Phosphofructokinase Mutants of Yeast
BIOCHEMISTRY AND GENETICS*$

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Mutants of Saccharomyces cerevisiae completely lacking the soluble glycolytic enzyme fructose-6-P kinase are described. The mutations are semidominant, do not complement one another, and define a gene PFK1 located 28-cm distal to rna1 on the extended right arm of chromosome XIII. Of 10 independent mutants, 3 can be suppressed by ochre suppressors. All mutants examined synthesize proteins that cross-react to the antibody against the purified yeast P-fructokinase. The enzyme in spontaneous revertants is distinguishable from the wild type enzyme with respect to thermostability and ATP inhibition. The locus PFK1 thus defines the structural gene of the enzyme.

The pfk1 mutants are not leaky in vivo. All the glucose consumed by a double mutant lacking both P-fructokinase and 6-P-gluconate dehydrogenase ends up as 6-P-gluconate, yet the pfk1 mutants can glycolyze and grow on glucose in air. The cell mass produced per unit of glucose also remains unchanged. Anaerobically, however, growth does not take place, nor does glycolysis. P-fructokinase is thus a dispensable enzyme for aerobic growth, but indispensable for anaerobic growth. The properties of pfk1 mutants suggest that yeast has an alternative mechanism for the aerobic metabolism of fructose-6-P, presumably through the recently reported particulate P-fructokinase (Lobo, Z., and Maitra, P. K. (1982) FEBS Lett. 137, 279-282).

Mutants lacking P-fructokinase are potentially interesting. This enzyme catalyzes an irreversible step in the Embden-Meyerhof pathway and is said to be a flux-determining enzyme in glycolysis (1). The kinetic properties of the enzyme from diverse sources are consistent with this interpretation. P-fructokinase from yeast has been implicated in particular in the Pasteur effect (2) and in the generation of glycolytic oscillations (3). A study of mutants of this enzyme is likely to shed light not only on such questions of cellular physiology but on mechanisms underlying allosteric regulation in general.

Mutants of Saccharomyces cerevisiae lacking this enzyme have been reported from several laboratories (4-7). An unexpected feature of all of these mutants is their ability to grow on glucose. The location of P-fructokinase in the glycolytic pathway suggests that the pentose phosphate pathway may act as a bypass in pfk1 mutants. The growth of these mutants on glucose might also reflect their leakiness in vivo. However, the recently discovered second P-fructokinase in S. cerevisiae (8) probably allows the growth of pfk1 mutants on hexose sugars.

We describe the results of some of our experiments which have a bearing on the problem of growth of pfk1 mutants on glucose. Genetic characteristics of the mutation are also described.

MATERIALS AND METHODS

Strains—A large number of S. cerevisiae strains which were used for mapping and suppression experiments, will be mentioned where appropriate in the text. All of these strains were given generously by the Yeast Genetic Stock Center at Berkeley. Other strains have been described: wild type strain haploid S. cerevisiae (9); pyruvate kinase mutants pykhl-4 and pykl-5 (10); 6-P-gluconate dehydrogenase mutant gnd (11); the P-fructokinase mutant allele pfkhl-1 was isolated earlier (4) as a glucose-negative mutant. Mutants lacking pyruvate decarboxylase pdc1 were isolated by H. M. Chikarmane of this laboratory. Media—The minimal medium and the YEP medium containing yeast extract, peptone, and carbon source have been described (9). The pfk1 and gnd mutants were maintained on YEPl medium containing ethyl alcohol.

Enzyme Assays—P-fructokinase activity was assayed fluorometrically in either cell-free extracts or Toluene lysates as before (12) in a reaction mixture containing 5 mM fructose-6-P, 1 mM ATP, 0.03 mM NADH, 1 unit each of aldolase and a-glycero-1-phosphate dehydrogenase, and 10 units of triose P isomerase. To avoid interference by the particulate enzyme activity which was present only in cultures grown on glucose but not on alcohol (8), glucose-grown cultures were examined only in cell-free supernatants for the soluble enzyme. Alcohol-grown cultures were assayed either in Toluene lysates or in centrifuged cell extracts. The segregation of P-fructokinase in tetrads was followed in Toluene lysates (9) in a discontinuous assay; 50 pl of the lysate were incubated overnight at 23 °C with 0.25 ml of an incubation mixture of 2 mm fructose-6-P, 0.5 mM ADP, and 1 mM ATP in 50 mm triethanolamine buffer containing 10 mm MgCl2, pH 7.4. Samples were heated at 65 °C for 10 min and fructose-1,6-bisP was assayed fluorometrically in 5 to 10 μl of the supernatants. The particulate P-fructokinase was practically inactive under conditions that allowed accumulation of fructose-1,6-bisP (8). Other methods have been described (9, 12).

RESULTS

Isolation of Mutants Lacking P-fructokinase—The allele pfkl-1 which has been used in most of the experiments unless mentioned otherwise was isolated accidentally as a glucose-negative mutant among clones resistant to 2-deoxyglucose (4). In crosses to wild type strains, the marker pfkl segregated independently of 2-deoxyglucose resistance and glucose negativity.

The majority of pfk1 mutants were obtained as spontaneous glucose-resistant revertants of pyk1 mutants (5) growing on YEP plates containing 150 mm ethanol and 50 mm glucose. The more than 120 independent pfk1 mutants which have been isolated by this method have been designated pfkl-2 through pfkl-200. A third method of isolation of pfk1 mutants

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was to revert pdcl mutants on glucose media; 3 mutants (alleles pfi1-201 to pfi1-203) were used for complementation studies. The pfk1 and pdcl markers were eliminated by crossing these isolates to a wild type strain; the resultant single gene mutants lacking P-fructokinase were all able to grow on glucose.

P-fructokinase Activity in Haploids and Diploids—The activity of P-fructokinase in the pfi1 mutants was generally less than 0.1% of the wild type level. A few mutants which had detectable activity (about 1%) were suppressed poorly for growth on alcohol + glucose medium. The enzyme levels in homoygous and heterozygous diploids carrying the allele pfi1-1 were examined in cell-free extracts from cultures growing exponentially on glucose. The P-fructokinase activity was as follows: PFK1, 290 nm/mg of protein; pfi1-1, <0.001 nm/mg of protein; PFK1/PFK1, 330 nm/mg of protein; PFK1/pfi1-1, 90 nm/mg of protein. In stationary cultures of glucose-to-alcohol-grown cultures, the enzyme activity was about a third of that of the wild type haploids or homohyous diploids. The mutation pfi1-1 thus behaved as semidominant. Heterozygous diploids pfi1/PFK1, however, grew on glucose azide plates as did the wild type strains.

For complementation studies, a number of mutants were crossed to one another in paired combinations. The diploids were isolated either by micromanipulation of the zygotes or by prototrophic selection and examined for P-fructokinase activity after growth on alcohol. In most of the hybrids no enzyme activity higher than that seen in the parents could be seen. A few combinations which indicated slightly more enzyme activity than in the parents were sporulated and the activity of P-fructokinase was examined in the haploid progenies. No spore was found to be P-fructokinase-positive. The enzyme activity in such diploids presumably came about by interallelic complementation, e.g. in the diploid pfi1-9/pfi1-6 (see “Nonsense Mutants”). Such experiments suggested that all the mutants tested lie in the same complementation group PFK1. We have, however, not examined all the paired combinations of mutants.

Nonsense Mutants—Of the 10 independent mutants examined in our collection, 3 could be suppressed by ochre suppressors SUP7 or SUP8. Mutants were crossed to the suppressor-bearing strains X2182-3D (a ade2-1, lys1-1, his5-2, trp5-48, met1-1 leu1-12 SUP7) and X2318-26C (ahis5-2, lys1-1, ade2-1, can1-100, trp5-48, ura3-1 SUP8) separately and the tetrad analysis of the sporulated diploids indicated coincident suppression of pfi1 along with known ochre-suppressible auxotrophic markers. Secondary crosses of pfi1-suppressed strains to wild type led to reappearance of P-fructokinase-negative spores as the suppressor was lost. We concluded from this that the following 3 alleles are nonsense mutants: pfi1-1, pfi1-16, and pfi1-17. No amber-specific suppression could be seen in any of the pfi1 mutants using the strain 4380-2C from Prof. G. R. Fink (Cornell University, Ithaca, NY) which carried the following markers: a met1-8a his4-64a leu2-1, lys1-1, SUP4-5a.

These experiments had generated recombinants carrying several nonsuppressible mutant alleles of pfi1 in conjunction with the nonsense suppressor SUP7 or SUP8 and a few of the suppressible auxotrophic markers. These strains were crossed to the test isolates of pfi1 mutant to examine their suppressibility in the diploid. For example, the hybrid pfi1-1 (non-suppressible) SUP7/pfi1-17 (nonsense) + was P-fructokinase-positive by enzyme assay, and its haploid progeny of the genotype pfi1-17 SUP7 was also enzyme-positive. One other diploid of the constitution pfi1-6 (nonsuppressible) SUP7/pfk1-1 (nonsense) +, was, however, found not to have any P-fructokinase activity. When this diploid was sporulated and the spores examined for the enzyme activity it was found that haploids carrying pfi1-1 SUP7 were in fact P-fructokinase-positive. In contrast, the enzyme-positive diploid pfi1-9 (nonsuppressible) +/pfi1-6 (nonsuppressible) SUP7 failed to produce a spore that had P-fructokinase activity. We interpret these observations as reflecting interaction of subunits in this oligomeric protein, providing an instance of negative complementation in the second case, and positive complementation in the last. We have not encountered a pfi1 mutant that is fully dominant with respect to its wild type allele PFK1.

Growth Properties—One of the features of pfi1 mutants that distinguish them from most other glycolytic lesions is their ability to grow on glucose, fructose, and mannose much as the wild type yeast. Results in Table I illustrate this with respect to the nonsense allele pfi1-1 growing, respectively, on alcohol and glucose as carbon sources. Loss of P-fructokinase reduced the rate of growth on alcohol only marginally, while on glucose it was halved. Restoration of enzyme activity by suppression of the nonsense mutation either by the unlinked tRNA suppressor SUP7 or by an intragenic suppressor R5 improved the rate of growth on glucose. However, the absence of any detectable enzyme activity in the soluble supernatants did not prevent growth.

How do pfi1 mutants lacking any measurable enzyme activity of P-fructokinase in soluble supernatants continue to grow on glucose? A reasonable explanation is that the pentose phosphate pathway acts as a bypass of this enzyme. Because of the stoichiometry

\[
\text{3-Glucose-6P + 6-NADP} \rightarrow \text{2-fructose-6-P} + \text{glyceraldehyde-3-P + 6NADP} + 3\text{CO}_2
\]

operative during the use of this pathway, half the glucose ends up as \(\text{CO}_2\) and is presumably lost from productive metabolism. The mutant allele pfi1-1 was grown aerobically on limiting glucose to stationary phase and the yield of cell mass, measured as \(E_{650}\), was determined. Fig. 1 shows a plot of the saturating growth for several concentrations of glucose using both the YEP medium and a minimal medium. The linearity between the cell yield and glucose added showed that they were proportional at low concentrations. However, the loss of P-fructokinase made hardly any difference to the efficiency of cell synthesis at limiting concentrations of glucose. Except for a reduction in the rate of growth, pfi1 mutants behaved very much like the wild type strain during aerobic growth on sugars.

Leakiness of the Nonsense Mutants—In order to examine

Table I

**Comparison of P-fructokinase activity with growth rate**

Haploid yeast cultures were grown on YEP glucose medium to the end of stationary phase and cell-free extracts were examined for P-fructokinase activity. The doubling periods were calculated during exponential growth on YEP medium containing glucose or ethanol. Speros T2A, T2B, and T2C were from a diploid heterozygous for pfi1-1 and SUP7. The strain pfi1-1 R5 was a spontaneous intragenic revertant of the pfi1-1 mutant.

<table>
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<th>Strain</th>
<th>Genotype</th>
<th>P-fructokinase</th>
<th>Doubling period on</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Alcohol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nm/mg protein</td>
<td>h</td>
</tr>
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<td>T2A</td>
<td>pfi1-1</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>T2B</td>
<td>pfi1-1</td>
<td>SUP7</td>
<td>45</td>
</tr>
<tr>
<td>T2C</td>
<td>+</td>
<td>+</td>
<td>115</td>
</tr>
<tr>
<td>pfi1-1 R5</td>
<td>pfi1-1 (R5)</td>
<td>+</td>
<td>140</td>
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</table>

1 H. M. Chikarmane, personal communication.
if the pfkl mutants were leaky in vivo, the nonsense allele pfkl-I was used to construct a double mutant pfkl gnd lacking the shunt pathway enzyme, 6-P-glucuronate dehydrogenase, in addition to lacking P-fructokinase. The double mutant did not grow on glucose, but grew on alcohol, and possessed no more than 5% of the dehydrogenase activity of the wild type strains. P-fructokinase activity measured either in the cell-free extracts or in toluene lysates was undetectable since the particulate activity was not induced by growth on alcohol (8). It was grown in YEP alcohol medium to stationary phase and a washed suspension of cells was treated with glucose. Aliquots were withdrawn into acid for estimation of 6-P-gluconate, respectively. Cultures were grown on a rotary shaker containing YEP and the wild type yeast during aerobic metabolism. The drop in the intracellular concentration of ATP indicated that the mutant strain was able to synthesize glucose-6-P. The levels of fructose-1,6-bisP (not shown) were low during the entire incubation period. The presence of oxygen on the other hand elicited a normal response. The effect of anaerobiosis was reversible. When N2 gas was replaced with O2, glucose utilization resumed and reached the aerobic rate. Mutants lacking P-fructokinase are thus characterized by having a negative Pasteur effect.

Revertants—Since we were interested in revertants that were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respiratory sufficient, the majority of the revertants were respira...
isolated as clones able to form colonies on YEP glucose plates containing azide. The spontaneous reversion frequency varied from $1 \times 10^{-5}$ to $5 \times 10^{-7}$ depending on the pfkl allele. The nonsense mutant pfkl-1 yielded both extragenic and intragenic revertants. On crossing to the wild type strain the revertant R1 gave enzyme-positive as also enzyme-negative revertants. The spontaneous reversion frequency varied.

The revertants from a number of pfkl mutants covered a wide spectrum of enzyme specific activity, thermolability, and kinetic property. Results in Fig. 5 illustrate this with respect to an intragenic revertant from a nonsense allele pfkl-1 and a SUP7-suppressed derivative pfkl-1 SUP7, and intragenic revertants pfkl-7 R13 and pfkl-7 R22 obtained from the non-suppressible allele pfkl-7. The enzyme from the revertant R22, for example, was not perceptibly inhibited by increasing concentrations of ATP unlike P-fructokinase from the revertant R13 or the wild type strain as seen in toluene lysates. When cell-free extracts were used, however, the reverted enzyme in R22 was found to be inhibited by high concentrations (4 mM) of ATP, while the wild type enzyme was inhibited at a much lower concentration of ATP (0.25 mM) under these conditions. No reverted enzyme was found to be completely insensitive to inhibition by ATP. The results in Fig. 5 also indicate that the reverted or the nonsense-suppressed enzyme could be either more stable or more labile to heat compared to the wild type enzyme. The inactivation was monophasic in every case studied.

Cross-reacting Material—Unfractionated cell-free extracts from several mutants were tested for the presence of proteins immunologically similar to P-fructokinase. Ouchterlony double-diffusion plates (16) containing rabbit antiserum raised against purified P-fructokinase from yeast were used in the central well; the peripheral wells contained either the crude extracts from the mutants, their revertants, or the wild type strain or the purified enzyme. Strong, single precipitin lines were seen in every case suggesting the presence of cross-reacting materials in the mutants, including two independent nonsense alleles of pfkl. The lines merged with one another to produce a complete hexagonal figure when a-hexagonal template was used. This suggested that mutants without enzyme activity synthesized P-fructokinase-like proteins. Since the soluble P-fructokinase of yeast is known to be composed of two nonidentical polypeptide chains (17, 18), it is possible that the precipitin lines were due not to the product of the gene PFK1, but reflected the presence of the second of the two constituent subunits.

Chromosomal Location—The mapping of pfkl proved laborious because its segregation had to be followed by assay of enzyme activity, and secondly, it was not linked to the centromere; the second division segregation frequency with respect to trpl was 0.69 (89/129). Table II gives the linkage analysis from meiotic data using three sets of diploids heterozygous for the marker pairs pfkl-1 rna1, pfkl-SUP8, and rna1-SUP8 on chromosome XIII; SUP8 was monitored by the homozygous ochre marker lysl-1. The nonsuppressible allele pfkl-3
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**DISCUSSION**

**Aerobic and Anaerobic Growth of pfk1 Mutants**—The property of pfk1 mutants to continue to grow on sugars contrasts with that of most of the glycolytic mutants studied in yeast (19) and in *Escherichia coli* (20). The possibility that this was due to their leakiness in *vivo* can be ruled out in view of the experiment using the double mutant pfhl *and* described in Fig. 2. The manner of their isolation suggested that loss of P-fructokinase allowed in some unknown manner the alleviation of growth inhibition caused by glucose in pfk1 or pfcl mutants. It is likely that growth under these conditions was permitted not so much by attenuation of metabolic flux through glycolysis (5) as by modulation of negative control such as catabolite repression. Since the pfk1 alleles constituted quite a tight metabolic block, the double mutant pfk1 pfcl somehow allowed the utilization of alcohol despite the presence of glucose. However, no relief of catabolic repression or catabolite inactivation could be seen in pfk1 mutants with respect to isocitrate lyase and the glucose-repressible alcohol dehydrogenase (21) or of fructose-1,6-bisphosphatase or P-enolpyruvate carboxykinase. It should, however, be noted that about two-thirds of the glucose-resistant mutants from pfk1 strains continued to be positive for P-fructokinase activity.

In the absence of oxygen, however, pfk1 mutants are unable to grow. Their rudimentary growth in standing cultures or in the presence of azide, the spontaneous selection of enzyme-positive revertants during their growth in the petite state (both nuclear and cytoplasmic), and the inability of stationary cultures of pfk1 mutants to metabolize glucose in absence of oxygen justify the conclusion that the loss of this enzyme leads to impairment of anaerobic growth. If pfk1 mutants grew anaerobically by way of the pentose phosphate pathway and obeyed the stoichiometry shown in Reaction 1, it is immediately apparent that anaerobic growth would not have been sustained; the shunt pathway produces too many reductant molecules for too few oxidant substrates. How then did single spores of the genotype pfk1 pet8 petx germinate on glucose to produce visible colonies? Was it due to some spore-specific P-fructokinase? Examination of spore suspensions of diploid progeny homozygous for pfk1 gave no indication of such an enzyme. Was it due to the diffusion of acetaldehyde as an electron acceptor from neighboring *PFK1* spores? This was ruled out by the observation that germination of spores was not affected by planting them far apart from one another. It is thus difficult to escape the conclusion that there must exist a *PFK1*-independent mechanism that allowed petite spores lacking P-fructokinase to undergo at least 20 cell divisions to produce a visible spore colony from a single micromanipulated cell. However, this was clearly inadequate to sustain the anaerobic growth of pfk1 mutants. Perhaps the recently reported particulate P-fructokinase serves this purpose (8) and is also responsible for the normal growth yield of pfk1 mutants from glucose.

**Glycolytic Behavior**—Two shades of behavior distinguished the P-fructokinase mutant from the wild type strain in aerobic incubation, one was the delayed rise of fructose-1,6-bisP and the attendant fall in the level of P-enolpyruvate as pyruvate dehydrogenase (21) or fructose-1,6-bisphosphatase or P-enolpyruvate carboxykinase. It should, however, be noted that the attendant fall in the level of P-enolpyruvate as pyruvate dehydrogenase (21) or fructose-1,6-bisphosphatase or P-enolpyruvate carboxykinase. It should, however, be noted that the absence of oxygen justify the conclusion that the loss of this enzyme leads to impairment of anaerobic growth. If pfk1 mutants grew anaerobically by way of the pentose phosphate pathway and obeyed the stoichiometry shown in Reaction 1, it is immediately apparent that anaerobic growth would not have been sustained; the shunt pathway produces too many reductant molecules for too few oxidant substrates. How then did single spores of the genotype pfk1 pet8 petx germinate on glucose to produce visible colonies? Was it due to some spore-specific P-fructokinase? Examination of spore suspensions of diploid progeny homozygous for pfk1 gave no indication of such an enzyme. Was it due to the diffusion of acetaldehyde as an electron acceptor from neighboring *PFK1* spores? This was ruled out by the observation that germination of spores was not affected by planting them far apart from one another. It is thus difficult to escape the conclusion that there must exist a *PFK1*-independent mechanism that allowed petite spores lacking P-fructokinase to undergo at least 20 cell divisions to produce a visible spore colony from a single micromanipulated cell. However, this was clearly inadequate to sustain the anaerobic growth of pfk1 mutants. Perhaps the recently reported particulate P-fructokinase serves this purpose (8) and is also responsible for the normal growth yield of pfk1 mutants from glucose.

was used when SUP8 was segregating; the nonsense allele pfhl-1 was used for the others. These results placed pfk1 on the extended right arm of chromosome XIII 28 centimorgans from rna1 in the order: centromere-SUP8-rna1-pfk1.

**TABLE II**

<table>
<thead>
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<th>Gene pair</th>
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<td>27.9</td>
</tr>
<tr>
<td>rna1-SUP8</td>
<td>Parental ditype: 8</td>
<td>37.0</td>
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<tr>
<td>pfk1-SUP8</td>
<td>Parental ditype: 24</td>
<td>56.3</td>
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**Fig. 5.** Effect on P-fructokinase of heat and ATP. Top, effect of ATP on the activity of P-fructokinase from revertants and a wild type strain. Alcohol-grown cultures of revertants R13 and R22 from pfk1-7 and a wild type strain haploid S. cerevisiae were examined at 1 mm fructose-6-P for P-fructokinase activity in toluene lysates. The enzyme activity is expressed in arbitrary units of NADH fluorescence. Bottom, heat-inactivation profile of P-fructokinase. Cell-free extracts of glucose-grown cultures of the intragenic revertant pfk722-I and a wild type strain haploid S. cerevisiae were examined for the remaining activity. The enzyme activity was used when SUP8 was segregating; the nonsense allele pfhl-1 was used for the others. These results placed pfk1 on the extended right arm of chromosome XIII 28 centimorgans from rna1 in the order: centromere-SUP8-rna1-pfk1.
practically disappeared. Under such conditions the mutant was almost as proficient in glycolysis as the wild type reflecting again the presence of the glucose-induced particulate enzyme (8).

Anaerobically, however, the situation was very different. Glucose metabolism was negligible and the kinetics of metabolites reflected blocked glycolysis (Fig. 4). The levels of ATP, unlike the aerobic experiment, were never restored following glucose addition. Reintroduction of oxygen reversed the changes seen in nitrogen.

Nature of pfk1 Mutation—The observation that pfk1 mutants obtained by three different procedures lie in the same complementation group suggests that the catalytic activity of P-fructokinase is coded possibly by a single locus. There is ample evidence for the conclusion that this gene defines the structural determinant for this enzyme. The suppressibility of three of these mutants by tRNA suppressors showed that the gene is translated. Further, the P-fructokinase activity restored in the majority of the revertants was perceptibly different from that in the wild type strains: some were more thermostabile while some others more stable than the unmaturated enzyme; some of them were altered with respect to ATP inhibition and sigmoidicity to fructose-6-P. Many of the reversion events leading to restoration of the enzyme activity mapped very close to or perhaps within the PFK1 gene. These results accord with the conclusion that the locus PFK1 specifies the structure of P-fructokinase. We have recently shown (18) that structural alterations in P-fructokinase come about by mutation in either of the genes PFK1 or PFK2 (8); lesions in the former cause loss of catalytic activity and those in the latter lead to loss of allosteric regulation (18). The observation that all the mutants described here were affected in the gene PFK1 rather than in PFK2 lends support to the idea that the former is the sole determinant that specifies the catalytic activity of P-fructokinase in S. cerevisiae.

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REFERENCES