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Regulation of Invertase Synthesis by Glucose in *Saccharomyces cerevisiae*

SALVADOR MORMENEO AND RAFAEL SENTANDREU*

Departamento de Microbiología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain

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Saccharomyces cerevisiae growing under repressible conditions (1% of glucose or more) produces a burst of external invertase when shifted to higher temperatures. The secretion of this invertase requires protein synthesis, but was found to be independent of RNA formation. The level of mRNA accumulated and translated was inversely proportional to the glucose present in the growth medium. These results are consistent with the hypothesis that invertase is continuously synthesized both in the presence and absence of glucose, but under repressible conditions is degraded before secretion takes place.

In yeasts, the synthesis of many enzymes is regulated by catabolite repression in a similar way to that described in bacteria (8). The molecular mechanism of the process is still unclear even in case of the most studied of repressible enzymes, invertase (EC 3.2.1.26). This enzyme was thought to be present in the cell in two main molecular forms: an internal or cytoplasmic form (5) and an external form (13) localized at the periplasmic space (10). Recently, it has been shown that there are three different invertases (16). Two are in the cytoplasm [one carbohydrate free and soluble in the cell sap (5) and the other partially glycosylated and membrane bound [16]). The third form is also glycosylated and is secreted to the cell exterior. A precursor-product relationship seems to be the link between the membrane-bound form and the periplasmic one (16). The relationship between the cell sap-soluble invertase and the others is unknown. A precursor-product relationship was initially suggested (1, 11, 13), but differences in amino acid composition (6) and in the kinetics of synthesis during the cell cycle (4) did not confirm that hypothesis. Moreover Ottolenghi (14) found differences in their K_m values for sucrose. Perlman and Halvorson (15) have recently described the existence of three mature mRNAs coded by individual *SUC* genes that direct the synthesis of three polypeptides. Two of these are the precursors of secreted glycoproteins, and the third one corresponds to the soluble cytoplasmic polypeptide. A different maturation step in the *SUC* gene product would be responsible for the final location of the invertase isoenzymes.

The level of enzyme secretion depends on the glucose concentration in the culture medium (7). Repression of the external invertase synthesis has been described as taking place at the level of transcription (3, 15), translation (3), and glycosylation (2) of the protein. In this last case, the non-glycosylated enzyme would be degraded by specific proteolytic enzymes (2). Specific degradation steps are also responsible for the glucose-dependent inactivation of the malate dehydrogenase of *Saccharomyces cerevisiae* (12).

In this paper we have studied the synthesis and secretion of external invertase under conditions of repression after incubation at higher temperatures than that of growth, and we propose that the regulation of its synthesis by glucose takes place at the levels of transcription of the corresponding genes, translation of the mRNA, and probably during maturation of the enzyme before secretion takes place.

MATERIALS AND METHODS

Chemicals. Cycloheximide, glucose oxidase, *o*-dianisidine, and concanavalin A were obtained from Sigma Chemical Co., St. Louis, Mo. Peroxidase was from Boehringer Mannheim, Mannheim, W. Germany, and Zymolyase 5000 was from Kirin Breweries, Gumma, Japan. Whenever possible other reagents used were of analytical grade.

Organisms and culture conditions. The organisms used were *S. cerevisiae* X2180-1A (a *SUC2 mal gal2 CUP1*), *S. cerevisiae* X2180-1B (α *SUC2 mal gal2 CUP1*), *S. cerevisiae* A364A (a *ade1 ade2 ura his7 lys2 tyl gal1*), *S. cerevisiae* 4059-358D (α *SUC1 MAL1 MGL ade1 ade2 leu1*), *S. cerevisiae* 136(Ts) (a gift from L. M. Hartwell, University of Washington, Seattle), and *S. cerevisiae* X2180-136(Ts) (*SUC2 MAL gal CUP1 rna1-1*), obtained in our department by crossing *S. cerevisiae* 2180-1B and *S. cerevisiae* 136(Ts). RNA synthesis of the last two mutants is blocked at the nonpermissive temperature of 37°C.

The cells were propagated in liquid YM-1 (9). Flasks

containing 100 ml of medium were inoculated with 1.5 mg (dry weight) of cells and incubated with shaking at 23°C to the early exponential phase.

The strains were maintained on slants of YM-1 medium containing 2% agar.

Derepression of invertase synthesis was carried out in YM-1 medium, but 50 mM maltose instead of glucose was used as a carbon source.

Protoplasts were incubated in YM-1 and YM-1-maltose media in the presence of 1 M sorbitol as an osmotic stabilizer.

Invertase assay. The invertase assay was essentially the same as that of Gascon and Lampen (5), but incubation was carried out at 30°C. One unit of invertase was the amount of enzyme which hydrolyzed 1 μmol of sucrose in 10 min at 30°C in sodium acetate buffer (pH 5) containing 0.125 M sucrose.

Protoplast preparations. Samples of 100 mg (dry weight) of cells were treated with 10 ml of 5 mM dithiothreitol in 100 mM Tris-hydrochloride (pH 8)–5 mM EDTA for 20 min at 23°C. After washing, the cells were suspended in 20 ml of 1 M sorbitol containing 20 mg of Zymolyase 5000 and incubated at 24°C for 30 min.

Precipitation with concanavalin A. Precipitation was carried out as described previously by Rodríguez et al. (16). Samples of dialyzate containing 0.2 U of invertase were treated with 300 μg of concanavalin to precipitate the glycosylated enzyme.

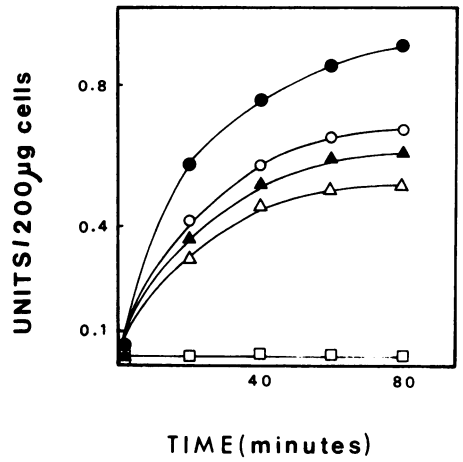

FIG. 1. Invertase activity in repressed cells: effect of the shift to a higher temperature. Exponentially growing cells of *S. cerevisiae* were collected, and samples were incubated in fresh YM-1 medium at 23 or 37°C. Samples were taken at the times indicated in the figure, and external invertase activity was measured. Symbols: *S. cerevisiae* 4059-358D (●), *S. cerevisiae* A364A (▲), *S. cerevisiae* 2180-1A (○), and *S. cerevisiae* 2180-1B (△) at 37°C, and all strains at 23°C (□).

RESULTS

Invertase activity in repressed cells: effect of the shift to a higher temperature. When *S. cerevisiae* X2180-1A was incubated under repressible conditions (1% glucose or more) at 23°C and then transferred to 37°C, a burst of external invertase was detected. This phenomenon did not take place when incubation was carried on at 23°C (Fig. 1).

To determine whether this behavior was generalized, the experiment was repeated with other *S. cerevisiae* strains (Fig. 1). Invertase was detected in all the cases tested. The enzyme appeared after a few minutes' delay at 37°C, and after about 40 min the rate of invertase secretion slowed down and finally came to a halt.

Invertase activity in repressed cells: effect of inhibition of RNA and protein syntheses. To find out whether the activity detected at 37°C was due to the activation of a proenzyme, to the translation of an already present mRNA, or to the transcription of a newly synthesized mRNA, the above-described experiment was repeated with *S. cerevisiae* 136(Ts) and *S. cerevisiae* 2180-136(Ts). Cultures of the two mutants were grown overnight at 23°C and then transferred to 37°C both in the presence and absence of cycloheximide.

Invertase activity was only detected in cultures incubated at 37°C in the absence of cycloheximide (Fig. 2). Since those mutants show inhibition of RNA formation at 37°C, the activity could only be due to the expression of an mRNA already present in the repressed cells. Moreover if a proenzyme had also accumulated, it would have been secreted both at 37°C and in the presence of cycloheximide.

Effect of glucose on the expression of invertase

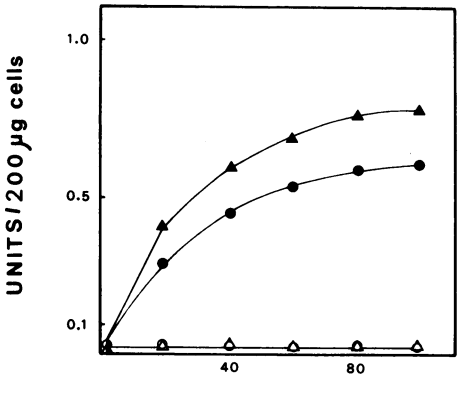

FIG. 2. Invertase activity in repressed cells: effect of inhibition of RNA and protein synthesis. Exponentially growing cells in YM-1 medium were collected and incubated in a fresh medium at 23°C (○), 37°C [▲ for *S. cerevisiae* 136(Ts) and ● for *S. cerevisiae* 2180-136(Ts)] and at 37°C in the presence of cycloheximide (△). Samples were taken, and the external invertase activity was determined.

mRNA. The influence of the concentration of glucose on the expression of the accumulated mRNA was studied in cells grown overnight in the presence of 1% glucose. The cells were transferred to a fresh medium with glucose at different concentrations (ranging from 0.25 to 5%) or in the presence of maltose at 37°C. With maltose, formation of invertase was continuous (at least for the duration of the experiment), although in the presence of glucose a plateau was reached in about 60 min. The total level of enzyme secreted was independent of the glucose concentration from 0.5 to 5%. At lower levels (0.25%) a significant increase in the invertase secreted was evident, suggesting that glucose interferes with the expression of the already accumulated mRNA (Fig. 3).

Effect of the temperature shift level on invertase formation. From the results reported above, it was of interest to discover the effect of the level of temperature shift on invertase formation. Samples of a culture of *S. cerevisiae* 2180-136(Ts) grown overnight at 23°C were incubated at different temperatures from 23 to 46°C. The kinetics of enzyme formation were similar in all cases, but the amount of enzyme found depended on the temperature of incubation. The higher the incubation temperature, the higher the total activity detected (Fig. 4). Interestingly enough, the enzyme found at 37°C and higher temperatures seemed to be the result of the translation of an already present mRNA, since as indicated above, RNA synthesis in this mutant is blocked under these conditions.

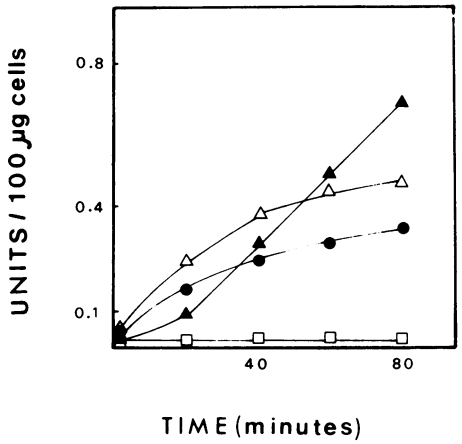

FIG. 3. Effect of glucose on the expression of invertase mRNA. Exponentially growing cells of *S. cerevisiae* 2180-136(Ts) grown at 23°C in YM-1 medium were transferred to a fresh medium with glucose at different concentrations (from 0.25 to 5%) or maltose. Symbols: 0.5% glucose (or higher concentrations) at 37°C (●) and at 23°C (□); 0.25% glucose at 37°C (△); and 2% maltose at 37°C (▲).

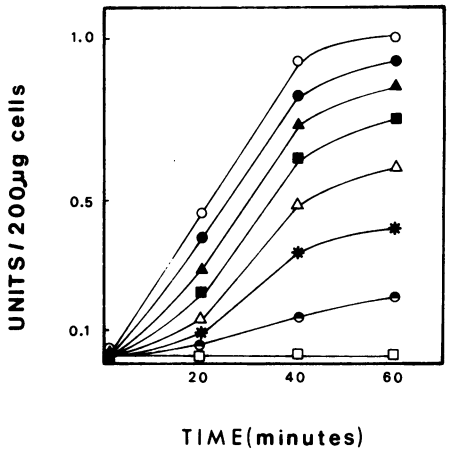

FIG. 4. Effect of the temperature shift level on invertase formation. *S. cerevisiae* 2180-136(Ts) repressed cells grown at 23°C in YM-1 medium were suspended in fresh medium and incubated at 23°C (□), 26°C (◒), 30°C (✱), 33°C (△), 37°C (■), 40°C (▲), 43°C (●), and 46°C (○). Samples were taken as indicated, and the external invertase activity was determined.

Similar results were obtained when this experiment was repeated with the other strains (data not shown).

Effect of glucose concentration on invertase mRNA accumulation. As described above, cells grown overnight and incubated at 37°C in glucose concentrations higher than 1% showed no differences in the amount of invertase produced, although nothing was known about the effect of glucose concentration on the invertase mRNA accumulation. When cells were grown overnight in the presence of 1, 2, 3, 4, or 5% glucose and then transferred to YM-1-glucose at 37°C, the level of invertase depended on the glucose present overnight. The mRNA present was highest in cells grown in 1% glucose; at other glucose concentrations less invertase mRNA accumulated (Fig. 5).

To determine whether the enzyme synthesized was glycosylated, protoplasts were incubated at 23°C for 180 min in YM-1 in the presence of 1 M sorbitol to allow protein synthesis to recover, after which they were transferred to YM-1 at 37°C and to YM-1 maltose at 24°C for 2 h. The supernatants of the protoplasts were dialyzed, and mannoproteins were precipitated with concanavalin A. Enzymatic activity in both cases decreased by 80 to 95% after treatment with concanavalin, suggesting that the enzyme secreted under repression conditions after the shift in temperature was glycosylated and similar to the enzyme synthesized by derepressed cells.

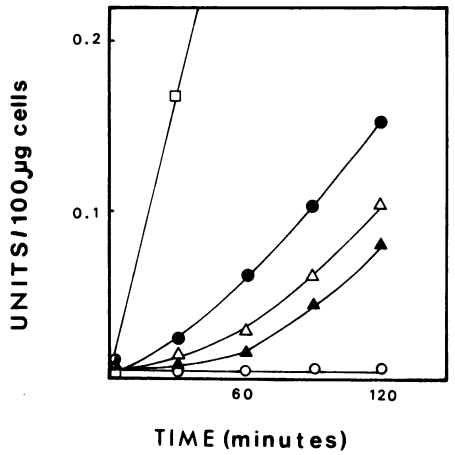

FIG. 5. Effect of glucose concentration on invertase mRNA accumulation. *S. cerevisiae* 2180-136(Ts) cells were grown with 1, 2, 3, and 4% glucose until the exponential phase. At an optical density at 600 nm of 0.3, cells were collected, washed, and inoculated in fresh YM-1 medium at 23 and 37°C. At the times indicated samples were taken, and the external enzyme activity was measured. Symbols correspond to activity detected at 37°C initially grown in 1% (□), 2% (●), 3% (△), and 4% (▲) glucose and the control at 24°C (○).

DISCUSSION

The synthesis of an enzyme is the result of the following events: transcription of a specific mRNA, translation of this mRNA into a protein, and in some cases posttranslational modifications that may involve proteolysis, phosphorylation, glycosylation, etc., followed by the secretion in case of an exocellular form.

Invertase is an extracellular enzyme, and regulation of its synthesis may take place at one or more of the steps outlined above.

Cells growing in glucose at a concentration of 1% or more do not produce extracellular activity, although some intracellular activity is found. Our results confirmed the above data, but we found that when the cells are shifted to a higher temperature, invertase secretion takes place (Fig. 1). This phenomenon might be due either to the activation of a proenzyme or to the de novo synthesis of invertase due to the expression of an already present or newly synthesized mRNA.

To discriminate between the above hypotheses, the synthesis of invertase was analyzed in cells in the presence of cycloheximide and at 37°C to block protein and RNA synthesis, respectively.

In the presence of cycloheximide no activity was detected, suggesting that the activation of an already present proenzyme was not a likely explanation for the new invertase secreted. But when the cells were incubated at 37°C, the temperature that blocks RNA synthesis in both *S. cerevisiae* 2180-136(Ts) and *S. cerevisiae* 136(Ts), activity was detected, suggesting that invertase secretion was probably the result of the translation of a specific RNA which was already present in the cells incubated in the presence of glucose. Moreover, this suggests that invertase synthesis at high temperatures is due to the expression of invertase mRNA molecules synthesized continuously in the presence of glucose.

The levels of mRNA present, determined as the total exocellular invertase detected at the higher temperature, depend on the glucose present in the growth medium. The higher the glucose present, the lower the invertase mRNA accumulated (Fig. 5). These results are in agreement with those reported previously, indicating that repression of invertase by glucose takes place at least at the level of transcription (3, 15).

Transfer of *S. cerevisiae* 136(Ts) or *S. cerevisiae* 2180-136(Ts) to a medium with either glucose or maltose at 37°C resulted in different levels of invertase (Fig. 4). The activity in cells incubated with 0.5% glucose or more was less than that found with 0.25% glucose or with maltose, suggesting that glucose interferes not only with transcription but also with the translation of the invertase RNA already accumulated. Perlman and Halvorson (15) also recently described that the action of glucose on invertase is at the level of transcription; however, Chu and Maley (2) did not detect this effect in one of the strains (ATCC 9763) employed by Perlman and Halvorson.

The appearance of enzyme activity after incubation of repressed cells at a higher temperature suggests that the invertase mRNA is constantly being transcribed in conditions of both repression and derepression. The invertase detected is higher under derepression conditions, suggesting that the glucose modifies only the amount of the mRNA accumulated, but not its presence.

Since transcription and translation seem to occur constantly, synthesis of the enzyme should also be constant. Therefore, the inability to detect enzyme activity in repressed cells must be due to a continuous degradation of the protein before its secretion.

We suggest that secretion of invertase in repressed cells after a temperature shift might be due to the uncoupling between synthesis and degradation of the protein molecules, since the amount of invertase detected is proportional to the amplitude of the shift (Fig. 3). When incubation at the new temperature is carried on, however, a new equilibrium between synthesis and degradation is reached after 60 min.

The results reported in this paper are in agreement with those of Chu and Maley (2); these

authors have suggested that the newly synthesized invertase in repressed cells is degraded before glycosylation takes place. The finding that the enzyme secreted under repression conditions is glycosylated suggests that if the degradation hypothesis is right, once the uncoupling phenomenon has taken place, glycosylation of invertase is normally carried out.

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