Sudden depletion of carbon source blocks translation, but not transcription, in the yeast *Saccharomyces cerevisiae*

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Received 6 June 1996

Abstract Expression of invertase in the yeast *Saccharomyces cerevisiae* is greatly delayed when derepression occurs in a medium that lacks a usable carbon source. The delay is not a consequence of defects in the transcription of the *SUC2* gene but is due to the impossibility of translating the normal levels of mRNA generated under derepressing conditions. The inhibition of translation in the absence of glucose has to be considered when reporter genes such as *E. coli lacZ* are used to measure transcription in conditions of carbon source starvation.

Key words: Catabolite repression; Carbon source starvation; Yeast; Transcription; Translation

1. Introduction

Carbon source starvation is one of the most common stress factors. It induces cells to enter into stationary phase, which allows long-term maintenance of viability [1]. However, not all starvations lead to stationary phase, many auxotrophic starvations and the abrupt withdrawal of glucose result in cell populations arrested at various positions in the cell cycle, causing a rapid loss in viability ([2], M. Martínez-Pastor and F. Estruch, unpublished results). It has been suggested that this could be a consequence of the inability to synthesize certain stage-specific proteins [2]. Entry into the stationary phase is characterized by a decrease in the growth rate, progressive reduction of RNA and protein synthesis and accumulation of glycogen and trehalose. Although protein synthesis is very low in stationary phase cells, the pattern of protein synthesis is similar to that in exponentially growing cells [3,4]. On the other hand, a number of genes are induced during entry into stationary phase. The expression of many of these genes is negatively regulated by the RAS-cAMP pathway through the stress response element (STRE) [5,6]. Recently, we have shown that the zinc finger transcriptional activators Msn2p and Msn4 are required for transcriptional induction mediated through STRE [7].

Yeast preferentially utilizes hexoses such as glucose and fructose as carbon source. In presence of these sugars, expression of genes involved in the utilization of alternate carbon sources, such as sucrose and galactose, is repressed at transcriptional level [8]. The *SUC2* (invertase) gene is one of the best characterized glucose repressible genes, it is exclusively regulated by glucose and does not require induction by sucrose or any other substrate [9]. The regulatory network responsible for glucose repression has been extensively studied (for review, see [10,11]) but many aspects remain unknown. It has recently been shown that glucose repression is not only regulated at the transcriptional level, and that mRNA stability modulates the expression of *SUC2* and other glucose repressible genes [12]. Moreover, an inhibition of protein synthesis caused by amino acid starvation and other stress conditions has been found in both mammalian and yeast cells [13].

We studied the response of the yeast *S. cerevisiae* to the sudden change of carbon source. Yeast cells exponentially growing in a medium containing glucose were transferred to a medium in which carbon source could not be used initially because the enzymes required to metabolize it were subjected to glucose repression. We found that in the complete absence of glucose, yeast cells require a long time before being able to use the new carbon source. This lag time is not a consequence of a defect in the transcription of the glucose-repressible genes but it is due to the complete inhibition of translation in the absence of a usable carbon source.

2. Materials and methods

2.1. Yeast strains, media and genetic methods

All experiments were done with *Saccharomyces cerevisiae* strain W303-1A (*MATa, SUC2, ade2, can 1, his3, leu2, trp1, ura3*) [14]. Standard methods were used for genetic analysis and transformation [15]. Synthetic medium (SM) is 0.67% (w/v) yeast nitrogen base without amino acids (Difco) supplemented with adenine (20 mg/l), uracil (20 mg/l), tryptophan (20 mg/l), histidine (20 mg/l), leucine (30 mg/l) and lysine (30 mg/l) and containing 2% dextrose (SD), 2% raffinose (SR), 2% raffinose+0.05% dextrose (SRd), 2% galactose (SG) or 2% galactose+0.05% dextrose (SGd).

2.2. Growth curves

Cells from stationary phase culture were inoculated to SD medium and grown exponentially for 24 h. Cells were then washed and diluted into non-repressing medium at a density of $2–5\times10^6$ cells/ml. Growth was monitored by measuring the optical density at 600 nm.

2.3. Preparation and Northern analysis of RNA

To prepare total RNA, cells were harvested, washed with RNase-free water and frozen at −80°C. Cells were broken by vortexing with glass beads in LETS buffer (0.1 M LiCl, 0.01 M Na_2-EDTA, 0.01 M Tris-HCl (pH 7.4), 0.2% SDS) and one volume of LETS saturated phenol. After two extractions with phenol:chloroform:isoamyl alcohol, the RNA was precipitated with one volume of 5 M lithium chloride and stored at −80°C for at least 2 h. The precipitate was washed with 70% ethanol and resuspended in RNase-free water. The amount of RNA was quantified by measuring the absorbance at 260 nm. RNA was fractionated on agarose gels containing formaldehyde and blotted on Hybond-N (Amersham) membrane.

2.4. Enzyme assays

Secreted invertase was assayed in whole cells as described in [16]. β-Galactosidase was assayed as described [17].

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PII S0014-5793(96)00683-7

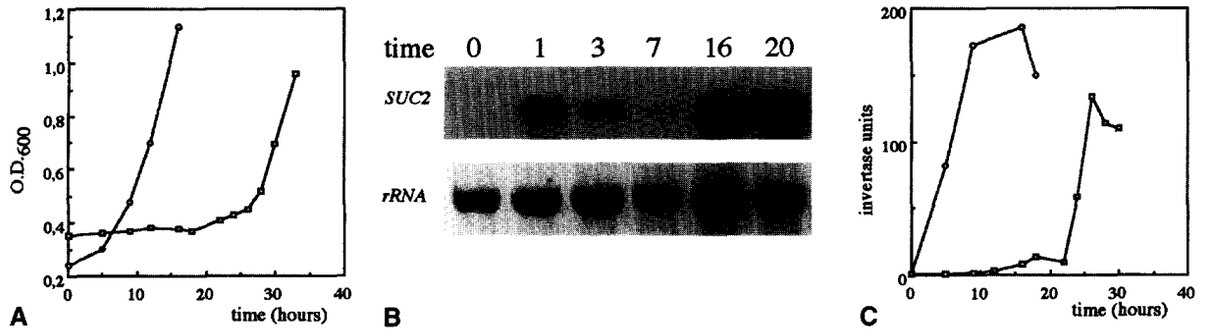

Fig. 1. Expression of *SUC2* mRNA and invertase along the growth curve. (A) Growth of yeast strain W303-1A in SR (squares) and SRd medium (circles). (B) *SUC2* mRNA at different times of incubation (in hours) in SR medium. Amounts of 4 μg of each sample were fractionated on 1.6% agarose gels and analyzed by Northern blot hybridization. The application and transfer of an equal amount of RNA were verified by ethidium bromide staining (rRNA). (C) Invertase activity at different times of incubation in SR (squares) and SRd (circles) medium. Invertase activity is expressed as μmol of glucose released/min/100 mg (dry weight) of cells

2.5. Uptake and incorporation of [^{35}S]methionine

The rate of uptake and protein synthesis was measured as described in [18].

3. Results

3.1. Yeast response to the change of carbon source

When exponentially growing wild type yeast cells were transferred from YPD to a fresh medium containing raffinose as carbon source (YPR), after a short initial phase of slow growth, cells resumed exponential growth (result not shown). However, when this experiment was repeated using synthetic minimal medium (SR), the lag phase, during which there was no significant increase in the optical density at 600 nm (OD_{600}) of the culture, exceeded 20 h (Fig. 1A). Differences between rich and minimal media are, in part, due to the presence of glucose or other fast metabolizable sugar in the rich medium, since the addition of a small amount (0.05%) of glucose to synthetic minimal medium (SRd) reduces the time required to start exponential growth to a similar level to that observed in rich medium (Fig. 1A).

It is reasonable to assume that the lag phase is the time required to derepress the *SUC2* gene and to synthesize invertase, the enzyme that allows the use of raffinose as carbon source. We investigated the levels of *SUC2* mRNA at different points in the growth curve when SR medium was used (Fig. 1B). For a short time after the shift to non-repressing conditions there is a transient expression of the *SUC2* gene. This initial expression does not produce measurable levels of invertase (Fig. 1C) and is not sufficient to support cell growth (Fig. 1A). A second activation of *SUC2* takes place shortly before exponential growth starts. In this case slightly higher levels of *SUC2* mRNA are observed, resulting in measurable levels of invertase activity (Fig. 1C) and cell growth (Fig. 1A). When a small amount of glucose was added to raffinose (SRd medium) no lag phase was observed for invertase activity (Fig. 1C).

The considerable time required to utilize raffinose as carbon source in the absence of glucose was unexpected. To check whether this behavior is common to other sugars we measured cell growth after transferring exponentially growing yeast cells to synthetic minimal medium with galactose as the only carbon source (SG). The results were similar to those obtained with raffinose, the lag phase being even longer. Similarly, the addition of 0.05% glucose to the galactose (SGd medium) eliminated the lag phase (results not shown). We have also measured in these media the expression of a *GAL10-lacZ* gene fusion through its β-galactosidase activity [19]. Activity was only detected shortly before resumption of growth. As occurred with invertase the delay was drastically reduced when 0.05% glucose was added to the medium (results not shown)

Therefore, glucose requirement for a fast expression is not limited to the *SUC2* gene and occurs in other glucose repressible genes.

3.2. Expression of SUC2 *gene in complete absence of glucose*

To try to explain the differences in invertase activity observed at short times between cells transferred to SR and SRd, we compared the kinetics of derepression of *SUC2* mRNA in both media by Northern blot. Fig. 2 shows that the level of *SUC2* mRNA at 30 min and 1 h after the transfer to the non-repressing conditions is not affected by the presence of glucose in the medium, since similar amounts of *SUC2* transcript were observed in cells derepressed in SR and SRd medium. At longer times there is a decay in the amount of *SUC2* mRNA when derepression occurs in SR medium (Fig. 2, lane 6; see also Fig. 1B).

A more detailed analysis of the invertase activity during this time period is shown in Fig. 3A. Invertase activity was detected only when derepression was performed in the presence of a low concentration of glucose. That is to say, in spite of the fact that equal amounts of *SUC2* mRNA are present in cells derepressed in SR and SRd at 30 min and 1 h, no invertase activity was observed in the complete absence of glucose. To exclude the possibility of a specific inactivation of invertase in the absence of any glucose, we examined the ex-

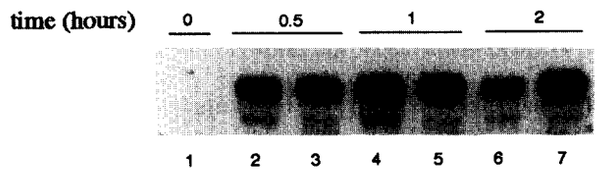

Fig. 2. Expression of *SUC2* mRNA does not depend on the presence of glucose in the medium. Total RNA was prepared from wild type W303-1A strain, previously grown on SD medium (lane 1), after incubation at the indicated times on SR (lanes 2, 4 and 6) or SRd medium (lanes 3, 5 and 7). The application and transfer of an equal amount of RNA were verified by ethidium bromide staining (not shown).

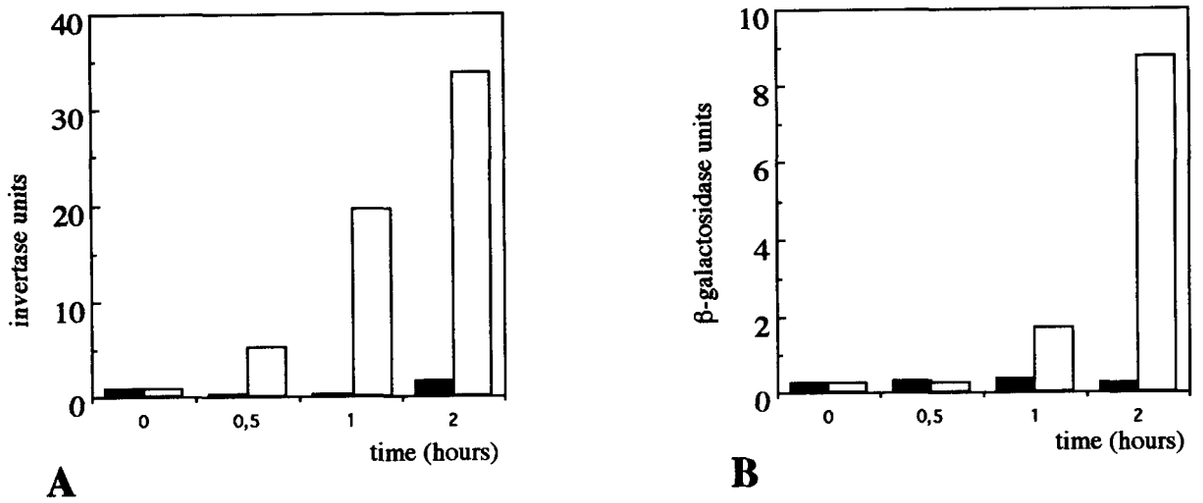

Fig. 3. Expression of invertase and *SUC2* promoter-directed β-galactosidase depends on the presence of glucose in the medium. Invertase (A) and β-galactosidase (B) activity at different times of incubation in SR (filled bars) and SRd (empty bars). Invertase activity is expressed as μmol of glucose released/min/100 mg (dry weight) of cells and β-galactosidase activity as described in [17].

pression of a *SUC2-LEU2-lacZ* gene fusion contained in the plasmid pLS11 [20] integrated at the chromosomal *URA3* locus. This gene fusion is normally regulated by glucose repression. Fig. 3B shows that β-galactosidase activity was only detected in SRd medium. As expected, the analysis of β-galactosidase by Western blot reveals the presence of this protein only when cells were derepressed in SRd medium (results not shown).

3.3. *Deprivation of glucose results in cessation of protein synthesis*

Since *SUC2* transcript levels in SR and SRd medium were similar but invertase activity was only observed in the presence of glucose, we investigated whether amino acid uptake and/or protein synthesis were affected by the absence of glucose during derepression. Over a short period, incubation of yeast in SR medium slightly reduces the rate of methionine uptake but the incorporation into proteins drops to the level observed when translation was inhibited with cycloheximide (Fig. 4). Therefore, the absence of invertase activity when cells are transferred to derepression medium devoid of glucose is the consequence of a general defect in protein synthesis. Translation capacity is restored after several hours of incubation, allowing synthesis of invertase when the second peak of *SUC2* expression occurs.

4. Discussion

The starting point of the present work was the observation that the expression of invertase was greatly delayed when derepression was performed in the complete absence of glucose. The finding that exponentially growing cells take about 20 h to use raffinose (or galactose) as carbon source was unexpected. The utilization of raffinose by yeast depends on the activity of invertase which is encoded by a gene, *SUC2*, regulated by glucose repression. A short time after the change to non-repressing conditions, transcriptional activation of *SUC2* was similar in minimal raffinose and in raffinose supplemented with a non-repressing amount of glucose. However, invertase activity was only detected for the first few hours in the glucose-containing medium. Our results indicate that inhibition of translation is responsible for the delay observed in the expression of invertase when derepression is performed in the complete absence of glucose. In the absence of a metabolizable carbon source the rate of methionine incorporation into protein was similar to that observed in the presence of inhibitory amounts of cycloheximide. The inhibition of translation is fully responsible for the absence of protein since in the presence of glucose the same level of mRNA gives detectable amounts of protein and enzyme activity.

In eukaryotic cells the translation initiation factor 2α (eIF-2α) has been involved in the regulation of translation in re-

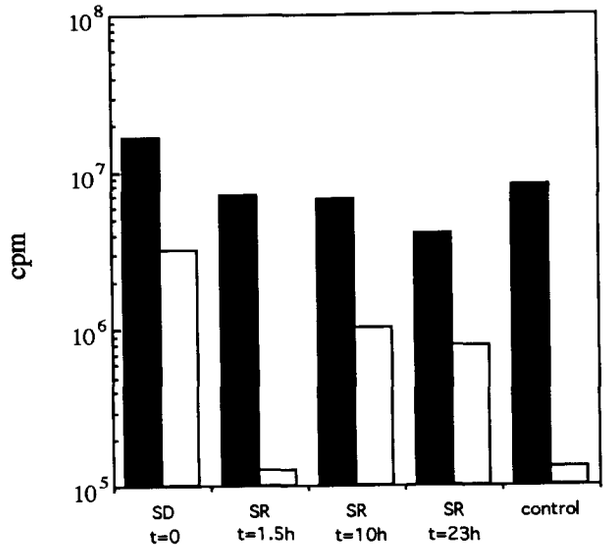

Fig. 4. [^{35}S]Methionine uptake and protein synthesis in the absence of glucose. Exponentially growing cells in SD medium were transferred to SR medium and [^{35}S]methionine uptake (filled bars) and [^{35}S]methionine incorporation into protein (empty bars) were measured after the indicated times of incubation in SR medium. Basal incorporation (control) was measured by adding to the cells 0.1 mg/ml of cycloheximide prior to the addition of [^{35}S]methionine.

sponse to stress conditions [13,21]. In yeast the phosphorylation state of eIF-2α has been analyzed by 2-D gel electrophoresis. The phosphorylated form of this protein is observed during logarithmic growth on fermentable and non-fermentable carbon sources, heat shock, starvation of essential amino acids, stationary phase and sporulation. Only with those cells that were starved for carbon source, was eIF-2α isolated in the non-phosphorylated state [22]. On the other hand, it has been shown that deprivation of amino acids activates the Gcn2p protein kinase which, in turn, phosphorylates eIF-2α. This reaction has an inhibitory effect in general protein synthesis that specifically stimulates translation of Gcn4p, a transcriptional activator of amino acid biosynthetic genes [13]. Taken together, these and our results would be consistent with a model in which eIF-2α would only be active in the phosphorylated form and a fine-tuning regulation of its activity would be achieved by further phosphorylation catalyzed by Gcn2p protein kinase.

The absence of translation in conditions of carbon source starvation has to be taken into account, especially when transcriptional induction is measured by the enzymatic activity of a reporter gene since, as our results show, absence of enzymatic activity is not always synonymous of the absence of transcription.

Acknowledgements: This work was supported by DGICYT grants PB92-0352 (to J.E Pérez-Ortín) and PB94-0994 (to F.E.) from the Spanish Ministry of Education. M.T.M.-P. was supported by a predoctoral fellowship of the 'Conselleria de Educació i Ciencia' (Generalitat Valenciana).

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