The cdc30 Mutation in \textit{Saccharomyces cerevisiae} Results in a Temperature-sensitive Isoenzyme of Phosphoglucose Isomerase

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(Received 8 July 1986)

The cdc30 mutation in the yeast \textit{Saccharomyces cerevisiae} causes cell cycle arrest late in nuclear division when cells are shifted from the permissive temperature of 25 °C to the restrictive temperature of 36.5 °C. Cell cycle arrest at 36.5 °C is dependent upon the carbon source used: a shift-up in glucose containing media results in cell cycle blockade, whereas a shift-up in ethanol, fructose, glycerol, glycerol plus ethanol, or mannose does not. Metabolite analyses showed accumulation of glucose 6-phosphate in a cdc30-bearing strain after a temperature shift-up in glucose-containing medium. Thermal denaturation studies and kinetic measurements indicate the existence of two isoenzymes of phosphoglucose isomerase (EC 5.3.1.9); one of which is apparently altered in the temperature-sensitive cell cycle mutant. We propose that the gene products of both the CDC30 and PGIl genes are required for cell cycle progression in glucose media and that the PGIl gene product has a regulatory function over the CDC30 gene product.

INTRODUCTION

Control of the cell division cycle is a fundamental problem in biology. In the yeast \textit{Saccharomyces cerevisiae}, the isolation and study of cell division cycle (cdc) mutants has provided much genetic and physiological information (Pringle & Hartwell, 1981). Biochemical understanding of regulation and control of the cell cycle has lagged behind genetic and physiological descriptions because the identities of most \textit{CDC} (wild-type) genes remain unknown. Defining the defect caused by a particular \textit{cdc} mutation identifies the biochemical function of the analogous \textit{CDC} gene product. This type of information is needed both to provide a molecular explanation for rate-limiting and essential features of the cell cycle, and to explain why altering a certain biochemical process results in a mutation with particular properties and appearance (Dickinson, 1984). In \textit{S. cerevisiae} the biochemical defect has been established in the following cases: cdc8 (thymidylate kinase) (Jong et al., 1984; Sclafani & Fangman, 1984); cdc9 (DNA ligase) (Johnston & Nasmyth, 1978; Barker et al., 1985); cdc19 (pyruvate kinase) (Kawasaki, 1979); cdc21 (thymidylate synthetase) (Game, 1976); cdc28 (protein kinase) (Reed et al., 1985) and cdc35 (adenylate cyclase) (Mason et al., 1984; Casperson et al., 1985).

Kawasaki (1979) attempted to determine the biochemical lesions in strains carrying mutations in each of the first 36 \textit{CDC} genes. This led to the discovery that cdc19 is a temperature-sensitive mutation in the pyruvate kinase structural gene and suggested that the cdc30 mutation also results in a glycolytic lesion, though no such defect was identified (Kawasaki, 1979). In this paper we present evidence that the cdc30 mutation results in a temperature-sensitive isoenzyme of phosphoglucose isomerase (EC 5.3.1.9; PGIl) and consider the requirement of the \textit{CDC30}-encoded function with the \textit{PGIl} gene product for cell growth on glucose.

METHODS

Strains, media and culture conditions. \textit{S. cerevisiae} strains A364A (MATa ade1 ade2 ura1 his7 lys2 tyr1 gal1) and 23015 (MATa ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc30-I) were obtained from the Yeast Genetic Stock Center.

Abbreviations: F6-P, fructose 6-phosphate; G6-P, glucose 6-phosphate; PGIl, phosphoglucose isomerase.
Berkeley, Calif., USA. Complex media (YEP/yeast source) contained 1% (w/v) yeast extract, 2% (w/v) bacteriological peptone, 0.01% (w/v) of both adenine and uracil, and the carbon source specified. The various carbon sources denoted were used at the concentration indicated: D, glucose (2% w/v); E, ethanol (1%, v/v); F, fructose (2%, w/v); G, glycerol (3%, v/v); M, mannose (2%, w/v). Fructose and mannose had contaminating glucose removed by pre-treatment with glucose oxidase as described by Maitra & Lobo (1971b). Agar (2%, w/v) was used to solidify media. For growth in liquid media, cells were shaken in conical flasks filled to 40% total volume.

**Assay of PGI activity.** Cells were harvested by filtration using cellulose acetate membrane filters (0.45 μm grade), and washed with buffer A (50 mM-potassium phosphate buffer, pH 7.4, containing 2 mM-EDTA and 2 mM-mercaptoethanol). The cells were resuspended in buffer A and disrupted by agitating the suspension (3 ml) at high speed for 35 s with an equal volume of 40-mesh acid-washed glass beads in a Braun homogenizer. The homogenate was decanted from the glass beads and centrifuged at 11 600 g for 15 s. Portions of the resulting supernatant were used immediately as the source of enzyme. The PGI assay was initiated by using fructose 6-phosphate (F6-P). The formation of glucose 6-phosphate (G6-P) was monitored by using G6-P dehydrogenase as a 'coupling enzyme' and following the reduction of NADP+ at 340 nm (Bergmeyer, 1963; Maitra & Lobo, 1971a).

**Assays of intracellular glycolytic intermediates.** Cells were harvested by filtration as described above, washed, and resuspended in buffer B (50 mM-potassium phosphate buffer, pH 7.0) at 8-10 times their original density. A stream of air was bubbled through the suspension. After 15 min a small sample was withdrawn for determination of the wet weight of cells; a further sample was removed as a 'zero time' sample for determination of intermediates and 60 μl 2 mM-glucose was added to the remaining cells. At various times thereafter further samples were removed for metabolite analysis. Material for metabolite analysis was added immediately to tubes containing perchloric acid to give a final concentration of 5% (w/v) perchloric acid. After 10 min, the material was centrifuged at 5000 g and the supernatants were neutralized with ice-cold potassium hydroxide. After further centrifugation to remove potassium perchlorate, the supernatants were analysed for glycolytic intermediates by using conventional spectrophotometric enzyme assays (Bergmeyer, 1963). Cells thus analysed were either in the early exponential phase of growth (wild-type at either 25 °C or 36.5 °C and mutant 23015 at 25 °C) or were the mutant cells which were cell cycle-arrested following a shift-up to 36.5 °C. The incubation temperature used for the glucose-fed cell suspension was the same as that for the previous cell culture (25 °C or 36.5 °C).

**Cell counting.** Cell number was determined using a Coulter counter.

**RESULTS AND DISCUSSION**

Fig. 1 shows the kinetics of cell cycle arrest of the cdc30-bearing strain after a shift-up in YEPD medium from the permissive temperature of 25 °C to the restrictive temperature of 36.5 °C. The mutant continued to grow and divide in the portion of culture which remained at 25 °C. Fig. 2 shows the typical morphology of strain 23015 after a 4 h shift-up. The cells arrested with a terminal phenotype characteristic of late nuclear division (Pringle & Hartwell, 1981). This confirms the work of Kawasaki (1979) on this strain.

Table 1 shows that strain 23015 arrested at 36-5 °C on YEPD but not on YEPE, YEPF, YEPG, YEPGE or YEPM. The cdc30-bearing mutant did arrest on YEPE if glucose was also present at concentrations of 0.5% or greater. This suggests a mutation in a glycolytic function, as many glycolysis mutants are inhibited by glucose (Fraenkel, 1982). The first clue as to the nature of the cdc30 mutation was that strain 23015 was able to grow and divide at 36-5 °C when either mannose or fructose was the carbon source. This was highly suggestive of a mutation in PGI (Clifton et al., 1978; Herrera & Pascual, 1978; Ciriacy & Breitenbach, 1979; Maitra, 1971). This conclusion was apparently contradicted by the finding that the cdc30 mutation is complemented by a pgil-f mutation, a result which was known previously (Kawasaki, 1979).

Metabolite analyses of the wild-type strain A364A and the cdc30-bearing strain 23015 in glucose at 36-5 °C revealed that the mutant accumulated G6-P, having a G6-P :F6-P ratio of 8-9 : 1 versus that of 2-4 : 1 for the wild type (Table 2). Accumulation of G6-P is not the same as in other pgil mutants described previously. Herrera & Pascual (1978) isolated 2 types of pgil mutant: their ‘type I’ mutant contained approximately 0-6 μmol G6-P (g wet wt of cells)−1, their ‘type II’ mutant contained approximately 2 μmol G6-P (g wet wt of cells)−1 after 24 h incubation in 9 mM-glucose. Maitra described a mutant defective in PG1 which accumulated 5-8 μmol G6-P (g wet wt of cells)−1 after a shift from acetate to 10 mM-glucose-containing medium. This latter strain accumulated approximately 21 μmol G6-P (g wet wt of cells)−1 in a 20 min period in an experiment analogous to the one described here (Maitra, 1971). It is not easy to compare the
Fig. 1. A typical experiment showing the effect of a temperature shift-up on the cdc30-bearing strain 23015. A culture was grown in YEPD medium at the permissive temperature of 25 °C and was then divided into two portions: one remained at 25 °C (○); the other was shifted to the restrictive temperature of 36-5 °C (●).

Fig. 2. Terminal phenotype of the cdc30-bearing strain 23015 4 h after a shift-up from the permissive temperature of 25 °C to the restrictive temperature of 36-5 °C in YEPD medium. The bar marker represents 10 μm (the magnification is the same in both photographs). Cells were viewed by differential interference contrast optics.

Table 1. Growth and cell division of wild-type (A364A) and mutant (23015) strains on different media at 36-5°C

The results are expressed as a seven-point scale from ++ + (wild-type on YEPD) to --- (mutant 23015 on YEPG + 1% D).

<table>
<thead>
<tr>
<th>Strain</th>
<th>YEPP at 25 °C</th>
<th>YEPP at 36-5 °C</th>
<th>YEPM</th>
<th>YEPF</th>
<th>YEPE</th>
<th>YEPP</th>
<th>YEPP</th>
<th>YEPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A364A</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>23015</td>
<td>++ ±</td>
<td>+ ±</td>
<td>++ ±</td>
<td>++ ±</td>
<td>++ ±</td>
<td>++ ±</td>
<td>++ ±</td>
<td>++ ±</td>
</tr>
</tbody>
</table>

Table 2. Concentrations of glycolytic intermediates in wild-type and mutant strains after 4 h at 36-5°C

Wild-type strain A364A and the cdc30-bearing mutant 23015 were grown in YEPD at 25 °C. The cultures were then shifted to 36-5 °C for 4 h and the concentrations of glycolytic intermediates were determined in each strain. The results shown were determined after 20 min incubation in the presence of fresh glucose and are the means of triplicate determinations.

<table>
<thead>
<tr>
<th>Glycolytic intermediate</th>
<th>Concentration [μmol (g wet wt of cells)^{-1}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-Phosphogluconate</td>
<td>0</td>
</tr>
<tr>
<td>Glucose 6-phosphate</td>
<td>1-40</td>
</tr>
<tr>
<td>Fructose 6-phosphate</td>
<td>0-57</td>
</tr>
<tr>
<td>Fructose-1,6-bisphosphate</td>
<td>0-93</td>
</tr>
<tr>
<td>Dihydroxyacetone phosphate</td>
<td>0-33</td>
</tr>
<tr>
<td>Glyceraldehyde 3-phosphate</td>
<td>0-20</td>
</tr>
<tr>
<td>3-Phosphoglyceric acid</td>
<td>0-29</td>
</tr>
<tr>
<td>2-Phosphoglyceric acid</td>
<td>0-07</td>
</tr>
<tr>
<td>Phosphoenolpyruvate</td>
<td>0-07</td>
</tr>
<tr>
<td>Pyruvic acid</td>
<td>2-30</td>
</tr>
</tbody>
</table>
Fig. 3. A typical experiment showing the effect on PGI activity of heating cell extracts of wild-type (▲) and mutant (●) strains at 55 °C. Activities corresponding to 100% were 1513 nmol min⁻¹ (mg protein)⁻¹ for the wild-type and 1003 nmol min⁻¹ (mg protein)⁻¹ for the mutant strain.

present results with those of Ciriacy & Breitenbach (1979), who expressed content of glycolytic intermediates on a dry weight basis. It is notable that strain 23015 contained less than did the wild-type of all the intermediates that occur after G6-P in the glycolytic sequence, except for phosphoenolpyruvate and pyruvic acid. This temporarily diverted attention towards examining pyruvate decarboxylase (EC 4.1.1.1) and pyruvate kinase (EC 2.7.1.40) activities in strain 23015. These enzymes elicited no structural defects in the mutant strain. The significance of the altered phosphoenolpyruvate and pyruvate levels is considered below.

Fig. 3 shows the effect of heating cell extracts of wild-type and mutant strains before assaying PGI activity. The PGI activity from strain 23015 was more thermolabile than that from the wild-type strain. Both plots are biphasic, indicating that two species of protein were denaturing. Extracts from the cdc30-bearing strain (grown at 25 °C) contained only two-thirds of the specific activity present in the wild-type when cells were harvested at similar densities.

Lineweaver–Burk plots constructed for PGI by assaying at 25 °C and 36 °C over a range of F6-P concentrations (0.01–10 mM) from wild-type and mutant strains were not linear, but hyperbolic (data not shown). This also indicates catalysis by two isoenzymes (Dixon & Webb, 1964). Eadie–Hofstee plots (Fig. 4) confirmed the existence of two isoenzymes. We propose to call these isoenzymes I and II (corresponding to the high- and low-Kₘ forms respectively). Isoenzyme I is apparently defective in the cdc30-bearing strain because increasing the assay temperature from 25 °C to 36-5 °C nearly doubled the maximal velocity for this isoenzyme from the wild-type strain, but resulted in a decrease in the maximal velocity in the case of isoenzyme I from the mutant (Table 3). There was little difference in the maximal velocity of isoenzyme II from mutant and wild-type strains assayed at the permissive and restrictive temperatures.

A widely held, but poorly documented opinion is that Hartwell’s cdc mutants often carry multiple mutations. As strain 23015 is a primary mutant isolate, one possible criticism of this work is that the biochemical defects reported here are a consequence of other mutations which are also present in the strain. However, neither we, nor Kawasaki, have detected any other such mutations associated with this cdc30-I-bearing strain or in a cdc30-2-bearing strain (J. R. Dickinson & A. S. Williams, unpublished data; Kawasaki, 1979). Furthermore, in crosses, reduced specific activity of PGI, increased thermolability of this enzyme and the ability to continue growth and cell division at 36-5 °C on non-fermentable substrates all co-segregated with the cdc30 phenotype.

Evidence was presented by Noltmann’s group that there are three isoenzymes of PGI which differ in net charge but not in Mᵣ (Nakagawa & Noltmann, 1967; Kempe et al., 1974a, b). These results have been largely disregarded because it has been shown by numerous workers that single mutations in the PGII gene give a complete phenotype (i.e. failure to grow on glucose and loss of enzyme activity) (Clifton et al., 1978; Herrera & Pascual, 1978; Ciriacy & Breitenbach, 1979;
Maitra, 1971). Furthermore, reversion studies were deduced to have shown that PGII is a structural gene (Maitra, 1971). The simplest explanation for the results presented here is that PGII and CDC30 genes are both structural genes for different isoenzymes of PGI and that the PGII gene product has both catalytic and regulatory function over the CDC30 gene product. For example, perhaps the PGII-encoded protein binds to the CDC30-encoded protein for activity, thus pgil mutations would render the CDC30-encoded isoenzyme inactive. Such a situation would not be unique in the yeast glycolysis system for it is known that the PFK2 gene determines not only the regulatory properties of the soluble enzyme (PFK1 gene product), but also the catalytic activity of particulate phosphofructokinase (Nadkarni et al., 1984). Other enzymes of the glycolytic pathway with multiple genes controlling their synthesis or catalytic activity are pyruvate decarboxylase (PDC1 and PDC2 genes) (Schmitt & Zimmermann, 1982), glucose-repressible alcohol dehydrogenase (ADR1, ADR2, ADR3, ADR4 and CCR1 genes) (Ciriacy, 1979), and hexokinase for which the HXK2 gene is both the structural gene for the hexokinase II isoenzyme and controls glucose repression (Entian & Frohlich, 1984; Entian et al., 1984; Frohlich et al., 1984).

The elevated levels of phosphoenolpyruvate and pyruvate in the mutant strain after a shift-up to 36-5 °C might be explained by the observation that the activities of pyruvate kinase and pyruvate decarboxylase are regulated at the transcriptional level and that this regulation is thought to be under the control of the PGII gene in an analogous fashion to the control exerted by the HXK2 gene product (Zimmermann, 1985).

Finally we must consider why a mutation in an isoenzyme of PGI should result in arrest of S. cerevisiae with a uniform terminal phenotype. The answer is not immediately apparent, but the question has been asked before in the case of cdc19 (pyruvate kinase) mutants. Presumably there are requirements for these two enzyme activities at specific times during the cell cycle. Alternatively, the CDC30 and CDC19 gene products have other functions (akin to the situation with HXK2) in addition to their catalytic activities.
We are grateful to Professor P. K. Maitra (Tata Institute of Fundamental Research, Bombay, India) for advice on the assay of glycolytic intermediates and for stimulating discussions.

REFERENCES


