C-6, C-3 and C-4 respectively: all were observed due to natural abundance.

Initial signal assignment was made by comparison of ¹³C chemical shifts with those of standard compounds recorded under comparable conditions. Additional information was obtained from further NMR experiments carried out on the perchloric acid extract. The DEPT method was used to establish the number of protons attached to each ¹³C nucleus and this confirmed CH or CH₂ for each signal in accordance with the assignments

Table 1. Specific activities of various enzymes in strains AAG2 (*pgi1Δ*) and S9 (*pgi1Δ spg29*)

Strain S9 was grown to stationary phase in YEPD. Strain AAG2 was grown to stationary phase in YEPE, harvested and transferred to YEPD for 24 h. The results are the means of duplicate determinations.

Enzyme*	Specific activity [mU (mg protein) ⁻¹]		Activity in suppressed strain relative to parent (%)
	AAG2	S9	
G6PDH	206.5	194.3	94.1
GND	81.9	169.1	206.5
RPE	1.0	2.1	200.7
TAL	74.4	117.6	158.0
NAD-GDH	24.4	57.2	234.5
NADP-GDH	42.7	86.3	202.0

* G6PDH, glucose-6-phosphate dehydrogenase; GND, 6-phosphogluconate dehydrogenase; RPE, ribulose-5-phosphate 3-epimerase; TAL, transaldolase; NAD-GDH, NAD-dependent glutamate dehydrogenase; NADP-GDH, NADP-dependent glutamate dehydrogenase.

given above. Further information was obtained from the ¹³C-¹H correlation spectrum. In particular this confirmed that the ¹³C at 65.3 is attached to protons at δH 3.47 and 3.55, as expected for glycerol. The carbon signals attributed to ethanol C-2, glutamate C-4, glutamine C-4 and β-D-glucose C-1 were shown to be connected to protons having chemical shifts of 1.3, 2.33, 2.45 and 4.6 respectively in accord with standard values.

Enzyme assays on the *pgi1Δ* parent and the *pgi1Δ spg29* suppressed double mutant

Phosphoglucose isomerase was undetectable in the parental strain AAG2 and in strain S9, confirming that both strains carry the *pgi1Δ* mutation and that suppression in S9 was not due to the regaining of this activity. The specific activity of glucose-6-phosphate dehydrogenase was virtually identical in both strains, but the specific activities of all of the other enzymes assayed were all increased in the bypass mutant to 150–235% of the levels present in the parental strain (Table 1). The greatest increase in specific activity occurred in the case of NAD-dependent glutamate dehydrogenase.

DISCUSSION

Spontaneous mutants were isolated carrying mutations which suppress the *pgi1Δ* mutation, thus allowing growth on glucose. In many respects these newly isolated *spg* mutants seem similar to those described by Aguilera (1987) and also the *rgl* mutants of Gamo *et al.* (1993). The reason for isolating these mutants was to establish the metabolic pathway used which enables a *pgi1Δ* suppressed

mutant to utilize glucose. ¹³C NMR studies clearly showed that the hexose monophosphate pathway was used to suppress the defect in phosphoglucose isomerase. Despite the fact that previous studies all suggested the hexose monophosphate pathway was used in suppressed *pgi1* strains (Aguilera, 1987; Gamo *et al.*, 1993; Boles *et al.*, 1993), this is the first time that the flux of carbon has actually been proved.

The specific activities of enzymes of the hexose monophosphate pathway were increased in the suppressed strain compared with the parental *pgi1Δ* strain with the exception of glucose-6-phosphate dehydrogenase. These results serve to confirm the NMR study and lead to the conclusion that glucose-6-phosphate dehydrogenase is not the limiting step of the metabolic bypass. Aguilera (1987) reported no significant differences in specific activities between *pgi1Δ* and *pgi1Δ spg1* double mutants. He also reported the specific activity of 6-phosphogluconate dehydrogenase as lower in the suppressed strains than in the *pgi1Δ* parents whereas we observed that the specific activity of this enzyme was double compared with the parental strain. The reason for the difference between our results is likely to be because Aguilera grew both sorts of strain in YEP containing 2% fructose and 0.1% glucose, whilst we grew the *pgi1Δ* strain in this medium (it will not grow in YEPD), and then transferred it to YEPD for 24 h before determining enzyme activities; mutant S9 was grown in YEPD. The very reason for doing this was the possibility that the activity of one or more enzymes of the hexose monophosphate pathway might be higher in the presence of (2%) glucose than (2%) fructose.

The largest increase recorded for a single enzyme was for NAD-dependent glutamate dehydrogenase. This observation confirms the work of Boles *et al.* (1993) on the importance of this activity in suppressing *pgi1Δ* mutations. However, it is worth commenting that the elevation of NAD-dependent glutamate dehydrogenase is nothing like as high as the massive overexpression achieved by Boles *et al.* (1993), who put the *GDH2* gene in a multi-copy plasmid. As demonstrated in the present study, *S. cerevisiae* does not have to achieve such a dramatic alteration to its normal metabolic activities: production of the large amounts of glycerol which were observed serves as an additional route for NAD regeneration. It is conceivable that other *spg* mutations result merely in the production of more glycerol without any increase whatsoever in the activity of NAD-dependent glutamate dehydrogenase, although it is not clear whether the extra glutamate produced (and not recycled to 2-oxoglutarate by NAD-dependent glutamate dehydrogenase) would be an advantage that allowed more rapid growth (glutamate is the major pool of nitrogen), or a detriment to the cell because of the depletion of an important intermediate of the tricarboxylic acid cycle.

In a study of carbon metabolism during sporulation in acetate it was noted that at a distinct time in the process metabolic flux around the hexose monophosphate pathway increased appreciably (Dickinson *et al.*, 1983). Per-

haps this up-regulation in sporulating diploids involves a gene which is mutated in *spg* mutants. Cloning of *SPG* genes will allow an examination whether the same genes are involved in controlling expression of hexose monophosphate pathway functions in sporulation and glycolytic bypass mutants.

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