Glucose-triggered signalling in *Saccharomyces cerevisiae*: different requirements for sugar phosphorylation between cells grown on glucose and those grown on non-fermentable carbon sources

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Addition of glucose or fructose to cells of the yeast *Saccharomyces cerevisiae* grown on a nonfermentable carbon source triggers within a few minutes post-translational activation of trehalase, repression of the *CTT1* (catalase) and *SSA3* (Hsp70) genes, and induction of the ribosomal protein genes *RPL1*, *RPL25* and *RPS33*. By using appropriate sugar kinase mutants, it was shown that rapid glucose- or fructose-induced activation of trehalase requires phosphorylation of the sugar. On the other hand, partial induction of *RPL1*, *RPL25* and *RPS33* as well as partial repression of *CTT1* and *SSA3* were observed in the absence of sugar phosphorylation. In glucose-grown nitrogen-starved yeast cells re-addition of a nitrogen source triggers activation of trehalase in a glucose- or fructose-dependent way, but with no apparent requirement for phosphorylation of the sugar. Repression of *CTT1* and *SSA3* under the same conditions was also largely dependent on the presence of the sugar and also in these cases there was a strong effect when the sugar could not be phosphorylated. Nitrogen induction of *RPL1*, *RPL25* and *RPS33* was much less dependent on the presence of the sugar, and only phosphorylated sugar caused a further increase in expression. These results show that two glucose-dependent signalling pathways, which can be distinguished on the basis of their requirement for glucose phosphorylation, appear to be involved in activation of trehalase, repression of *CTT1* and *SSA3* and induction of ribosomal protein genes. They also show that nutrient-induced repression of *CTT1* and *SSA3* is not a response to improvement of the growth conditions because the addition of nonmetabolizable sugar does not ameliorate the growth conditions. Similarly, the upshift in ribosomal protein synthesis cannot be a response to increased availability of energy or biosynthetic capacity derived from glucose, but it is apparently triggered to a significant extent by specific detection of glucose as such.

Keywords: glucose signalling, trehalase, catalase, Hsp70, ribosomal protein genes

INTRODUCTION

The addition of glucose, fructose or a related rapidly fermented sugar to cells of the yeast *Saccharomyces cerevisiae* growing on a nonfermentable carbon source, like glycerol or ethanol, triggers an extensive series of regulatory responses at both the post-translational and transcriptional level (recent review: Thevelein & Hohmann, 1995).

For glucose-induced activation of cAMP synthesis (Beullens *et al.*, 1988), inactivation of fructose-1,6-bisphosphatase (Gancedo & Gancedo, 1979; Entian *et al.*, 1983) and most other non-cAMP-mediated responses, such as glucose repression (Entian, 1986), activation of plasma membrane H⁺-ATPase (Becher dos Passos *et al.*, 1992) and induction of *PDC1* (Boles *et al.*, 1993), phosphorylation of the glucose is essential, as shown by

loss of the response in mutants unable to phosphorylate glucose. The addition of glucose or fructose to cells growing on a nonfermentable carbon source is well known to trigger also a rapid increase in the growth rate, which is preceded by a characteristic upshift in the synthesis of ribosomal RNA and protein (Kief & Warner, 1981; Donovan & Pearson, 1986; Mager & Planta, 1991). Requirement of sugar phosphorylation for this upshift has not been studied up to now.

Starvation of yeast cells on a glucose-containing medium for any other essential nutrient, such as nitrogen, phosphate or sulfate, will cause them to arrest in the cell cycle and enter a resting state called G0. These cells continue to ferment glucose, but at a lower rate, and they show accumulation of trehalose and glycogen, induction of the *CTT1* gene, encoding catalase, several genes encoding heat shock proteins, such as *SSA3*, and also repression of the whole spectrum of ribosomal protein genes (review: Werner-Washburne *et al.*, 1993). Re-addition of the missing nutrient triggers rapid activation of trehalase and mobilization of trehalose (Hirimburegama *et al.*, 1992), activation of glycogen phosphorylase, inactivation of glycogen synthase and mobilization of glycogen (François *et al.*, 1988), repression of *CTT1* and *SSA3* (Bissinger *et al.*, 1989; Werner-Washburne *et al.*, 1989) and induction of the ribosomal protein genes (Pearson & Haber, 1980; Mager & Planta, 1991). All the effects associated with the re-induction of growth under these conditions are at least in the long term dependent on the presence of both a fermentable carbon source and a complete growth medium. Therefore, we have called the signalling pathway involved in these phenomena the 'fermentable-growth-medium-induced pathway' (Thevelein & Hohmann, 1995). At present, it is unclear whether more than one independent pathway is involved in triggering the different target processes.

The metabolic changes measured in this work, i.e. activation of trehalase, repression of *CTT1* and *SSA3*, and induction of the ribosomal protein genes *RPL1*, *RPL25* and *RPS33*, are clearly correlated under a variety of conditions with growth induction or growth stimulation (Pearson & Haber, 1980; Thevelein, 1984; Thevelein & Beullens, 1985; Bissinger *et al.*, 1989; Werner-Washburne *et al.*, 1989; Mager & Planta, 1991; Hirimburegama *et al.*, 1992). Whether glucose-triggered signalling involved in growth induction or growth stimulation itself requires phosphorylation of glucose is difficult to measure directly because glucose also provides through its metabolism energy and intermediates which are an essential requirement for growth. In order to investigate the nutrient-induced signalling requirements for growth induction, one is limited to studying growth-related events as markers. Interestingly, it has recently been shown that a number of growth-related events in yeast are triggered in a non-cAMP-mediated way by glucose which could not be phosphorylated (Granot & Snyder, 1991, 1993). In addition, incubation of stationary-phase cells for a certain period of time in the presence of unphosphorylated glucose shortened the time required for bud emergence when the cells were subsequently

transferred to a complete growth medium (Granot & Snyder, 1991, 1993). This might indicate a lack of sugar phosphorylation requirement for the control of growth, although indirect mechanisms cannot be excluded.

In *S. cerevisiae* three enzymes are known to be capable of phosphorylating glucose. Glucokinase, encoded by the *GLK1* gene, is specific for glucose, whereas hexokinases PI and PII, encoded by *HXK1* and *HXK2*, respectively, phosphorylate both glucose and fructose (Fraenkel, 1982). Requirement of sugar phosphorylation is easily studied in cells of triple kinase or double hexokinase mutants, grown on a nonfermentable carbon source, by adding glucose or fructose, respectively. Similarly, requirement of sugar phosphorylation for responses in glucose-grown cells can easily be studied in cells of a *hxxk1 hxxk2* double mutant by washing glucose away and re-incubating the cells with fructose (e.g. Gancedo & Gancedo, 1979; Beullens *et al.*, 1988; Walsh *et al.*, 1991; Becher dos Passos *et al.*, 1992; Boles *et al.*, 1993). This approach is more reliable than the use of glucose analogues since these might not be detected properly by glucose-sensing systems.

METHODS

Strains, media and growth conditions. The following isogenic sets of yeast strains were used: (1) WAY.5-4A (*MATa ura3-52 his3- δ 1 MAL2-8^c MAL3 SUC3 lcr1*), WAY.14-1A (*MATa ura3-52 his3- δ 1 leu2-3,112 hxxk2::LEU2 glk1::LEU2 MAL2-8^c MAL3 SUC3 lcr1*), WAY.glk1-1A (*MATa ura3-52 his3- δ 1 leu2-3,112 hxxk1::HIS3 hxxk2::LEU2 MAL2-8^c MAL3 SUC3 lcr1*), WAY.glk1-5C (*MATa trp1-289 his3- δ 1 leu2-3,112 hxxk1::HIS3 glk1::LEU2 MAL2-8^c MAL3 SUC3 lcr1*) (generously provided by K. D. Entian) and PD1501 (*MATa his3- δ 1 ura3-52 leu2-3,112 hxxk1::HIS3 hxxk2::LEU2 glk1::LEU2 MAL2-8^c MAL3 SUC3 lcr1*); (2) YSH327 (*MATa leu2-3/112 ura3-1 trp1-1 his3-11/15 ade2-1 can1-100 GAL SUC2 hxxk1::HIS3 hxxk2::LEU2*) and YSH757 (*MATa leu2-3/112 ura3-1 trp1-1 his3-11/15 ade2-1 can1-100 GAL SUC2 hxxk1::HIS3 hxxk2::LEU2 glk1::LEU2*) (both strains are isogenic to W303-1A; they were used for the experiment shown in Fig. 4a). Cells were grown at 30 °C on YPD [4% (w/v) glucose, 2% (w/v) peptone and 1% (w/v) yeast extract] or YPglycerol [3% (v/v) glycerol, 2% (w/v) peptone and 1% (w/v) yeast extract]. Nitrogen starvation was carried out with exponential-phase cells grown on YPD and subsequently starved for nitrogen at 30 °C on a medium containing 4% glucose and 0.17% Difco Yeast Nitrogen Base without amino acids and without ammonium sulfate, for a period of 24 h at a cell density of 4–8 mg cells (wet weight) ml⁻¹. At the end of the starvation period the remaining glucose level was at least 20 mM.

Activation of trehalase. Exponential-phase YPglycerol-grown cells were collected by centrifugation, washed twice with ice-cold 25 mM MES/KOH buffer (pH 6) and resuspended at a cell density of 25 mg ml⁻¹ in 25 mM MES/KOH buffer (pH 6). The cells were pre-incubated at 30 °C for 10 min before the first sample was taken. Activation of trehalase was triggered by addition of 4% (w/v) glucose or fructose. Nitrogen-starved cells were collected by centrifugation, washed twice with ice-cold 25 mM MES/KOH buffer (pH 6) and resuspended at the same cell density in fresh nitrogen starvation medium with either glucose or fructose (each 4%). They were pre-incubated at 30 °C for 30 min before the first sample was taken. For nitrogen-induced activation of trehalase 10 mM asparagine and

the amino acids for which the strain is auxotrophic were used as nitrogen source.

Sampling, preparation of crude extracts and trehalase assay.

Samples containing 75 mg (wet weight) cells were taken at the indicated times and quickly mixed with 45 ml ice-cold water. The cells were collected by centrifugation for 3 min at 4 °C. The pellet was resuspended in 0.5 ml ice-cold 50 mM MES/KOH buffer (pH 7) containing 50 μ M CaCl_2 . The cells were broken by vigorous mixing for 90 s in a small pre-cooled test tube containing 1.5 g glass beads (0.5 mm diameter). The crude enzyme extract was centrifuged for 3 min at 13000 r.p.m. in a microcentrifuge at 4 °C. The supernatant was dialysed overnight at 4 °C in a microdialysis apparatus against 5 mM MES/KOH buffer (pH 7) containing 50 μ M CaCl_2 . For trehalase assay, 10 μ l dialysed extract was incubated with 140 μ l water and 100 μ l substrate buffer (250 mM trehalose in 62.5 mM MES/KOH buffer, pH 7, with 125 μ M CaCl_2) for 30 min at 30 °C. The reaction was stopped by boiling for 3 min at 100 °C. The glucose liberated was assayed by the glucose oxidase/peroxidase method. Protein was determined by the Lowry procedure. Specific activity of trehalase is expressed as nmol glucose liberated min^{-1} (mg protein) $^{-1}$.

RNA extraction and Northern blot analysis. Growth and starvation conditions were the same as those used for the trehalase activation experiments. Total RNA was extracted essentially as described previously by shaking cells with glass beads in the presence of acid, water-equilibrated phenol (Sherman *et al.*, 1986). Equal amounts of RNA (A_{260}) were separated in 1% (w/v) agarose gels, containing formaldehyde, transferred to a nylon membrane and hybridized with ^{32}P -labelled DNA fragments of the coding region of *CTT1*, *SSA3*, *RPL1*, *RPL25* or *RPS33*. The filters were then analysed using phosphorimager technology (Fuji, BAS1000; Software, PCBAS 2.0). In all cases equal amounts of RNA were loaded. Actin mRNA levels were used as standards but in stationary-phase cells actin mRNA levels were also lower compared to exponential-phase cells.

Chemicals. The fructose used was from Merck (quality: extra pure, cat. no. 5321).

RESULTS

When wild-type *S. cerevisiae* cells grow on a nonfermentable carbon source, they accumulate a high level of trehalose. Addition of a rapidly fermented sugar like glucose or fructose triggers an approximately fivefold activation of trehalase within a few minutes, resulting in rapid mobilization of trehalose (Van der Laat, 1974; Thevelein & Beullens, 1985). When glucose or fructose was added to glycerol-grown cells of a *hxx1 hxx2 GLK1* strain, which is only able to phosphorylate glucose and not fructose, only glucose triggered activation of trehalase. Fructose had no effect (Fig. 1). In a strain able to phosphorylate both glucose and fructose (*hxx1 HXX2 glk1*), activation of trehalase was observed with both sugars (Fig. 1). In a triple sugar kinase mutant (*hxx1 hxx2 glk1*) neither glucose nor fructose caused activation of trehalase (Fig. 1).

On the other hand, in stationary-phase cells obtained in the presence of glucose, no requirement for phosphorylation of the sugar was observed. When yeast cells are starved for nitrogen on a glucose-containing medium, they enter the stationary phase G0 and accumulate a high level of trehalose. Re-addition of nitrogen triggers

Fig. 1. Trehalase activity after addition of a fermentable sugar to exponential-phase glycerol-grown cells. ●, ○, Strain lacking both hexokinase PI and PII but not glucokinase (*hxx1 hxx2*) (●, addition of 4% glucose; ○, addition of 4% fructose). ▲, △, Strain lacking glucokinase and hexokinase PI (*hxx1 glk1*) (▲, addition of 4% glucose; △, addition of 4% fructose). ■, □, Strain lacking glucokinase and both hexokinase PI and PII (*hxx1 hxx2 glk1*) (■, addition of 4% glucose; □, addition of 4% fructose).

Fig. 2. Trehalase activity after addition of a nitrogen source (10 mM asparagine and essential amino acids) in the presence of a fermentable sugar to nitrogen-starved glucose-grown cells. ●, ○, ■, Strain lacking both hexokinase PI and PII (*hxx1 hxx2*) (●, presence of 4% glucose; ○, presence of 4% fructose; ■, no sugar present). ▲, △, ■, Strain lacking glucokinase and hexokinase PI (*hxx1 glk1*) (▲, presence of 4% glucose; △, presence of 4% fructose; □, no sugar present).

approximately fivefold activation of trehalase within 10–20 min, causing rapid mobilization of trehalose concomitant with the initiation of growth. This nitrogen-induced activation is largely dependent on the presence of a rapidly fermented sugar like glucose or fructose (Hirimburegama *et al.*, 1992; Durnez *et al.*, 1994; Fig. 2).

Fig. 3. (a) Northern blot analysis of the expression of *CTT1* (catalase T), *SSA3* (heat shock protein Hsp70) and the ribosomal protein genes *RPL1*, *RPL25* and *RPS33* after addition of 2% glucose (G) or 2% fructose (F) for 20 min or 60 min to exponential-phase glycerol-grown cells (gly) of yeast strains either lacking both hexokinase PI and PII but not glucokinase (*hxxk1 hxxk2 GLK1*) or lacking glucokinase and hexokinase PI (*hxxk1 HXXK2 glk1*). Actin messenger levels are shown as control. (b) Quantification of the results in (a). Actin messenger levels were used for calibration.

When a nitrogen source was added to nitrogen-starved cells of a *hxxk1 hxxk2 GLK1* strain incubated in the presence of glucose or fructose, rapid activation of trehalase was not only observed with glucose but also with fructose, which cannot be phosphorylated in this strain (Fig. 2). Also in a strain able to phosphorylate both glucose and fructose (*hxxk1 HXXK2 glk1*) both sugars caused activation of trehalase (Fig. 2). In all cases the activation was dependent to a large extent on the presence of both the nitrogen and the sugar (Fig. 2), as reported previously (Hirimburegama *et al.*, 1992; Durnez *et al.*, 1994). [Since a triple sugar kinase mutant (*hxxk1 hxxk2 glk1*) cannot grow on glucose or fructose, the experiment in which nitrogen is added to glucose-grown nitrogen-starved cells could not be performed with this strain.] These results show that activation of trehalase in glucose-grown cells by a combination of fermentable sugar and nitrogen, i.e. through the fermentable-growth-medium induced pathway, does not depend on phosphorylation

and by extension not on metabolism of the fermentable sugar.

When wild-type yeast cells are growing on a nonfermentable carbon source, expression of the genes *CTT1* and *SSA3* is much higher than on a rapidly-fermented sugar like glucose or fructose (Bissinger *et al.*, 1989; Werner-Washburne *et al.*, 1989). Addition of glucose for 20 min or 60 min to cells of strains (*hxxk1 hxxk2 GLK1* and *hxxk1 HXXK2 glk1*) able to phosphorylate the glucose caused complete disappearance of the *CTT1* and *SSA3* messengers (Fig. 3). The *CTT1* messenger was somewhat more sensitive than the *SSA3* messenger (Fig. 3). However, in the case of fructose complete disappearance of the *CTT1* and *SSA3* messengers during this time period was only observed in the *hxxk1 HXXK2 glk1* strain, which can phosphorylate the fructose. In the *hxxk1 hxxk2 GLK1* strain, a strong signal remained for both *CTT1* and *SSA3* after 20 min and 60 min in the presence of fructose

Fig. 4. (a) Northern blot analysis of the expression of *SSA3* (heat shock protein Hsp70), *CTT1* (catalase T) and the ribosomal protein genes *RPL1*, *RPL25* and *RPS33* after addition of 2% glucose for 5, 10, 30 and 60 min to exponential-phase glycerol-grown cells of yeast strains (W303-1A background) either lacking both hexokinases (*hxx1 hxx2 GLK1*) or lacking both hexokinases and also glucokinase (*hxx1 hxx2 glk1*). Actin messenger levels are shown as control. (b) Quantification of Northern blot analysis of the expression of *SSA3* (heat shock protein Hsp70), *CTT1* (catalase T) and the ribosomal protein genes *RPL1*, *RPL25* and *RPS33* after addition of 2% glucose for 10, 30 and 60 min to exponential-phase glycerol-grown cells of a yeast strain (WAY.glk1-1A) lacking only both hexokinases (*hxx1 hxx2 GLK1*) or a strain (PD1501) lacking both hexokinases and also glucokinase (*hxx1 hxx2 glk1*). Actin messenger levels were used for calibration.

(Fig. 3). In a triple sugar kinase mutant (*hxx1 hxx2 glk1*) there was a significant delay in the glucose-induced disappearance of the messengers compared to the strain with an active glucokinase (*hxx1 hxx2 GLK1*; Fig. 3), which can phosphorylate the glucose (Fig. 4). This was also observed in another strain background (W303-1A) (Fig. 4). These results suggest the existence of two pathways causing repression, one pathway dependent on sugar phosphorylation and the other independent of it.

Expression of all ribosomal protein genes is tightly coupled to the growth rate. In cells growing slowly on a nonfermentable carbon source the expression is low. Addition of glucose causes, within a few minutes, a rapid 5–10-fold induction ('upshift') of the whole spectrum of ribosomal protein genes (Pearson & Haber, 1980; Kief & Warner, 1981; Donovan & Pearson, 1986; Mager & Planta, 1991). We measured the upshift of *RPL1*, *RPL25* and *RPS33* (Fig. 3). The genes *RPL1* and *RPL25* are controlled by Rap1 whereas *RPS33* is controlled by Abf1.

Addition of glucose or fructose for 20 min or 60 min to cells of the *hxx1 hxx2 GLK1* or *hxx1 HXX2 glk1* strains triggered a significant upshift in expression of the ribosomal protein genes *RPL1*, *RPL25* and *RPS33*. However, when the sugar cannot be phosphorylated (fructose in the *hxx1 hxx2 GLK1* strain) the upshift was only partial (about two- to threefold). This was also observed for the glucose-induced upshift in two different strain backgrounds in the time-course experiment shown in Fig. 4.

In stationary-phase yeast cells previously grown on YPD and then starved for nitrogen on a glucose-containing medium, the expression of *CTT1* and *SSA3* was much higher while the expression of *RPL1*, *RPL25* and *RPS33* was much lower than in cells growing exponentially on glucose (Fig. 5). This was observed both in a *hxx1 hxx2 GLK1* strain and in a *hxx1 HXX2 glk1* strain. Addition of a nitrogen source for 60 min in the presence of glucose or fructose to these strains caused disappearance of the

Fig. 5. Northern blot analysis of the expression of *SSA3* (heat shock protein Hsp70), *CTT1* (catalase T) and the ribosomal protein genes *RPL1*, *RPL25* and *RPS33* after addition of a nitrogen source [10 mM $(\text{NH}_4)_2\text{SO}_4$ and essential amino acids] for 60 min in the presence of glucose (G+NS $\rightarrow$ G+N) or fructose (G+NS $\rightarrow$ F+N) to cells starved for nitrogen on a glucose-containing medium (G+NS) of strains either lacking both hexokinases but not glucokinase (*hxx1 hxx2 GLK1*) or lacking glucokinase and hexokinase PI (*hxx1 HXX2 glk1*). The expression in the absence of sugar (G+NS $\rightarrow$ N) and in exponential-phase (ex) and stationary-phase (st) glucose-grown (G) and glycerol-grown (gly) cells is shown for comparison. Actin messenger levels are shown as control.

CTT1 and *SSA3* messengers and a strong induction of the *RPL1*, *RPL25* and *RPS33* messengers (Fig. 5). Addition of nitrogen alone also caused disappearance of the *CTT1* and *SSA3* messengers to some extent but much less than in the presence of (phosphorylated or unphosphorylated) sugar (the *CTT1* message dropped to about 52% instead of to about 7% while the *SSA3* message dropped to about 24% instead of to about 5%). For the upshift in ribosomal gene expression there was no significant difference between addition of nitrogen alone and addition of nitrogen plus unphosphorylated sugar.

DISCUSSION

The results presented in this paper show that in G0 cells starved for nitrogen on a glucose-containing medium,

none of the nitrogen-induced and glucose- (or fructose-) dependent regulatory responses is entirely dependent on phosphorylation of the sugar. In addition, fermentable-sugar-induced repression of *CTT1* and *SSA3* and upshift of ribosomal protein synthesis in cells growing on a nonfermentable carbon source also to a significant extent does not require phosphorylation of the sugar. These are the first biochemically defined regulatory responses in yeast cells which depend on the presence of a rapidly fermented sugar without the need for any metabolism of the sugar. They might be involved in the previously described physiological and cell cycle effects caused by unphosphorylated glucose (Granot & Synder, 1991, 1993). All glucose-induced regulatory responses studied up to now depended at least on phosphorylation of the sugar by one of the three known glucose-phosphorylating

enzymes (Gancedo & Gancedo, 1979; Entian, 1986; Beullens *et al.*, 1988; Becher dos Passos *et al.*, 1992; Boles *et al.*, 1993).

The lack of a requirement for sugar phosphorylation in activation of the fermentable-growth-medium-induced pathway indicates that the regulatory responses induced by this pathway are not triggered by enhanced availability of energy or biosynthetic capacity derived from the glucose. Apparently, the dependence on glucose is due to specific detection of the glucose in the medium.

We have obtained evidence previously that the glucose dependency of nitrogen-plus-glucose-induced activation of trehalase is not due to a requirement of glucose-induced activation of the Ras-adenylate cyclase pathway (Thevelein & Beullens, 1985; Thevelein, 1991; Hirimburegama *et al.*, 1992; Durnez *et al.*, 1994). This is also suggested by the present finding that there is no requirement for phosphorylation of the sugar, since glucose-induced activation of the Ras-adenylate cyclase pathway requires glucose phosphorylation (Beullens *et al.*, 1988). When glucose is added to cells grown on a nonfermentable carbon source, phosphorylation of the sugar is required for activation of trehalase (Fig. 1) and for the rapid repression of *CTT1* and *SSA3* (Fig. 3). These results suggest that glucose-induced activation of the Ras-adenylate cyclase pathway might be involved. The time-course experiments of glucose-induced *CTT1* and *SSA3* repression (Fig. 4) suggest a model in which glucose-induced, phosphorylation-dependent activation of the Ras-adenylate cyclase pathway causes a rapid effect while the fermentable-growth-medium-induced, sugar-phosphorylation-independent pathway affects the same targets but more slowly. Obviously, the two pathways together can also cause a stronger final response and not just a faster one. There has been controversy concerning the involvement of the Ras-adenylate cyclase pathway as mediator of the glucose-induced upshift in ribosomal protein gene expression (Kraakman *et al.*, 1993; Griffioen *et al.*, 1994; Klein & Struhl, 1994). Our result that the upshift is to a significant extent independent of glucose phosphorylation is in contradiction with at least exclusive mediation by the Ras-adenylate cyclase pathway, since glucose phosphorylation is required for stimulation of this pathway (Beullens *et al.*, 1988).

Up to now, nutrient control of *CTT1*, *SSA3* and several co-regulated genes has mainly been interpreted in the light of the existence of an alternative stress-induced (STRE) pathway which is different from the classical heat shock factor (HSF) controlled pathway (Kobayashi & McEntee, 1990, 1993; Wieser *et al.*, 1991; Marchler *et al.*, 1993). In this view, induction of this series of genes in stationary-phase cells is considered to be a response to 'starvation stress'. Any deviation from the optimal growth condition would lead to induction and hence the major control on expression would be exerted by a stress-controlled pathway rather than a nutrient-controlled pathway. A consequence of this model is that suboptimal nutrient conditions are in some way detected by a stress-sensing system. Our results, on the other hand, show that nutrient-induced repression of *CTT1* and *SSA3* is not a

response to termination of physiological starvation because the addition of nonmetabolizable glucose is unable to lift physiological starvation. It is clear that the presence of nonmetabolizable glucose instead of metabolizable glucose is a strong deviation from the optimal growth condition and still the cell reacts as if the optimal growth condition were present. Therefore, this result tentatively points to a nutrient-controlled pathway as the major control system, in which the presence of the nutrients is detected by specific sensing systems and the stress conditions only secondarily or even unspecifically interfere with the proper functioning of the signalling pathway. Since addition of nitrogen alone also causes partial effects, it appears that different nutrients trigger different signalling pathways which finally converge to give the full response.

It is not surprising that the upshift in ribosomal protein synthesis which occurs upon addition of a rapidly fermentable sugar to cells growing on a nonfermentable carbon source or upon addition of a nitrogen source to nitrogen-starved cells has always been interpreted in relation to the induction or stimulation of growth under these conditions (Pearson & Haber, 1980; Kief & Warner, 1981; Mager & Planta, 1991; Raue & Planta, 1991; Kraakman *et al.*, 1993; Klein & Struhl, 1994). The glucose-induced upshift in cells growing on a nonfermentable carbon source might consist of two phases, an initial rapid phase independent of metabolism and a long-term maintenance effect dependent on metabolism (Griffioen *et al.*, 1994). Our results (Fig. 4) are in agreement with this view. They also show that the initial upshift in ribosomal protein synthesis is not a consequence of a change in the cellular growth rate, causing, for example, a higher demand for protein synthesis, since unphosphorylated glucose cannot support faster growth. These data support the view that the growth rate in yeast is regulated by mechanisms independent of growth itself. Using different approaches, Ju & Warner (1994) have recently arrived at a similar conclusion.

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