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Glycogen metabolism in a *Saccharomyces cerevisiae* phosphoglucose isomerase (*pgi1*) disruption mutant

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Disruption of the gene *pgi1* of *Saccharomyces cerevisiae*, which codes for phosphoglucose isomerase, results in a dramatic increase in the amount of intracellular glycogen in early exponential cultures. The level of glucose 6-phosphate was much higher in mutant than in wild-type cells. Phosphorylase *a* activity and the state of activation of glycogen synthase were also investigated. Phosphorylase *a* activity was rather low along the culture in wild-type cells, whereas it was consistently higher in mutants. Glycogen synthase was mostly in the active form in early-medium exponential cultures in wild-type cells whereas the activation state of this enzyme in mutant cells, although lower at the earlier steps of the culture, did not differ from wild-type cells at later stages. The fact that the intracellular levels of UDP-glucose are markedly increased in mutant cells suggest that the observed accumulation of glycogen results from a rise in substrate availability rather than from the activation of the enzyme responsible for the synthesis of the polysaccharide.

Phosphoglucose isomerase mutant; Gene disruption; Glycogen accumulation; Phosphorylase; Glycogen synthase; *Saccharomyces cerevisiae*

1. INTRODUCTION

The interconversion of glucose 6-phosphate and fructose 6-phosphate in yeast is catalyzed by the enzyme phosphoglucose isomerase (PGI) (see [1] for review). Mutants with reduced PGI activity were isolated as a result of their ability to grow in fructose as carbon source but not in glucose [2]. Yeast phosphoglucose isomerase is encoded by the gene *pgi1* [3]. Deletion of *pgi1* results in no measurable PGI activity and failure to grow in fructose as a sole source of carbon [4,5]. Instead, addition of trace amounts of glucose was necessary to support growth in fructose-supplemented media. Under these conditions, mutant cells accumulate large amounts of glucose 6-P, as it was previously observed for mutants with residual PGI activity [6,7].

Glycogen is the major energy reserve in yeast (see [8] for review) and glycogen synthase and glycogen phosphorylase are the key enzymes controlling glycogen metabolism. In yeast, as well as in many other organisms, both enzymes are regulated by phospho–dephosphorylation reactions [9–14]. Phosphorylation activates phosphorylase and inactivates synthase, while dephosphorylation provokes the opposite effects. Both enzymes can also be under the control of allosteric modulators. For instance, glucose 6-P is able to activate glycogen synthase in spite of the phosphorylation state of the enzyme [9,10,15–17]. The role of glucose 6-P is especially interesting because this sugar phosphate is also a metabolic precursor of UDP-glucose, the glucose-donor substrate for glycogen synthase. In this context, the availability of *pgi1* deletion mutants provides with a useful tool to test how the accumulation of glucose 6-P affects glycogen metabolism in yeast cells. Our work demonstrates that a *pgi1* deletion mutant accumulates large amounts of glycogen, most probably as a result of an increase in the intracellular levels of UDP-glucose.

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2. MATERIALS AND METHODS

2.1. *Strain and media*

The haploid strain UTL-7A (*MAT a leu 2-3/112 ura 3-52 trp1*) was used to construct strain EBY-UTL23, which carries a disrupted *pgi1* gene. Wild-type and *pgi1* cells were grown in medium containing yeast extract 1%, peptone 2%, glucose 0.1% and fructose 2% at 30°C with continuous shaking (250 strokes/min). Growth was monitored by measuring the optical density of the cultures at 660 nm.

2.2. *Disruption of* pgi1 *gene*

The one-step gene disruption method [18] was used. Briefly, the 2.68 kbp *ScaI/Eco*RV fragment carrying the gene *pgi1* from plasmid pPGI1-1 [3] was subcloned into plasmid pUC18. The resulting plasmid, pEB2, was digested with *Cla*I and *Kpn*I to remove a 1.25 kbp fragment of the *pgi1* gene. This fragment was replaced with a 1.11 *ClaI/Kpn*I fragment containing the entire *URA3* gene to give plasmid pEB23. This plasmid was digested with *Dra*I and *Bam*HI and the

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insert used to transform UTL-7A cells as described [19]. Uracil prototrophic transformants were selected and the disruption confirmed by Southern blot.

2.3. *Preparation of extracts*

At the appropriate time, culture samples containing approx. the same amount of cells were rapidly collected by vacuum filtration. For enzyme activity determinations, the cell cake was washed once with 10 ml of cold water and immediately resuspended in 500 μl of ice-cold buffer containing 50 mM Tris-HCl (pH 7.4), 0.6 M sucrose, 100 mM KF, 5 mM EDTA, 2 mM EGTA, 2 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride and 0.5 mM benzamidine. The same volume of acid-washed glass beads was added and extracts prepared as described in [20].

For metabolite determination the cell cake (about 30 mg dry weight) was immediately frozen in liquid nitrogen. Acid extracts for determination of glucose 6-P and UDP-glucose were prepared as in [20]. For glycogen measurements, about 30 mg of cells (dry weight) were collected and immediately resuspended in 1 ml of cold perchloric acid and the same volume of glass beads. The mixture was immediately frozen in liquid nitrogen. When appropriate, samples were thawed in ice and acid extracts prepared by five pulses of vigorous vortexing during one min. After each pulse, samples were kept on ice for one min. Glass beads were removed by low-speed centrifugation and supernatants saved for glycogen determination. Alkaline extracts were prepared by resuspending the cell cake (about 15 mg dry weight) in 1 ml of sodium hydroxide 54 mM prewarmed at 80°C and incubating the cells at this temperature for 15 min. The suspension was then centrifuged at 12,000 rpm in a microfuge and the supernatant used for fructose 2,6-bisphosphate measurements.

2.4. *Measurement of glycogen synthase and phosphorylase 'a' activities*

Glycogen synthase activity was determined by measuring the amount of [^{14}C]glucose incorporated from UDP-[^{14}C]glucose into glycogen as described in [21]. The assay mixture was as described in [20]. The active form of glycogen synthase was determined in the absence of exogenous glucose 6-phosphate. Total glycogen synthase activity was measured in the presence of 6.6 mM glucose 6-phosphate in the assay mixture. Glycogen phosphorylase *a* was measured as in [20]. The assays were performed at 30°C. One unit is the amount of enzyme that catalyses the incorporation of 1 μmol of [^{14}C]glucose per min into glycogen.

2.5. *Determination of metabolites*

Glycogen, glucose 6-P and fructose 2,6-bisphosphate levels were essentially measured as described in [20]. UDP-glucose was determined spectrophotometrically by using UDP-glucose dehydrogenase essentially as described in [22]. In all cases metabolite concentrations are referred to grams of cells (dry weight).

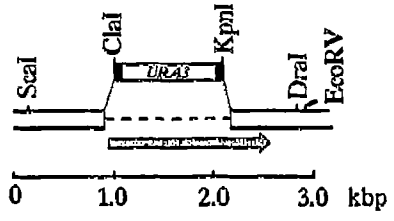

Fig. 1. Disruption of the *pgi1* gene. A 1.25 kbp *Cla*I-*Kpn*I fragment containing most of the coding region of gene *pgi1* (depicted as arrow) was removed and replaced by a 1.11 kbp fragment containing the entire *URA3* gene. Haploid UTL-7A cells were transformed and transformants selected in synthetic medium lacking uracil. See the main text for additional details.

2.6. *Other determinations*

Protein concentration was measured in buffered extracts using the Biuret method [23] with bovine serum albumin as standard. Fructose concentration in the medium was measured after removal of the cells as described in [24].

3. RESULTS

3.1. *Disruption of the* pgi1 *gene results in accumulation of glycogen*

The gene *pgi1* encoding the glycolytic enzyme phosphoglucose isomerase was disrupted in vitro and the construct used to transform haploid UTL-7A cells to produce a disruption mutant EBY.UTL23 strain (Fig. 1). Mutant *pgi1* cells lacked detectable phosphoglucose isomerase activity (not shown). Wild-type and *pgi1* cells were grown in rich medium containing 0.1% glucose and 2% fructose as carbon source. Under these conditions, the rate of growth of EBY.UTL23 mutants at early exponential phase was lower than UTL-7A wild-type cells (doubling time of about 270 min vs. 90 min). However, a noticeable increase in the doubling time was observed in mutant cells at later stages of the culture. In addition, very high levels of glycogen at the early exponential period of culture were observed in mutant cells. The concentra-

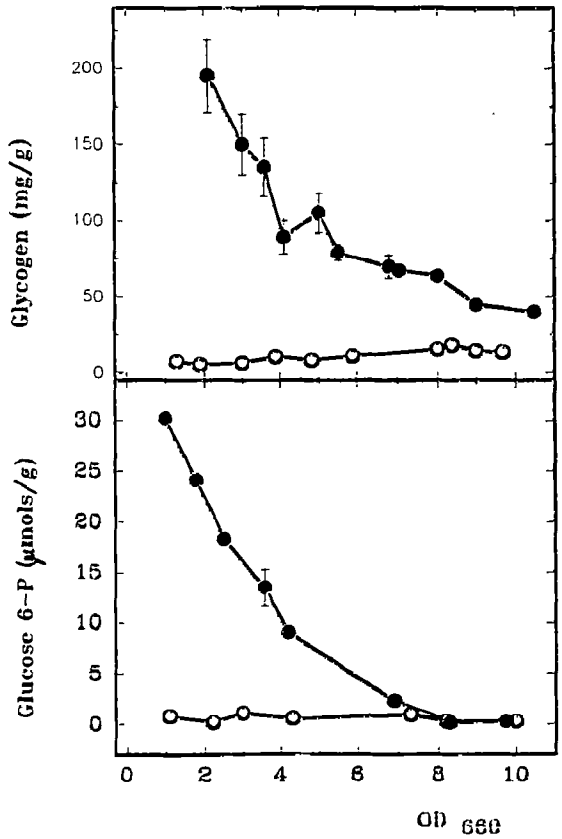

Fig. 2. Glycogen and glucose 6-P levels during the growth of wild type (○) and *pgi1* mutant (●) strains. Values are mean ± S.E.M. from at least 3 independent experiments.

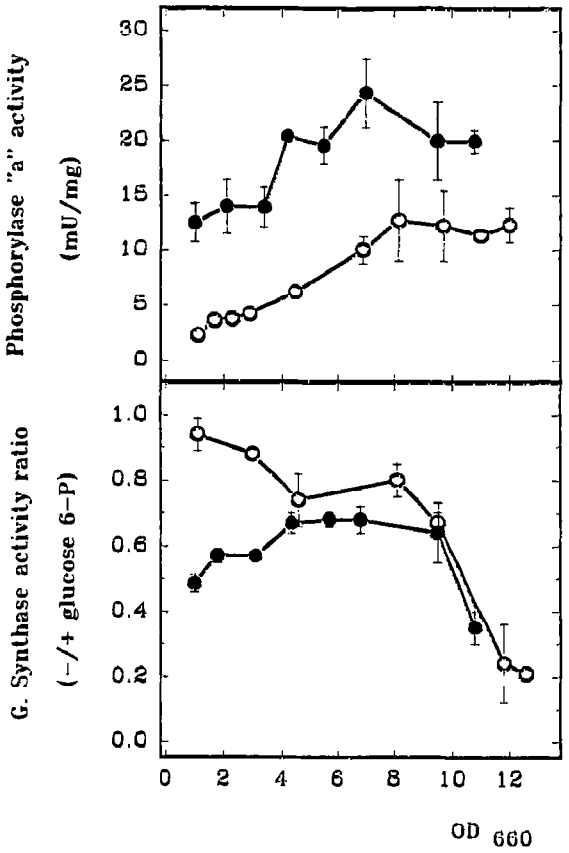

Fig. 3. Glycogen phosphorylase *a* and glycogen synthase activity ratio in wild-type (○) and *pgi1* mutant (●) cells. Glycogen synthase activity was measured in the absence (active form) or the presence (total enzyme) of exogenous glucose 6-P and the activation state of the enzyme is presented as the −/+ glucose 6-P activity ratio. Values are mean ± S.E.M. from 3–5 independent experiments.

tion of the polysaccharide decreased rapidly when the culture approached the stationary phase. In wild-type cells the concentration of glycogen was steadily lower (Fig. 2A). The levels of glucose 6-P were also determined. The profile obtained for this sugar phosphate was very similar to the one obtained for glycogen (Fig. 2B). At early exponential phase, the amount of glucose 6-phosphate in *pgi1* mutants was about 20–30-fold higher than in wild-type cells. In addition, the concentration of the sugar phosphate sharply decreased, reaching baseline values once the culture entered late exponential phase. When fructose 2,6-bisphosphate was measured, mutant cells were found to contain very low levels of this phosphorylated sugar when compared with the isogenic wild-type strain (1.2 ± 0.3 vs. 10.3 ± 0.9 nmol/g cells in early exponential cultures).

3.2. *Glycogen synthase and phosphorylase activities in the* pgi1 *mutant*

In order to understand the basis for the accumulation of glycogen observed in the *pgi1* mutant, glycogen

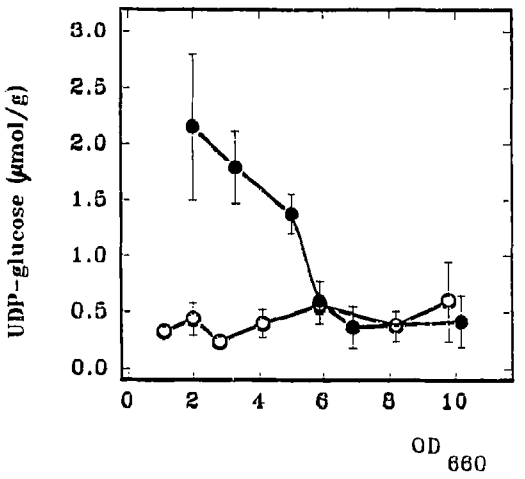

Fig. 4. Intracellular levels of UDP-glucose during the growth of wild type (○) and *pgi1* mutant (●) yeast cells. Values are mean ± S.E.M. from 3 independent experiments.

phosphorylase and glycogen synthase activities were determined. Glycogen phosphorylase *a* in wild-type cells was rather low, increasing at the end of the exponential phase. In mutants, phosphorylase appears to be more active along the culture (Fig. 3A). Both the active form and the total amount of glycogen synthase were measured in wild-type and mutant cells, and the activation state of the enzyme was expressed as the active form/total activity ratio. In wild-type cells the enzyme is almost fully active during early-medium exponential phase. As the carbon source is nearly depleted, glycogen synthase activity ratio decreases. The same profile is observed in mutant cells, although the enzyme is less active at early exponential phase (Fig. 3B). The intracellular concentration of the glycogen synthase substrate, UDP-glucose, was measured along the culture (Fig. 4). UDP-glucose levels in wild-type cells were rather constant, ranging from 0.2–0.5 μmol/g. On the contrary, in the *pgi1* mutants, UDP-glucose levels were much higher at early exponential phase (about 6-fold when compared to wild-type cells) and they decreased steadily, reaching values similar to those observed in wild-type cells when the culture approached stationary phase.

4. DISCUSSION

In this paper we show that during the exponential phase, yeast cells lacking phosphoglucose isomerase accumulate a large amount of glycogen, whereas the levels of the polysaccharide decrease during the culture. This situation is completely different from the previously described for other strains [25,26], in which the amount of polysaccharide is almost negligible and it accumulates only at the end of the exponential phase. In order to understand the basis for the accumulation of glycogen, it is important to consider the characteristic accumulation of glucose 6-P in the *pgi1* mutant. Glucose 6-P has

been found to be a stimulator of glycogen synthase [9,10,15–17] and an inhibitor of phosphorylase [12,27]. Our results with the *pgi1* disruption mutant show that in early exponential cultures the intracellular concentration of glucose 6-P can be as high as 30 μmol/g cells. In these conditions one might expect that the accumulation of glycogen could be the result of the activation of glycogen synthase caused by the rise in intracellular glucose 6-P. However, it has to be considered that, although the experimental conditions do preserve the covalent modifications of the enzyme affecting its activity (i.e. phosphorylation state), the non-covalent allosteric effect of intracellular glucose 6-P cannot be observed during the enzymatic assays, simply because the experimental procedures that allow the determination of the activity do not preserve the intracellular levels of this sugar phosphate, which are known to be very sensitive to the sampling techniques [28].

Our results demonstrate that, in early exponential cells, the activity of synthase in *pgi1* mutants is somewhat lower than in wild-type cells, despite the difference in glucose 6-P levels. This is in contrast to what has been found in glycogen synthase from rat hepatocytes, where increase in intracellular levels of glucose 6-P correlates with the activation of the enzyme [29,30]. This effect has been attributed to a dephosphorylation of synthase induced by glucose 6-P. In this context, it has been recently shown that glucose 6-P is able to stimulate the in vitro dephosphorylation of rabbit muscle glycogen synthase by protein phosphatases. This effect was shown to be rather specific for synthase [29], suggesting that it was most likely due to an allosteric configuration change of this enzyme which facilitates its dephosphorylation, rather than to an effect on the phosphatases. Therefore, our results indicate that the yeast enzyme does not respond to glucose 6-P in the same way as the mammalian enzyme does. A most interesting issue would be to understand the molecular basis for such different behavior, since the yeast isoforms of glycogen synthase, although structurally related to the mammalian enzymes, are only 48–50% identical to the rabbit muscle enzyme [31,32].

An alternative explanation for the accumulation of glycogen in the presence of high levels of glucose 6-P could be an increase in the levels of UDP-glucose, the glucose-donor substrate for glycogen synthase. Such an increase could be expected since the reactions catalyzed by phosphoglucomutase and UDPG-pyrophosphorylase are believed to be near the equilibrium. This is consistent with our finding of increased levels of UDPG in *pgi1* cells. Therefore, the emerging picture would be that glycogen would accumulate at early exponential phase in *pgi1* cells mostly as a result of increased substrate availability and not as a result of the effect of glucose 6-P on glycogen synthase. It is important to consider that since glycogen synthase is already almost fully active in wild-type cells, the expected allosteric activation due to the rise in glucose 6-P cannot account for the large accumulation of glycogen found in mutants. In this regard, it is suggestive that, in mutant cells, the glycogen content decreases during the growth of the culture. This change parallels the decrease in UDPG and glucose 6-P. The reduced availability of UDPG and the relatively high phosphorylase activity found in mutant cells could account for the much lower levels of glycogen found at late exponential phase in the phosphoglucose isomerase mutant. In this regard, the possibility cannot be ruled out that the fact that *pgi1* mutants can grow in the presence of trace amounts of glucose could be explained, at least partially, by its ability to accumulate glycogen during the initial phases of the culture.

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REFERENCES

[1] Fraenkel, D.G. (1982) Carbohydrate Metabolism, in: The Molecular Biology of the Yeast *Saccharomyces*. Metabolism and Gene Expression (Stratern, N.J., Jones, E.W. and Broach, J.R. eds.) pp. 1–37, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[2] Maitra, P.K. (1971) J. Bacteriol. 107, 759–769.
[3] Aguilera, A. and Zimmermann, F.K. (1986) Mol. Gen. Genet. 202, 83–89.
[4] Aguilera, A. (1986) Mol. Gen. Genet. 204, 310–316.
[5] Green, J.B.A., Wright, A.P.H., Cheung, W.Y., Lancashire, W.E. and Hartley, B.S. (1988) Mol. Gen. Genet. 215, 100–106.
[6] Maitra, P.K. and Lobo, Z. (1977) Mol. Gen. Genet. 156, 55–60.
[7] Gancedo, J.M. and Gancedo, C. (1979) Eur. J. Biochem. 101, 455–460.
[8] Gancedo, C. and Serrano, R. (1989) Energy-Yielding Metabolism, in: The Yeast, vol. 3, 2nd edn, pp. 205–259, Academic Press.
[9] Rothman-Denes, L.B. and Cabib, E. (1970) Proc. Natl. Acad. Sci. USA 66, 967–974.
[10] Rothman-Denes, L.B. and Cabib, E. (1971) Biochemistry 10, 1236–1242.
[11] Huang, K.P. and Cabib, E. (1972) Biochem. Biophys. Res. Commun. 49, 1610–1616.
[12] Fosset, M., Muir, L.W., Nielsen, L.D. and Fisher, E.H. (1971) Biochemistry 10, 4105–4113.
[13] Becker, J.U., Wingerden-Drissen, R. and Schiltz, E. (1983) Arch. Biochem. Biophys. 225, 667–678.
[14] Hwang, P.K. and Fletterick, R.J. (1986) Nature 324, 80–84.
[15] Rothman, L.B. and Cabib, E. (1966) Biochem. Biophys. Res. Commun. 25, 644–650.
[16] Rothman, L.B. and Cabib, E. (1967) Biochemistry 6, 2107–2112.
[17] Huang, K.P. and Cabib, E. (1974) J. Biol. Chem. 249, 3851–3857.
[18] Rothstein, R.J. (1983) Methods Enzymol. 101, 202–211.
[19] Schiestl, R.H. and Gietz, R.D. (1989) Curr. Genet. 16, 339–346.
[20] Fernández-Bañares, I., Clotet, J., Ariño, J. and Guinovart, J.J. (1991) FEBS Lett. 290, 38–42.
[21] Thomas, J.A., Schlender, K.K. and Larner, J.J. (1968) Anal. Biochem. 25, 486–499.
[22] Keppler, D. and Decker, K. (1974) in: Methods in Enzymatic Analysis, vol. 4 (H.U. Bergmeyer ed.) pp. 524–529.
[23] Layne, E. (1957) Methods Enzymol. 3, 450–451.

[24] Beutler, H.-O. (1984) in: Methods in Enzymatic Analysis, vol. VI (H.U. Bergmeyer ed.) pp. 321–327, Varlag Chemia
[25] Lillie, S.H. and Pringle, J.H. (1980) J. Bacteriol. 143, 1384–1394.
[26] François, J., Villanueva, M.E. and Hers, H.-G. (1988) Eur. J. Biochem. 174, 551–559.
[27] Sagardia, F., Golay, I. and Rodriguez, M. (1971) Biochem. Biophys. Res. Commun. 42, 829–835.
[28] Sáez, M.J. and Lagunas, R. (1976) Mol. Cell. Biochem. 13, 73–78.
[29] Ciudad, C.J., Carabaza, A. and Guinovart, J.J. (1986) Biochem. Biophys. Res. Commun. 141, 1195–1200.
[30] Carabaza, A., Ciudad, C.J., Baqué, S. and Guinovart, J.J. (1992) FEBS Lett. 296, 211–214.
[31] Villar-Palasí, C. (1991) Biochim. Biophys. Acta 1095, 261–267.
[32] Farkas, I., Hardy, T.A., DePaoli-Roach, A.A. and Roach, P.J. (1990) J. Biol. Chem. 265, 20879–20886.
[33] Farkas, I., Hardy, T.A., Goebl, M.G. and Roach, P.J. (1991) J. Biol. Chem. 266, 15602–15607.