The **gcr** (Glycolysis Regulation) Mutation of Saccharomyces cerevisiae*

(Received for publication, April 17, 1981)

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**gcr** is a mutation considerably decreasing the assayed amounts of most glycolysis enzymes in Saccharomyces cerevisiae (Clifton, D., Weinstock, S. B., and Fraenkel, D. G. (1978) Genetics 88, 1-11). We show here that although in the wild type strain the amounts of these enzymes do not greatly differ between cells from different media, in the **gcr** mutant strain most of the enzyme amounts are 5% or less, relative to wild type, from cells grown without sugars, but 20-50% from cells grown with sugars. Lower relative values were found for phosphoglycerate mutase and enolase. A corresponding alteration in the mutant in the intensities of several major protein bands could even be seen in stained gels after electrophoresis of crude extracts; the profiles were otherwise normal. Results of titration of phosphoglycerate kinase with antibody accorded with activity. Transfer of cells between the two types of media did not lead to a more rapid adjustment of enzyme amounts than expected from the steady state levels. **gcr** is not allelic to **gpm** (the gene for phosphoglycerate mutase) or to **RNA1** (which affects transport of RNA from the nucleus). Translation of total RNA in a rabbit reticulocyte lysate gave a pattern of polypeptides similar to the in vivo one. Thus, **gcr** is likely to affect somehow mRNA synthesis or lifetime for a discrete number of proteins.

Enzymes of the glycolytic pathway comprise a major fraction of yeast-soluble protein, and mutations are known for most of them (1). Such mutations affect single enzymes, with one exception, **gcr** ("glycolysis regulation"), which seems to decrease the level of most enzymes of the pathway (2). This paper is about the **gcr** mutation.

**EXPERIMENTAL PROCEDURES**

**Strains and Media**—Both the "wild type" strain DFY1 (a **gcr** **ts**1 and the mutant strain DFY67 (a **gcr-1 leu2 lys1 trpl**) were described (2). DFY112 (a **ade6 gcr-1 trpl**) is a segregant from a cross of DFY67 and DFY58 (a **ade6**, Ref. 2). DFY159 is strain 381-la from K.-B. Lam (1). Strain A364A (a **ade1 ade2 ura3 trpl his7 lys2 gal1** and its **rva1-1** mutant strain ts364 (4) were from A. Hopper (Hershey Medical Center, Hershey, PA). As described (2), the minimal medium was M53 supplemented with a vitamin mixture, while enriched medium was M63 with 10 g/liter of Bacto-tryptone, 4 g/liter of Bacto-yeast extract, and added carbon source, 1% as indicated.

**Enzyme Assays**—Extracts were prepared as previously (2), using cells resuspended in extract buffer (50 mM KH2PO4, 2 mM Na-EDTA, and 2 mM mercaptoethanol, pH 7.4), usually supplemented with 2 mM phenylmethylsulfonyl fluoride. Enzyme assays were also as described previously (2) with the exception of aldolase (5). Protein was assayed by the biuret method (6) unless otherwise specified.

**Electrophoresis**—Polyacrylamide gel electrophoresis was according to Laemmli (7) but with slabs (10 × 14 × 0.15 cm) (8), 5% stacking, and 8-12% or 8-14% separating gels. Samples of extract were pre-treated by 2-min boiling in treatment buffer (7). Electrophoresis was 16 h, 25 V. Protein staining was according to Fairbanks et al. (9). Fixing solution was 3.46% sulfosalicylic acid and 11.3% trichloroacetic acid.

**In Vitro Translation**—Total RNA was extracted by the method of McAlister and Finkelson (10) from 2-ml cultures of cells in growth (A600 of approximately 3) on enriched medium with lactate plus glycerol or lactate plus glycerol plus maltose after addition of cycloheximide to 50 µg/ml. After two precipitations from LiCl plus ethanol, the ethanol was removed by drying under N2. The reticulocyte lysate and other incubation reagents, prepared according to Pelham and Jackson (11), were kindly supplied by B. E. Roberts (Department of Biological Chemistry, Harvard Medical School). Total incubation volumes of 0.025 ml contained the treated reticulocyte cell-free extract, master mix, 80 mM KAc; 0.6 mM Mg(Ac)2, 25 µCi of L-[35S]methionine, and RNA as specified. Incubation was 60 min, 37°C.

**RESULTS**

**Growth**—The **gcr** mutant strain DFY67 was obtained originally by selection for glucose resistance in a pyruvate kinase mutant (**pyk**) and subsequent outcrossing of the **pyk** mutation. Strain DFY67 was reported to grow very slowly on enriched medium with glucose but normally on enriched medium with pyruvate, and cells from the latter "permissive" medium had low assayed amounts of most glycolysis enzymes (2).

More data on growth are shown in Table I. In the usual enriched medium (which allows limited growth in the absence of additional carbon source), the mutant strain grew normally on gluconeogenic carbon sources, grew more slowly than normal on maltose, and barely grew at all on glucose or fructose. Glucose was inhibitory in an otherwise adequate medium (i.e., one containing a gluconeogenic carbon source, such as lactate), while maltose was not inhibitory.

In the above characteristics, the **gcr** mutant strain did not differ markedly from some other known glycolysis mutants. The pattern of growth on minimal medium was somewhat different, however, being relatively less impaired on sugars but more impaired on lactate (Table I). Glycolysis enzyme levels were, therefore, assayed after growth in a variety of media.

**Inducibility of Enzymes**—Table II, columns 1-4, shows data from four cultures: the wild type and mutant strains grown in enriched medium with lactate plus glycerol or lactate plus glycerol plus maltose. As is seen by normalizing to the values in the wild type strain from medium with lactate plus glycerol, the mutant had 1-10% levels of most glycolysis enzymes. (Similar results were reported for growth with pyruvate (2).) In the cultures also containing maltose, on the other hand, although the wild type profile did not change much (compare columns 2 and 1), in the mutant the enzyme levels were about 5-fold higher (compare columns 4 and 3).
Similar experiments were done with cells grown in other media. Table II shows that the average profile from three gluconeogenic media (column 7) and from nine media containing sugars (column 8) resembled the gluconeogenic and glycolytic profiles of columns 3 and 4, respectively. Again (columns 5 and 6), the wild type patterns differed little between the two types of media. Other wild type strains, including DFY56, the other parental strain used in construction of strain DFY67 (gcr) (2), had the usual wild type profile (data not shown).

The general pattern of the gcr effect could also be seen in sodium dodecyl sulfate gels of total soluble protein, stained with Coomassie blue (Fig. 1). For the wild type strain in the two standard conditions, the protein patterns were similar, although there were some clear differences, one of which probably reflects the inducible enzyme, maltase, size 63,000 (12). By comparison with migration of known standards, some of which are yeast glycolysis enzymes, and the knowledge that these enzymes comprise an appreciable fraction of yeast-soluble protein (1), some of the prominent bands of Fig. 1 may be tentatively assigned to particular glycolysis proteins or mixtures thereof. In the extracts of the gcr mutant strain grown on lactate plus glycerol, those prominent bands were considerably less intense, while in the extract from the mutant grown with maltose, they were stronger (see, for example, the band migrating like glyceraldehyde-3-P dehydrogenase (Gld)).

Fig. 1 also shows that although one set of proteins was clearly affected by the gcr mutation, most other protein bands were of similar intensity in wild type and mutant strains. (Two enzymes known by assay to be unaffected are glucose-6-P dehydrogenase and isocitrate dehydrogenase [Table I].) Thus, it is possible that the gcr mutation affects only a small set of proteins.

Although the gel patterns make it unlikely that the enzymes are present in normal amount but have lower activity, one would also like to assess the amount of antigen. This was done for phosphoglycerate kinase (Fig. 2). Antibody titration showed that the amount of activity matched the amount of antigen in the three cases of wild type (high activity), mutant with uninduced low activity, and mutant with induced moderate activity.

The gcr mutation has not yet been mapped, but the earlier work (2), as well as experiments to be reported, has shown the phenotype of impaired growth on sugars to segregate 2:2 in crosses with wild type strains, as expected for a single gene mutation. To the degree it has been tested, the phenomenon of apparent partial inducibility of the affected enzymes by sugars is a consequence of the same mutation. For example, in three tetrads from the cross DFY1 (GCR) × DFY112 (gcr) giving 2:2 segregation of growth on glucose, the inducibility phenomenon was seen in all six glucose-negative segregants (gcr) but not in the others (GCR). Interestingly, however, induction was not observed with maltose itself in all cases, presumably because one of the parental strains (DFY1) carries a MAL gene, and the other (DFY112) does not. This result would be consistent with induction requiring metabolism of the sugar.

**Altered Synthesis or Altered Degradation of the Proteins**—
The experiments above do not exclude the possibility that the gcr mutation affects protein turnover. Phenylmethylsulfonyl fluoride did not markedly affect the enzyme profile, but it was

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**Table I**

**Growth on plates**

The strains were streaked on enriched or minimal medium supplemented with the indicated carbon source (1% each) and average colony size measured after incubation at 30 °C for 3 days (enriched medium), 5 days (minimal medium with glucose or maltose), or 7 days (minimal medium with lactate).

|                | DFY1 (Lactate + glycerol) | DFY1 (Lactate + glycerol + maltose) | DFY67 (Lactate + glycerol + maltose) | Colony size (mm) |
|----------------|--------------------------|-------------------------------------|-------------------------------------|-----------------|
| Enriched medium plus |                          |                                     |                                     |                  |
| Glucose        | 2.5                      | 0.2                                 | 109                                 | 109             |
| Fructose       | 2.0                      | 0.1                                 |                                     |                  |
| Maltose        | 1.5                      | 0.6                                 |                                     |                  |
| Pyruvate       | 0.7                      | 0.7                                 |                                     |                  |
| Lactate        | 0.6                      |                                     |                                     |                  |
| Lactate + glycerol |                        | 0.3                                 | 0.5                                 | 0.5             |
| Lactate + glycerol + glucose |          | 2.0                                 | 0.1                                 | 0.1             |
| Lactate + glycerol + maltose |          | 1.5                                 | 0.7                                 | 0.7             |
| (No addition)  | 0.3                      |                                     |                                     | 0.3             |

**Minimal medium**

|                | DFY1 (Lactate + glycerol) | DFY1 (Lactate + glycerol + maltose) | DFY67 (Lactate + glycerol + maltose) | Colony size (mm) |
|----------------|--------------------------|-------------------------------------|-------------------------------------|-----------------|
| Glucose        | 0.8                      |                                     |                                     | 0.8             |
| Maltose        | 1.2                      |                                     |                                     | 1.2             |
| Lactate        | 0.5                      |                                     |                                     | 0.5             |

*These minimal plates also contained lysine, leucine, and tryptophan, each 25 μg/ml.

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**Table II**

**Enzyme activities**

In the first four columns, cultures were grown in liquid-enriched medium supplemented with the indicated carbon sources, 1% each, and harvested in logarithmic growth or early stationary phase. Enzyme activities are given as per cent of value in column 1; values in parentheses are units/mg of protein. In the second four columns, cells were harvested from growth on plates so that occasional revertant clones from DFY67 could be excluded. The three "gluconeogenic" media (column 7) contained as supplements lactate, lactate plus glycerol, or ethanol plus glycerol; the "glycolytic" media (column 8) were glucose, glucose plus glycerol, glucose plus lactate, glucose plus glycerol plus lactate, maltose (twice), maltose plus glycerol, maltose plus lactate, maltose plus lactate plus glycerol, and galactose. Values in columns 6-8 were normalized to column 5.
...proteins being characteristic in each medium from the time of the transfer.

**Nonidentity with Other Mutants**—We first considered the possibility that gcr might affect a single, known glycolytic enzyme, with the pleiotropic phenotype being an indirect consequence of that protein’s alteration. The two most likely candidate enzymes might be phosphoglycerate mutase and enolase since their levels are most affected (Table II). As mentioned, most glycosylation enzyme mutants do have the phenotype of defective growth in enriched medium with glucose.

Enolase mutants have not been reported. But there are mutants lacking phosphoglycerate mutase (gpm) and they have normal profile of other enzymes (3, 17, a result we have confirmed with a gpm mutant sent by K.-B Lam (3)). Nonetheless, to test whether gcr might be a special allele of gpm, a cross was performed between DFY67 (gcr) and strain DFY159 (gpm). The diploid grew normally on glucose and had normal glycolysis enzyme levels. If the two mutations were actually allelic, segregants would be 2:2 gpm/gcr. In fact, the usual tetrads contained one haploid with wild type enzyme profile and three others, which by assay and backcrossing were found to be gpm, gcr, and the double mutant gpm gcr. These results accord with gcr and gpm being in different genes.

**FIG. 1.** Protein profile of crude extracts. Polyacrylamide gel electrophoresis (7) used an 8–12.5% separating gel, and staining was with Coomassie blue (see “Experimental Procedures”). Lanes 1–4 contained crude extracts (100 µg of protein) (13) from wild type (DFY1, indicated as 1) and the mutant (DFY67, indicated as 67) in the usual two media (lactate plus glycerol or lactate plus glycerol plus maltose). The other lanes contained known proteins, as follows; their nominal subunit sizes are also given. Lane 5: m, β-galactosidase from *Escherichia coli* (116,000) (14), Sigma G5635, 1 µg; n, phosphofructokinase from rabbit muscle (80,000) (15), Sigma 6877 1 µg. Zaf, glucose-6-P dehydrogenase from yeast (51,000) (1), Boehringer 127-035, 0.7 µg. Eno, enolase from yeast (44,000) (1), Sigma E6126, 0.5 µg. o, aldolase from rabbit muscle (40,000) (15), Boehringer 102-652, 2 µg. Gld, glyceraldehyde-3-P dehydrogenase from yeast (36,000) (1), Sigma G8037, 0.3 µg. Tpi, triose-P isomerase from yeast (37,000) (1), Sigma T2507, 1 µg. Lane 6: p, phosphorylase a from rabbit muscle (93,000) (15), Sigma P1261, 1.3 µg. Tkt, transketolase from bakers yeast (80,000) (1), Sigma T6133, 0.3 µg. Pgi, phosphoglucone isomerase from yeast (60,000) (1), Sigma P9010, 0.2 µg. Pgkp, phosphoglycerate kinase from yeast (40,000) (1), Boehringer 102-430, 1.3 µg. Adh, alcohol dehydrogenase from yeast (36,000) (1), Boehringer 102-709, 1.3 µg. Gpm, phosphoglycerate mutase from rabbit muscle (27,000) (16), Boehringer 108-464, 3.3 µg. Lane 7 contained a mixture of two sets of molecular weight standards from Pharmacia (HMW 17-0445-01 and LMW 17-0446-01, sizes according to Pharmacia): a, ferritin (220,000); b, phosphorylase b (94,000); c, albumin (67,000); d, catalase (60,000); e, ovalbumin (43,000); f, lactate dehydrogenase (36,000); g, carbonic anhydrase (30,000); h, trypsin inhibitor (21,000); i, α-lactalbumin (14,000).

usually included in the extraction buffer. Change of medium experiments was also done (Table III). In the first one, growth was in gluconeogenic medium with lactate, and maltose was added for a 3-h incubation. During this time, amounts of the affected enzymes (in this experiment glyceraldehyde-3-P dehydrogenase and triose-P isomerase were assayed) increased, as expected, and the increases did not occur in the presence of cycloheximide. In the second experiment, cultures were transferred from a medium containing maltose and lactate to one without maltose. In this case, there was relatively little change in the enzyme profile over the 3 h. These and similar experiments did not reveal a rapid change in enzyme activities which might have indicated modification of preformed proteins; the data would best fit with the rate of synthesis of the affected proteins being characteristic in each medium from the time of the transfer.

**FIG. 2.** Titration of phosphoglycerate kinase activity versus antiserum. Incubation mixtures (0.2 ml) contained crude extract adjusted to 0.05 unit of phosphoglycerate kinase, 1 mg of bovine serum albumin, 0.1 ml of extract buffer, and the indicated quantity of 1:100 diluted phosphoglycerate kinase antiserum (kindly supplied by J. Thorner, Department of Microbiology and Immunology, University of California, Berkeley, CA). Activity was assayed after 30 min at 37°C. Growth was in enriched medium with glucose (●, DFY1; ○, DFY67) or lactate (□, DFY67).

**TABLE III**

Enzyme activities after a shift to new medium

Cultures of strain DFY67 (gcr) were grown in enriched medium with lactate or lactate plus maltose, transferred to the indicated media, and enzymes assayed after 3-h further incubation.

| Enzyme activities | Triose-P isomerase | Glyceraldehyde-3-P dehydrogenase | Glucose-6-P dehydrogenase |
|-------------------|--------------------|---------------------------------|--------------------------|
| Lactate           |                    |                                 |                          |
| To lactate        | 0.25               | 0.12                            | 0.14                     |
| To lactate + maltose | 0.63               | 1.03                            | 0.13                     |
| To lactate + maltose + CHX* | 0.18               | 0.10                            | 0.11                     |
| Lactate + maltose |                    |                                 |                          |
| To lactate + maltose | 1.04               | 1.75                            | 0.12                     |
| To lactate        | 1.04               | 1.75                            | 0.12                     |
| To lactate + CHX  | 1.23               | 1.39                            | 0.11                     |

*CHX, cycloheximide, 50 µg/ml. 
Another test for whether gcr might directly affect phosphoglycerate mutase or enolase was to examine the temperature lability of these enzymes in a revertant strain. The revertant chosen, DFY67R, contained approximately 50% of the wild type levels of phosphoglycerate mutase and enolase, and their labilities in crude extracts did not clearly differ from wild type, being for phosphoglycerate mutase, 50% loss of activity after 2 min at approximately 65 °C and for enolase, 50% loss at approximately 50 °C. These results give no support to the idea that gcr actually affects the structure of one of those two enzymes.

The rna1 mutation (4) is thought to affect RNA transport from the nucleus. The strain ts136 (rna1) did show some indication of being more temperature sensitive in growth with glucose than in gluconeogenic growth, but assay showed it to contain a normal profile of glycolytic enzymes, and a diploid between ts136 and DF112 (gcr) also gave a normal profile as well as growing well even at 37 °C on glucose minimal medium (data not shown). Hence, gcr and rna1 are unlikely to be allelic.

mRNA—If the gcr mutation does not affect the structure of glycolysis enzymes, it is likely to affect their synthesis. To test whether or not mRNA for these enzymes was present in normal amount, total RNA was obtained from the two mutants in the two standard growth conditions and used in an in vitro translation system from rabbit reticulocytes (Fig. 3). Total incorporation of radioactivity to hot trichloroacetic acid-insoluble material was similar in the four incubations (see legend), but the labeled polypeptide profiles differed. In the mutant, several prominent bands were missing or much less intense in the culture grown without maltose but more normal from the culture with maltose, while in the wild type strain, the two profiles were similar.

Thus, the in vitro polypeptide pattern was similar qualitatively to the in vivo pattern, and the deficiency of glycolytic enzymes is associated with a deficiency in their messages, at least as assessed in the heterologous translation system.

**DISCUSSION**

**Lesion of the gcr Mutant**—The gcr mutation reduces the amount of most glycolysis enzymes. The several types of experiments in this paper suggest that the enzymes themselves may be normal and that the lesion is likely to affect gene expression at the level of mRNA synthesis or stability. The effect may be on synthesis of a limited class of proteins, which includes most of the glycolysis enzymes. The exact lesion is not known.

It is tempting to speculate that gcr affects some regulatory component in glycolysis gene expression. However, it is uncertain even whether glycolysis enzyme levels are normally highly regulated. The work of Maitra and Lobo (18, 19), who studied differential enzyme synthesis after glucose addition to gluconeogenically grown cells, suggested large induction factors depending on such metabolites as glucose-6-P, while the type of data given in Table II for the wild type strain does not show large differences in enzyme level in the steady state. Perhaps there is induction in all strains, but thresholds differ, so it is revealed by the gcr mutation in strains otherwise "constitutive."

**Growth of the gcr Mutant**—The growth phenotype of the gcr mutant generally accords with its enzyme levels. In the usual enriched medium with sugars, the levels of phosphoglycerate mutase and enolase are low, and it may be that the growth impairment primarily reflects these blocks. In enriched medium with gluconeogenic carbon sources such as lactate, glycolysis is a biosynthetic pathway and certain end products are supplied by the medium, so perhaps the minimal levels of the several glycolysis enzymes are adequate. It is not clear, however, to what degree the phenotype of the gcr mutant will eventually prove to be explained in detail by the glycolysis enzyme pattern.

**Acknowledgments**—We are grateful for help from B. E. Roberts, P. C. Tai, and J. Thorner.

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**Fig. 3. Polypeptides made by translation of mRNA in a reticulocyte lysate.** See "Experimental Procedures." Incubation mixtures of 0.025 ml contained (C) no added RNA or 0.15 A260 unit of total RNA obtained from I, DFY1 grown on lactate plus glycerol; I + M, DFY1 from the medium supplemented with maltose; 67, DFY67 from lactate plus glycerol; and 67 + M, DFY67 from the medium supplemented with maltose. After the incubations, 0.001-ml portions were assayed for radioactivity insoluble in boiling 0.3 M trichloroacetic acid. Values from the five incubations were 5,100, 37,200, 39,800, 30,500, and 39,700 cpm, respectively. Portions of 0.01 ml (0.013 ml for incubation sample 67) were boiled 2 min in treatment buffer (7) supplemented with 1 mM phenylmethylsulfonyl fluoride and subjected to electrophoresis (8-14% gel). The two outside lanes contained a mixture, courtesy of P. C. Tai of this department, of 14C-methylated proteins (New England Nuclear Co.), 15,000 cpm in left lane and 30,000 cpm in right lane. The proteins are a, phosphorylase b, 92,500; b, bovine serum albumin, 69,000; c, ovalbumin, 46,000; d, carbonic anhydrase, 30,000; e, cytochrome c, 12,500. After electrophoresis and fixation, the gel was treated with Enhance (New England Nuclear), washed with 1% glycerol, dried, and autoradiographed on Kodak X-Omat R film at −70 °C overnight.
Glycolysis Regulation Mutation (ger) of S. cerevisiae

Saccharomyces (Strathern, J. N., Jones, E. W., and Broach, J. R. eds) Cold Spring Harbor Laboratory, New York, in press

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