GLYCOLYSIS MUTANTS IN *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Mutants have been isolated in *S. cerevisiae* with the phenotype of growth on pyruvate but not on glucose, or growth on rich medium with pyruvate but inhibition by glucose. Screening of mutagenized cultures was either without an enrichment step, or after enrichment using the antibiotic netropsin (YOUNG *et al.* 1976) or inositol starvation (HENRY, DONAHUE and CULBERTSON 1975). One class of mutants lacked pyruvate kinase (*pyk*), another class had all the enzymes of glycolysis, and one mutant lacked phosphoglucose isomerase (*pgi*, MAITRA 1971). Partial reversion of pyruvate kinase mutants on rich medium containing glucose gave double mutants now also lacking hexokinase (*hxk*), phosphofructokinase (*pfk*), or several enzymes of glycolysis (*gcr*). In diploids the mutations were recessive. *pyk*, *pgi*, *pfk*, and *gcr* segregated 2:2 from their wild-type alleles. *PYK hxk*, *PYK pfk*, and *PYK gcr* segregants grew on glucose.

ALTHOUGH glycolysis in *Saccharomyces cerevisiae* has been the source of much biochemical knowledge, few mutants have been studied. SKOOG and LINDEGREN (1947) reported mutants which grew on organic acids but not on glucose; it is possible that some had lesions in glycolysis. MAITRA (1971) described a phosphoglucose isomerase mutant, obtained by nitrosoguanidine mutagenesis and screening for clones able to grow on fructose but not glucose. The same author (MAITRA 1970) used a hexokinase mutant, selected for resistance to 2-deoxyglucose, to study glucokinase. MAITRA and LOBO (1971a) showed that some glycolytic enzymes were present in much greater amounts under conditions of glycolysis than gluconeogenesis, and the use of mutants led them to suggest glucose 6-phosphate as a possible inducer (1971b). For at least one enzyme of the pathway, fructose diphosphatase, glucose causes inactivation, probably by proteolysis (MOLANO and GANCEDO 1974); there is a *S. carlsbergensis* mutant unable to grow on sugars and thought to be affected specifically in the inactivation (VAN DE POLL, KERKENAAR and SCHAMHART 1974). Mutations affecting alcohol dehydrogenases have also been described (CIRIACY 1975). A listing (PLISCHKE *et al.* 1975) of *S. cerevisiae* mutations includes some of the above, but no others primarily affecting glycolysis.

We have begun a study of yeast glycolysis and here report several mutants: hexokinase, phosphoglucose isomerase, phosphofructokinase, pyruvate kinase, and one that apparently affects several enzymes. Certain such mutants, and

others, have recently been found in other laboratories (SPRAGUE 1977; LAM and MARMUR 1977; LOBO and MAITRA 1977; MAITRA and LOBO 1977; J. K. BHATTACHARJEE, personal communication).

MATERIALS AND METHODS

Yeast strains: The two parental strains used for mutant selection were D585–11C (**a** *lys1*, "DFY1"), from the Cold Spring Harbor yeast course, and strain MC-6A ("DFY22") from S. HENRY (HENRY, DONAHUE and CULBERTSON 1975). M. BRANDRISS and D. BOTSTEIN supplied strains D583–42B (*α leu2–1 trp1–1 tyr7–1*, "DFY56"), D500–2B (*α ade6*, "DFY58"), and D502–33 (**a** *ade6*, "DFY59").

Media: The minimal medium was M63 (COHEN and RICKENBERG 1956), (0.1 M KH_2PO_4, 15 mM $(NH_4)_2SO_4$, 0.8 mM $MgSO_4$, 2 μM $(Fe)_2(SO_4)_3$, adjusted to pH 7.0 with KOH). It was always supplemented with 0.4 mg/l each of thiamine-HCl, pyridoxine, and pantothenate, 0.002 mg/l biotin, and 2 mg/l inositol (the latter omitted for inositol starvation). The carbon source was 10 g/l, and any amino acid supplements were usually 25 mg/l. Plates also contained 20 g/l Bacto agar (Difco). Rich medium was usually M63 supplemented with 10 g/l Bacto tryptone and 4 g/l Bacto yeast extract; it also contained an added carbon source, 10 g/l (*e.g.*, rich-pyruvate, rich-glucose, etc.). Other types of rich media (all with 2% agar) were the following (FINK 1970): YPD (20 g/l peptone, 10 g/l yeast extract, 20 g/l glucose); presporulation medium (3 g/l peptone, 8 g/l yeast extract, 100 g/l glucose); and sporulation medium (10 g/l potassium acetate, 1 g/l yeast extract, 0.5 g/l glucose). Incubations were at 30°, aerobic; New Brunswick Gyrotory shakers were used for liquid cultures, with flasks ⅕ full.

Comments on media: The pH 7 minimal medium was compared with some more commonly used media for yeast [including one buffered with citrate at pH ca. 5 (OLSON and JOHNSON 1949)], and Yeast Nitrogen Base without amino acids (which has trace elements, PFAFF, MILLER, and MRAK 1966), assessing rate of colony development and/or growth in liquid medium on carbon sources such as glucose, lactate, pyruvate, and acetate. It generally gave growth rates and cell yields similar to the other media. For vitamins, with strain D585–11C only biotin was essential, but colonies were larger and of more uniform size with the mixture. Media such as YPD, YP-Pyruvate, etc., could usually be substituted for the M63-based rich media, although the latter were used for the data of Tables 1 and 2.

Enzyme assays: Initial screenings of enzyme activities used cells made permeable by a method slightly modified from SERRANO, GANCEDO and GANCEDO (1973). To 1 ml of culture was added 4 ml extract buffer (50 mM K_2HPO_4, 2 mM Na-ethylenediamene tetraacetic acid, 2 mM 2-mercaptoethanol, pH 7.4) and 1 ml solution C (1 vol solution A (ethanol:toluene:triton X-100,10:2:1, v/v/v, kept frozen) and 14 vol solution B (ethanol:toluene 5:1, v/v, fresh)) and the mixture was treated 1 min with a vortex mixer at room temperature. The cells were centrifuged, washed twice with extract buffer, and assayed the same way as extracts (below).

The enzyme activity values of Table 2 were measured from extracts made with a French press. Cultures were centrifuged and the cells washed once with water. The pellets were resuspended (1g wet wt/3 ml) in extract buffer, passed once through a French press cell at 20,000 psi, and centrifuged 2 hr at 35,000 rpm in a Spinco roter 50 Ti. The resulting crude extract was passed through a small column of Sephadex G-50, coarse (Pharmacia), 1 vol extract/4 vol resin; samples of 1 vol were collected; typically about ⅔ of the protein emerged in fraction 3, which was used for assays, and was free of the metabolites such as fructose diphosphate, sometimes present in large amounts in untreated extracts.

Assays were according to MAITRA and LOBO (1971a) with minor changes. 1 ml incubation mixtures all contained 50 mM triethanolamine and 10 mM $MgCl_2$, pH 7.4. The additional components (other than extract) for the complete reactions were as follows (quantity of reagent is mentioned only once if the same in other assays; auxilliary enzymes were from Boehringer or Sigma). Hexokinase (*Hxk*): 0.3 mM *NADP*+, 1 mM ATP, 5 mM glucose or fructose (as indicated), 4 μg glucose 6-phosphate dehydrogenase (Zwf), and 2 μg phosphoglucose isomerase

(Pgi). Zwf: $NADP^+$, and 1 mM glucose 6-phosphate. Pgi: $NADP^+$, 1 mM fructose 6-phosphate, and Zwf. Fructose diphosphatase (Fdp): $NADP^+$, 0.5 mM fructose 1,6-diphosphate, Pgi, and Zwf. Phosphofructokinase (Pfk): 0.2 mM NADH, ATP, 5 mM fructose 6-phosphate, 40 μg fructose diphosphate aldolase (Fda), 3 μg triose phosphate isomerase (Tpi), and 30 μg α-glycerophosphate dehydrogenase (Gpd). Fda: NADH, 1 mM fructose 1,6-phosphate, Tpi, and Gpd. Tpi: NADH, ca. 0.4 mM DL-glyceraldehyde 3-phosphate (Sigma), and Gpd. Glyceraldehyde 3-phosphate dehydrogenase (Gld): NADH, 1 mM glycerate 3-phosphate, 5 mM cysteine-HCl, ATP, and 20 μg phosphoglycerate kinase (Pgk). Pgk: like Gld, but with 20 μg Gld instead of Pgk. Phosphoglycerate mutase (Pgm): NADH, glycerate 3-phosphate, 1 mM ADP, 10 μg enolase (Eno), 20 μg pyruvate kinase (Pyk), and 20 μg lactate dehydrogenase (Ldh). Eno: NADH, glycerate 3-phosphate, ADP, 10 μg Pgm, Pyk, and Ldh. Pyk: NADH, 1 mM phosphoenolpyruvate, 1 mM fructose 1,6-diphosphate, ADP and Ldh. Reactions were followed at 340 nm at 25° with a Gilford recording spectrophotometer. Rates were calculated from slopes with corrections for the rate without substrate, etc.; for phosphofructokinase and aldolase, 2 NADH were assumed oxidized per substrate used. Rates are in μmoles/min, mg protein. Protein was determined by the biuret reaction (LAYNE 1957) after precipitation of the sample with 0.5 M trichloracetic acid; the standard was bovine serum albumin, as measured by absorbance (DANIEL and WEBER 1966).

Chemicals: Netropsin was a kind gift of E. L. PATTERSON, Lederle Laboratories, Pearl River, N.Y. Glusulase was from Endo Laboratories. Other chemicals were from the usual suppliers.

RESULTS

Isolation of mutants: In *Escherichia coli*, where many glycolytic mutants are known (BACHMANN, LOW and TAYLOR 1976; FRAENKEL and VINOPAL 1973), different growth substrates ultimately enter the glycolytic pathway at different points, so mannitol is a permissive carbon source for phosphoglucose isomerase mutants, fructose for phosphofructokinase mutants, etc. *Saccharomyces cerevisiae* uses fewer carbon sources (Figure 1); the usual sugars (glucose, fructose, mannose, galactose, disaccharides) enter at one end of the pathway and gluconeogenic compounds (ethanol, pyruvate, lactate, acetate) at the other. Laboratory strains do not all have the same range of usable carbon sources. We surveyed a few (D587–4B, S288C, and D585–11C) and chose, mainly on the basis of colony sizes on minimal plates, strain D585–11C (**a**, *lys*)—to be called DFY1—for initial experiments. It grows on glucose, fructose, mannose, pyruvate, lactate, and acetate.

Mutants were isolated with the phenotypes of (a) no growth on glucose but growth on pyruvate, or (b) inhibition in media containing glucose. The first phenotype might be thought restrictive to mutants lacking enzymes used in glycolysis and not gluconeogenesis (*e.g.*, phosophofructokinase and pyruvate kinase); however, *E. coli* mutants in other steps have a similar phenotype (because of "leakiness" etc.). The rationale for mutants inhibited by glucose also came from the work with prokaryotes, where sugar inhibition of such mutants is thought to be related to the accumulation of toxic levels of normal metabolites. In both types of selection potential mutants were screened on other media and then assayed for the enzymes of glycolysis. (Almost all the strains mentioned in the remainder of the paper are shown in Table 1, which gives growth characteristics, and Table 2, which gives activities of the glycolytic enzymes.)

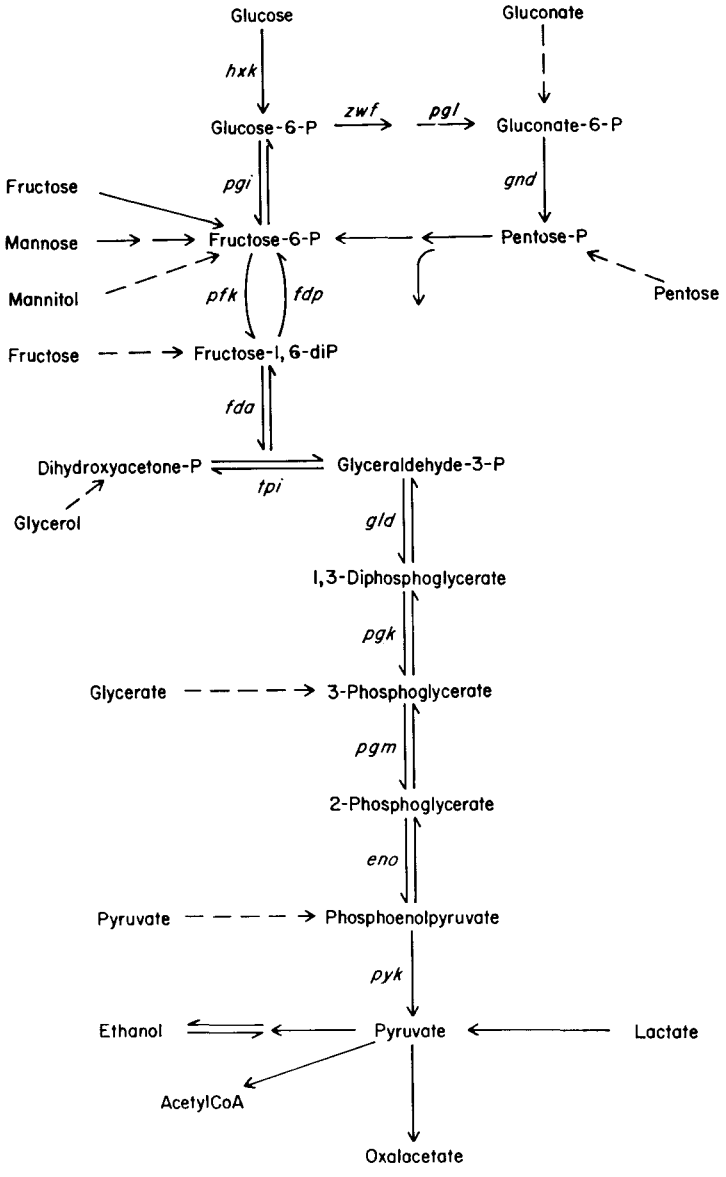

FIGURE 1.—Pathways of sugar metabolism. The gene abbreviations are those used for *E. coli* (1) with the exception of *gld* for glyceraldehyde 3-P dehydrogenase (see legend to Table 2 for enzyme names). Pathways indicated by dashed arrows are known in *E. coli*, but in Saccharomyces are either not commonly found or might be used only at high substrate concentrations (*e.g.*, GANCEDO, GANCEDO and SOLS 1968).

(i) *An isolation without enrichment*: Strain DFY1 was grown to stationary phase in YPD medium, washed and resuspended in salts solution M63 and treated 1 min and 2 min with ultraviolet irradiation (Sylvania germicidal lamp G15T8). Survivals were *ca.* 30% and 10%, respectively, and in the latter case

TABLE 1

Growth on plates

Strain and origin		Growth (colony size, mm) Rich glucose 3 days	Rich pyruvate 4 days	Minimal glucose 4 days	Minimal pyruvate 7 days	Other markers
DFY1	(is strain D585–11C)	>3.0	2.0	0.6	0.7	a *lys1*
DFY3	*pyk-1* mut. of DFY1	0.0	1.6	0.0*	0.7	a *lys1*
DFY7	a class 2 mutant of DFY1	0.0	1.6	0.0	0.6	a *lys1*
DFY22	(is strain MC6A, ref. 8)	2.5	1.3	0.8	0.5	a *ino1–13 ino4–8*
DFY23	*pyk-4* mut. of DFY22	0.0	0.8	0.0	0.5	a *ino1–13 ino4–8*
DFY34	*pgi-1* mut. of DFY22	0.0†	0.6	0.0†	0.0	a *ino1–13 ino4–8*
DFY56	(is strain D583–42B)	2.5	1.5	0.8	0.2	α *leu2–1 trp1–1 tyr7–1*
DFY60	diploid DFY1 × DFY56	>3.0	1.2	1.3	0.3	(prototroph)
DFY61	diploid DFY22 × DFY56	>3.0	1.5	1.2	0.5	(prototroph)
DFY62	*hxk-1 pyk-1*, from DFY3	0.3	1.3	0.1	0.2	a *lys1*
DFY63	diploid DFY62 × DFY56	>3.0	2.5	>3.0	1.5	(prototroph)
DFY64	*hxk-1* seg. of DFY63	2.1	1.3	1.6	0.5	*leu2–1 tyr7–1*
DFY65	*gcr-1 pyk-1*, from DFY3	0.2	1.2	0.0	0.1	a *lys1*
DFY66	diploid DFY65 × DFY56	>3.0	1.7	>3.0	1.8	(prototroph)
DFY67	*gcr-1* seg. of DFY66	0.5	1.3	0.6	0.2	a *leu2 lys1 trp1*
DFY68	*pfk-1 pyk-4*, from DFY23	0.1	0.9	0.0	0.4	a *ino1–13 ino4–8*
DFY69	diploid DFY68 × DFY56	>3.0	2.0	>3.0	1.8	(prototroph)
DFY70	*pfk-1* seg. of DFY69	2.0	1.1	0.7	0.2	α *ino leu2*

Approximate average colony size was measured after incubation at 30° for the times indicated. For comparison of all the strains in one experiment, the minimal plates were supplemented with inositol, leucine, lysine, tryptophan, tryosine, and phenylalanine. However, it should be noted that growth of some of the strains on minimal plates was partially inhibited by non-essential supplements. Rich medium was M63 + 10 g/l Bacto tryptone + 4 g/l Bacto yeast extract + 10 g/l glucose or pyruvate.

* No growth on fructose or mannose either.

† Grows on rich fructose but not minimal fructose.

the frequency of lysine prototrophy increased from 1×10^{-6} to 129×10^{-6}. The treated cultures were either directly spread to minimal plates with acetate (and lysine), or first grown up in YP medium (no added carbon source) before spreading to the acetate plates. After 7 days incubation colonies were patched to minimal medium with glucose and minimal medium with acetate. Three glucose-negative strains were found among 3900 clones, one of them being DFY3. It was also unable to grow on fructose or mannose, or glucose with pyruvate (both 1%), or rich medium with glucose; it grew on minimal lactate, minimal pyruvate, and minimal acetate. Strain DFY3 was later identified as lacking pyruvate kinase (a class 1 mutant).

(ii) *Isolations using the antibiotic netropsin* (see YOUNG *et al.* 1976): The protocol was developed with the parental strain DFY1 and the glucose-negative mutant DFY3. In a minimal medium permissive for the wild-type strain (1%

TABLE 2

Enzymes (specific activity in crude extracts)

Strain and lesion	Hxk		Pgi	Zwf	Pfk	Fdp	Fda	Tpi	Gld	Pgk	Pgm	Eno	Pyk
	Glu	Fru											
DFY1 (haploid)	1.01	1.46	1.00	0.19	0.24	0.06	0.14	10.41	3.56	2.33	0.64	0.58	1.16
DFY3 (pyk-1)	0.61	0.75	2.08	0.13	0.10	0.07	0.07	4.79	2.66	2.19	0.22	0.37	0.00
DFY7 (?)	0.92	1.15	2.61	0.16	0.17	0.08	0.19	7.70	5.05	3.99	0.80	0.56	1.72
DFY22 (haploid)	0.60	0.84	1.84	0.10	0.09	0.02	0.06	8.26	2.92	3.70	0.17	0.58	1.38
DFY23 (pyk-4)	0.58	0.65	2.08	0.09	0.12	0.03	0.07	5.80	3.15	1.65	0.39	1.03	0.00
DFY34 (pgi-1)	1.07	1.23	0.00	0.12	0.08	0.01	0.03	8.76	3.17	2.08	0.12	0.29	1.30
DFY56 (haploid)	0.59	0.81	1.53	0.08	0.06	0.02	0.09	7.22	2.29	1.42	0.29	0.33	1.12
DFY60 (diploid)	0.52	0.74	1.13	0.09	0.05	0.02	0.11	3.34	2.07	1.17	0.19	0.22	0.72
DFY61 (diploid)	0.88	1.29	2.81	0.11	0.15	0.04	0.16	8.92	5.85	4.76	0.78	1.17	1.98
DFY62 (pyk-1 hxk-1)	0.35	0.01	2.21	0.16	0.12	0.07	0.05	6.90	3.05	2.72	0.20	0.38	0.00
DFY63 (pyk-1 hxk-1/+ +)	0.48	0.68	1.87	0.16	0.10	0.03	0.11	7.04	3.08	3.12	0.30	0.34	0.40
DFY64 (hxk-1)	0.38	0.02	2.38	0.19	0.14	0.06	0.07	9.01	3.75	2.26	0.26	0.49	0.93
DFY65 (pyk-1 gcr-1)	0.46	0.44	0.24	0.09	0.05	0.03	0.02	0.16	0.03	0.64	0.00	0.00	0.00
DFY66 (pyk-1 gcr-1/+ +)	0.90	1.48	2.69	0.16	0.08	0.06	0.14	7.57	2.22	1.93	0.32	0.31	0.40
DFY67 (gcr-1)	0.70	0.68	0.23	0.12	0.06	0.02	0.01	0.61	0.09	0.27	0.00	0.00	0.05
DFY68 (pyk-4 pfk-1)	0.57	0.66	1.77	0.08	0.00	0.03	0.05	4.53	3.15	1.49	0.31	1.00	0.00
DFY69 (pyk-4 pfk-1/+ +)	0.91	1.22	3.20	0.12	0.09	0.05	0.18	9.96	5.87	6.50	0.70	1.26	1.08
DFY70 (pfk-1)	1.28	1.85	2.94	0.14	0.00	0.02	0.15	8.06	5.05	5.59	0.80	1.72	3.41

The cells were grown to stationary phase in rich medium with pyruvate, extracts made, and enzymes assayed as described in MATERIALS AND METHODS. The enzyme abbreviations are: Hxk, hexokinase (with glucose or fructose); Pgi, phosphoglucose isomerase; Zwf, glucose 6-P dehydrogenase; Pfk, phosphofructokinase; Fdp, fructose 1,6-diphosphate phosphatase; Fda, fructose 1,6-diphosphate aldolase; Tpi, triose phosphate isomerase; Gld, glyceraldehyde 3-phosphate dehydrogenase; Pgk, phosphoglycerate kinase; Pgm, phosphoglycerate mutase; Eno, enolase; and Pyk, pyruvate kinase. The origin of the strains, and other markers, are shown in Table 1. Values less than 10% of wild type are underlined.

glucose plus 1% pyruvate), 5 μg/ml netropsin gave 0.06% survival after 18 hr; in the same medium there was 40% survival of the mutant. Without the antibiotic, the mutant also survived in a minimal glucose plus pyruvate medium (5 hr treatment, 91%, and 3 days treatment, 76%); in a minimal medium with glucose alone the mutant eventually lost viability (5 hr treatment, 60% survival, and 3 days treatment, 1% survival). Therefore, it seemed that a treatment with netropsin in minimal medium with pyruvate and glucose, or glucose alone, would serve to considerably enrich a culture for glucose-negative mutants, at least of the DFY3 type.

Accordingly, strain DFY1 was again treated with ultraviolet light (3% survival) and grown out both in minimal medium with pyruvate and in rich medium with pyruvate. The cells were washed and suspended at *ca.* 2×10^6/ml in minimal medium with glucose. Netropsin, 5 μg/ml, was added after 1 hr and the incubation continued 18 hr. There was generally a small increase in A_{580}

(*e.g.*, from 2.0 to 3.0), and survival was 0.1 — 1%. Survivors were allowed to form colonies on minimal medium with pyruvate; of 200 tested, ten had the phenotype glucose-negative pyruvate-positive. Two of them (class 1 mutants) lacked pyruvate kinase. Several of the others, of superficially similar phenotype but containing pyruvate kinase, will be called class 2 mutants (*e.g.*, DFY7).

One netropsin enrichment was done on a nonmutagenized culture of strain DFY1. Zero mutants were found among 552 survivors.

(iii) *Isolations using inositol starvation*: We found strain MC-6A (here called DFY22: *ino1–13*, *ino4–8*, **a**), (HENRY, DONAHUE and CULBERTSON 1975) to grow well on minimal medium (supplemented with inositol) containing glucose, mannose, or fructose; on pyruvate, lactate, and acetate, colonies were smaller than those of DFY1, although these compounds considerably improved cell yield in rich medium. Accordingly, we selected the mutant phenotype of growth on rich medium with pyruvate but not on rich medium with glucose (*i.e.*, glucose inhibition). Mutagenesis was with ethyl methane sulfonate (HENRY, DONAHUE and CULBERTSON 1975); there was no detectable killing. The mutagenized culture was subcultured in rich medium with pyruvate, washed and incubated in inositol-free minimal medium containing pyruvate and glucose and the amino acid and base supplement used by HENRY, DONAHUE and CULBERTSON (1975). After 24 and 50 hr starvation (*ca.* 0.1% and 0.01% survival, respectively) the survivors were grown out as colonies on rich medium with pyruvate. Twelve of *ca.* 200 clones grew little if at all on rich glucose; ten of these mutants also did not grow on minimal glucose or minimal fructose. Seven of the ten, including DFY23, lacked pyruvate kinase (*i.e.*, class 1 mutants). One mutant, DFY34, had a phenotype slightly different from the others, growing well on rich fructose but not rich glucose, and not growing on minimal fructose, glucose, or pyruvate. It proved by assay to lack phosphoglucose isomerase (*pgi*).

Enzymes: The mutants were screened by assay for all the enzymes of the glycolytic pathway between glucose and pyruvate (see MATERIALS AND METHODS). Representative data are shown in Table 2. DFY3 and DFY23 are class 1 mutants: they completely lack pyruvate kinase according to the usual assay. One class 2 mutant is shown, strain DFY7; growth was superficially similar to class 1 strains but normal levels of pyruvate kinase and the other glycolytic enzymes were present. Class 2 mutants, which may include many different types, will not be further discussed. DFY34 is a phosphoglucose isomerase mutant. In all cases mixed extracts of mutant and parental strain gave additive activities and no evidence of inhibition. The data also show that in the two types of mutants with identified lesion (*pgi* and *pyk*) levels of other enzymes were not greatly affected.

Revertants of pyruvate kinase mutants: Growth of the pyruvate kinase mutants is inhibited by glucose, so derivatives resistant to glucose might have an earlier block. Using one pyruvate kinase mutant from each parental strain (DFY3 and DFY23), *ca.* 10^6 cells were spread on several plates of the nonpermissive medium rich pyruvate plus glucose; some plates were then exposed to ultraviolet light.

After 1 week of incubation, "revertants" were apparent over a background of slower growth. Strains DFY62, DFY65, and DFY68 are three of them.

Strain DFY62 came from ultraviolet treated strain DFY3 (*pyk-1*). It still contained the pyruvate kinase defect, fructose phosphorylation was almost zero, and glucose phosphorylation was *ca.* half normal. The new lesion will be called *hxk* ("hexokinase"). Strain DFY65 also came from ultraviolet treatment of strain DFY3. Assay showed strain DFY65 to be affected in the levels of several enzymes of glycolysis (other than pyruvate kinase): phosphoglycerate mutase and enolase were absent and several others had very reduced levels. The new mutation is tentatively named *gcr-1* (glycolysis regulation). The third revertant type, strain DFY68, came from strain DFY23 (*pyk-4*) without mutagenesis. It lacked both pyruvate kinase and phosphofructokinase (*pfk-1*).

Diploids and segregation: Parental strains, and presumed double mutants (DFY62, 65, and 68) were crossed with strain DFY56 (glucose-positive, mating type α) and diploids (DFY63, 66, and 69) were selected (MATERIALS AND METHODS). Random spores were obtained on rich medium with pyruvate, and tested on rich medium with glucose and assayed. From diploid DFY63 (*hxk pyk/HXK PYK*), of 22 colonies from rich pyruvate medium 11 grew poorly on rich glucose. All 11 lacked pyruvate kinase, and 5 also lacked fructose kinase (*e.g.*, *HXK pyk* and *hxk pyk*, respectively). Of the 11 which grew on rich glucose, 9 had both enzymes, and 2 lacked fructose kinase but had pyruvate kinase (*i.e.*, *HXK PYK* and *hxk PYK*, respectively). The latter was a new combination, and isolates were tested for other markers and enzymes (*e.g.*, strain DFY64). Similarly, segregants of four types were found from the other diploids of interest: DFY67 (*gcr PYK*) from DFY66 (*gcr pyk/GCR PYK*), and DFY70 (*pfk PYK*) from DFY69 (*pfk pyk/PFK PYK*). DFY67 showed the low levels of several glycolysis enzymes already seen in the original parental strain (DFY65) with the difference of containing pyruvate kinase, although at much lower level than the wild type. DFY70 is a phosphofructokinase mutant in a pyruvate kinase positive background, and only that enzyme was absent. It may be concluded that the mutations were recoverable from diploids and segregated from *pyk*. The new isolates DFY64 (*hxk*), DFY67 (*gcr*) and DFY70 (*pfk*) grew on glucose (Table 1).

Results of tetrad dissection were as follows. For *pgi/PGI*, 4 complete tetrads segregated 2:2 according to growth on rich medium with glucose and assay for phosphoglucose isomerase. For *gcr/GCR*, three complete tetrads showed 2:2 segregation according to growth on rich medium with glucose and by assay of enolase and phosphoglycerate mutase (*i.e.*, either both present or both very low). For strain DFY69 (*pfk pyk/PFK PYK*), three complete tetrads showed by assay 2:2 segregation of each of phosphofructokinase and pyruvate kinase (1 PD, 1 NPD, and 1 T). A spore from the last cross, DFY138 (*α leu2 pfk-1 trp1 tyr7*), was crossed with DFY1 (**a** *lys1*). Of 60 complete tetrads, 58 showed (by assay) 2:2 segregation of *pfk-1*. PD:NPD:T ratios were 5:11:37 for *pfk/leu*, 5:12:39 for *pfk/lys*, 12:8:38 for *pfk/trp*, and 11:9:36 for *pfk/tyr*. Thus *pfk-1* was unlinked to

these markers or to a centromere. (Ratios for the centromere markers *leu2/trp1* were 22:22:10.)

These data accord with the markers *pgi-1*, *gcr-1*, *pfk-1*, and *pyk-4* being single nuclear gene mutations. (A map position *pyk-1* on chromosome *I* has been reported for pyruvate kinase by SPRAGUE (1977) and MAITRA and LOBO (1977). Each group showed independent mutants not to complement. The pyruvate kinase mutants described here have a phenotype similar to *pyk1* mutants and may also be in that locus.)

Analysis of *hxk* has shown the phenotype to depend on *two* independent mutations separately affecting two known hexokinase isoenzymes (GANCEDO, CLIFTON and FRAENKEL 1977; LOBO and MAITRA 1977).

DISCUSSION

The pyruvate kinase mutants have the "simplest" phenotype: they do not grow on sugars, so there seems to be no other productive pathway of phosphoenolpyruvate metabolism. Glucose inhibits their growth. There are several possible mechanisms for this (*e.g.*, repression of cytochromes or other enzymes of pyruvate metabolism, or their inactivation (*e.g.*, DUNTZE, NEUMANN and HOLZER 1968), ATP depletion, or toxicity of an accumulated metabolite). SPRAGUE (1977) has shown that glucose causes adenine nucleotide levels to fall in a pyruvate kinase mutant, and MAITRA and LOBO (1977) found phosphoenolpyruvate accumulation. The fact that earlier blocks in glycolysis lessen glucose inhibition may help in studies of glucose effects.

The phosphoglucose isomerase mutant is similar to the one described by MAITRA (1971): it fails to grow on glucose and glucose is inhibitory. The differences between the two mutants are difficult to assess. (MAITRA's mutant grew on fructose in minimal medium and seemed to produce petites at unusually high frequency; ours did not grow on fructose or any minimal medium; it did grow on rich fructose. MAITRA showed that even in growth on fructose the amount of acid hydrolyzable glucose was near normal, and suggested "leakiness" of the lesion. It is also possible that yeast, unlike *E. coli* (VINOPAL *et al.* 1975) cannot grow normally completely lacking glucose; in that case apparent differences in growth between different mutant isolates and media might be related to glucose contamination.) The lack of growth on glucose of yeast *pgi* mutants shows that the hexose monophosphate shunt is not available. *E. coli pgi* mutants differ in this respect, growing slowly on glucose via the shunt, but it is not known what is rate limiting there either (FRAENKEL and VINOPAL 1973).

None of the mutations reported here are yet known to be in the structural genes for the enzymes in question; *gcr* in particular might be regulatory. The growth on glucose of the strain apparently lacking phosphofructokinase (*pfk*) or several glycolytic enzymes (*gcr*) is, of course, surprising. It may be that in these cases the mutations are more "leaky" than shown by assay. But other pathways should be considered too.

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