Constitutive glucose-induced activation of the Ras-cAMP pathway and aberrant stationary-phase entry on a glucose-containing medium in the *Saccharomyces cerevisiae* glucose-repression mutant *hex2*

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Addition of glucose to cells of the yeast *Saccharomyces cerevisiae* growing on a nonfermentable carbon source triggers a rapid, transient increase in the cAMP level. The occurrence of this cAMP spike appears to be correlated inversely with the glucose-repression state of the cells. This was also observed for the *hex2* mutant, which is deficient in glucose repression and which displayed the cAMP signal constitutively. When cells of the *hex2* mutant were starved for nitrogen on a glucose-containing medium, they rapidly lost viability, similarly to mutants with overactivation of the Ras-adenylate cyclase pathway. Flow cytometry measurements showed that G₁ arrest of the *hex2* mutant under such conditions was incomplete. Trehalose accumulation, a typical feature of cells entering the stationary phase G₀, was very short-lived in the *hex2* mutant under the same conditions. These results are in agreement with the presence of continuous glucose-triggered activation of cAMP synthesis in *hex2* cells on a glucose-containing nitrogen-starvation medium. In the course of these experiments a spontaneous suppressor mutant, *shx* (for suppressor of *hex2*), was isolated which survived nitrogen starvation on a glucose-containing medium much better than the *hex2* strain. It also showed normal G₁ arrest and much longer accumulation of trehalose. The suppressor mutation also caused inability to grow on nonfermentable carbon sources and absence of invertase derepression, and it was epistatic to *hex2* for these characteristics also. The isolation of this epistatic derepression mutation supports the idea that the defect in glucose repression of the *hex2* mutant is the cause of its rapid loss of viability during nitrogen starvation on a glucose-containing medium. Substitution of glucose for glycerol partially abolished the rapid loss of viability in the *hex2* mutant. These results suggest that the glucose-repressible character of the pathway involved in glucose-triggered activation of cAMP synthesis might have a physiological role in preventing overstimulation of cAMP synthesis and allowing proper entrance into the stationary-phase G₀ in a medium containing ample glucose but lacking another essential nutrient for growth. Such a situation might be quite common in the glucose-rich natural environment of *S. cerevisiae*.

Keywords: yeast, signal transduction, nitrogen starvation, cell cycle, glucose-sensing

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Abbreviation: cAPK, cAMP-dependent protein kinase.

INTRODUCTION

In the yeast *Saccharomyces cerevisiae*, the synthesis of cAMP is controlled by an elaborate regulatory pathway (for recent reviews see Broach & Deschenes, 1990; Thevelein, 1991, 1992; Tatchell, 1993). The main components known in this pathway are adenylate cyclase, which is controlled by the Ras1 and Ras2 proteins (Toda *et al.*, 1985), the GDP/GTP exchange factor of the Ras proteins, Cdc25 (Camonis *et al.*, 1986), and the Ras-GTPase activating proteins, Ira1 and Ira2 (Tanaka *et al.*, 1989, 1990). cAMP causes activation of cAMP-dependent protein kinase (cAPK), which is composed of catalytic subunits encoded by the genes *TPK1*, *TPK2* and *TPK3* (Toda *et al.*, 1987b) and regulatory subunits encoded by the gene *BCY1* (Toda *et al.*, 1987a). The *PDE1* and *PDE2* genes encode the two phosphodiesterase enzymes which are responsible for cAMP hydrolysis (Sass *et al.*, 1986; Nikawa *et al.*, 1987b). Another major factor downregulating cAMP synthesis is feedback inhibition by cAMP-dependent protein kinase (Nikawa *et al.*, 1987a). By analogy with the signalling function of the Ras proteins and other G-class proteins in mammalian cells, the yeast Ras-adenylate cyclase pathway is also considered to function as a signalling pathway. However, the actual signalling function of the yeast Ras pathway is not well understood.

Only two stimuli, fermentable sugars and intracellular acidification, have clearly been identified as potent stimulators of Ras-pathway-dependent cAMP synthesis *in vivo* (reviewed by Thevelein, 1991, 1992). Glucose and related fermentable sugars cause a Ras-dependent rapid, transient increase in the cAMP level ('cAMP signal') when added to cells growing on a nonfermentable carbon source, such as glycerol or ethanol, or to stationary-phase cells deprived of glucose (Thevelein & Beullens, 1985; Mbonyi *et al.*, 1988). Intracellular acidification triggered for example by protonophore addition at low extracellular pH causes a much larger cAMP increase in the same cell types but also in cells growing exponentially on glucose (Thevelein *et al.*, 1987; Argüelles *et al.*, 1990). Glucose-induced stimulation of cAMP synthesis, on the other hand, is largely absent in cells growing exponentially on glucose. This is apparently related to the glucose-repression state of the cells (Beullens *et al.*, 1988; Argüelles *et al.*, 1990). In yeast cells growing exponentially on glucose, a large number of genes involved in respiration, gluconeogenesis, transport and catabolism of alternative carbon substrates and other processes are repressed. An extensive number of mutants deficient in glucose repression, e.g. *hex2* and *hex2*, or deficient in derepression, e.g. *cat1/snf1* and *cat3/snf4*, have been isolated and characterized (for recent reviews see Entian & Barnett, 1992; Johnston & Carlson, 1992). The glucose-repression-deficient mutant *hex2* displays a glucose-induced cAMP signal also in cells growing on glucose, while mutants unable to show derepression, *cat1/snf1* and *cat3/snf4*, do not show a glucose-induced cAMP signal either in stationary-phase cells or in cells growing on glucose. Acidification-induced cAMP synthesis, on the other hand, can be triggered in all cell types, also in the derepression mutants (Beullens *et al.*, 1988; Argüelles *et al.*, 1990).

A tentative explanation for these observations is the presence of a glucose-repressible protein in the glucose-triggered activation pathway of Ras-adenylate cyclase, upstream of the point where it is triggered by intracellular acidification (Thevelein, 1991). Such a protein, however, has not been identified yet.

The functioning of glucose as a stimulator of the Ras-adenylate cyclase pathway is consistent with the observation that upon addition of glucose to cells grown on a nonfermentable carbon source, several enzymes and processes are activated or inactivated which are known to be regulated by cAMP-dependent protein phosphorylation (reviewed by Thevelein & Hohmann, 1995). Cells growing exponentially on glucose also display the same set of phenotypic characteristics as mutants with elevated cAMP levels or cAPK activity, e.g. low trehalose and glycogen levels, low heat resistance, and low expression of heat-shock protein genes and other genes, such as *CTT1* and *UBI4*. However, if cells are starved on a glucose-containing medium for any other essential nutrient, they arrest in the G₁ phase of the cell cycle, accumulate in G₀ and acquire all properties which are typical of mutants with a low level of cAMP or low activity of cAPK in spite of the continuous presence of glucose (Lillie & Pringle, 1980; Bissinger *et al.*, 1989; Hirimburegama *et al.*, 1992; Werner-Washburne *et al.*, 1993). Apparently, under such conditions glucose is unable to stimulate cAMP synthesis or at least not to such an extent that the phenotype resembles the high-cAMP phenotype. Yeast cells that are starved on a glucose-containing medium for another essential nutrient remain glucose-repressed. Therefore, the presence of a glucose-repressible protein in the glucose-induced activation pathway of cAMP synthesis would explain the absence of stimulation of cAMP synthesis under conditions where ample glucose is present but another essential nutrient is lacking. Since it has been demonstrated that other essential nutrients are able to cause similar activation of enzymes and processes as glucose or high cAMP, but in a non-cAMP-dependent way, for instance in cells lacking the regulatory subunit of cAPK (Thevelein & Beullens, 1985; Cameron *et al.*, 1988; Belazzi *et al.*, 1991; Durnez *et al.*, 1994), we have proposed that a novel nutrient-induced pathway activates directly the free catalytic subunits of cAPK (Thevelein, 1991). This pathway, which we recently called the 'fermentable-growth-medium-induced pathway' (Thevelein & Hohmann, 1995) would be responsible for the 'high-cAPK phenotype' during growth on glucose, while the glucose-induced activation pathway of cAMP synthesis would be responsible for the stimulation of the transition from gluconeogenic/respiratory growth to fermentative growth. The function of the glucose-repressible character of the latter pathway would be to prevent stimulation of cAMP synthesis in cells which are deprived of an essential nutrient in a medium containing glucose.

In the present paper we show that the glucose-repression mutant *hex2* displays characteristics of a high-cAMP phenotype when starved for nitrogen on a glucose-containing medium, which is consistent with this idea.

The *hex2* mutant is not only deficient in glucose repression but shows a pleiotropic phenotype. One distinctive feature is its inability to grow on maltose when a constitutive *MAL* gene is present. This is apparently due to misregulation of maltose uptake (Entian, 1980). The Hex2 protein is located in the nucleus, possibly indicating a direct role in the control of transcription (Niederacher & Entian, 1991). The *hex2* mutation was also isolated as a second-site suppressor (*srn1*) of a mutation affecting RNA processing, but the mechanism involved is unclear (Tung *et al.*, 1992).

METHODS

Yeast strains and growth conditions. The *Saccharomyces cerevisiae* strains used in this study are shown in Table 1. The cells were grown in YPD (1%, w/v, yeast extract, 2%, w/v, bacto-peptone, 2%, w/v, glucose) or YPG (1%, w/v, yeast extract, 2%, w/v, bacto-peptone, 3%, v/v, glycerol) at 30 °C. For nitrogen-starvation experiments, cells were pregrown on either YPD or YPG until mid-exponential phase and transferred to a medium containing 4% (w/v) glucose or 5% (v/v) glycerol and 0.167% (w/v) Difco Yeast Nitrogen Base without amino acids and without ammonium sulfate. This medium was renewed regularly in order to prevent depletion of the glucose and/or other nutrients.

Determination of survival. To estimate survival percentages, known quantities of cells were plated on YPD. After a few days of incubation at 30 °C, the number of colonies was counted and compared to the number of cells which had been plated. The initial survival ratio was always taken as 100%.

Determination of cAMP and trehalose content, and invertase activity. For cAMP measurements, the cells were pre-incubated at 30 °C for 10 min in 25 mM MES buffer pH 6.0 at a density of 30 mg ml⁻¹. Samples were taken before and after addition of 100 mM glucose. Extraction and determination of cAMP were

performed as described previously (Thevelein *et al.*, 1987). Extraction and determination of trehalose were carried out according to Neves *et al.* (1991). Invertase activity was determined by standard methods (Goldstein & Lampen, 1975; Celenza & Carlson, 1984) and protein by the Lowry procedure.

Flow cytometry. DNA content was determined by flow cytometry using propidium iodide as a DNA stain (Popolo *et al.*, 1982).

Genetic analysis. Tetrad analysis was performed according to standard methods (Sherman *et al.*, 1986).

Reproducibility of the results. All experiments were repeated at least three times. Representative results are shown.

RESULTS

Previous work has shown that the glucose-repression mutant *hex2* displays a glucose-induced cAMP signal also in exponential-phase glucose-grown cells, as opposed to wild-type cells, which only display such a signal when grown on nonfermentable carbon sources (Beullens *et al.*, 1988). The results in Fig. 1 show that this is also true for the *hex2* glucose-repression mutant. The absence or presence of the glucose-induced cAMP signal is not related to the activity of mitochondrial respiration, which is a glucose-repressible function, since addition of 10 p.p.m. antimycin A did not impair the cAMP response (results not shown). We have chosen the *hex2* mutant for further studies on the physiological consequences of the constitutive character of glucose-induced activation of cAMP synthesis, because the sugar kinases, like hexokinase 2, are themselves also involved in the generation of the cAMP signal (Beullens *et al.*, 1988).

When cells of the *hex2* mutant and the corresponding wild-type strain were starved for nitrogen on a glucose-

Table 1. *Saccharomyces cerevisiae* strains used in this work

Strain*	Relevant genotype	Complete genotype	Source
SMC-19A	Wild-type; isogenic to SMC-1B/3	<i>MATα leu1 MAL2-8^c MAL3 SUC3</i>	K. D. Entian
SMC-1B	Wild-type; isogenic to SMC-1B/3	<i>MATα his4 MAL2-8^c MAL3 SUC3</i>	K. D. Entian
SMC-1B/3	<i>hex2</i>	<i>MATα hex2 his4 MAL2-8^c MAL3 SUC3</i>	K. D. Entian
FD11003	Revertant of SMC-1B/3	<i>MATα his4 MAL2-8^c MAL3 SUC3</i>	This work
FD11001	<i>hex2 shx</i>	<i>MATα hex2 shx his4 ura3 ΔMAL2-8^c MAL3 SUC3</i>	This work
JS.cat3-2A	<i>cat3/snf4</i>	<i>MAα leu2-3, 112 ura3-52 ade1 cat3-37 MAL2-8^c</i>	K. D. Entian
HRX.42-13C	<i>cat1/snf1</i>	<i>MATα trp1 ade1 his4 cat1-42 MAL2-8^c MAL3 SUC3</i>	K. D. Entian
YSH371	<i>cat1/snf1Δ</i>	<i>MATα leu2-3,112 ura3-52 trp1-92 snf1Δ::LEU2 SUC GAL</i>	S. Hohmann
YM1871	<i>grr1</i>	<i>MATα ura3-52 his3-200 ade2-101_{oc} lys2-80 grr1-1121 LEU::pRY181</i>	M. Johnston
YM2061	Wild-type for YM1871	<i>MATα ura3-52 his 3-200 ade2-101_{oc} lys2-801</i>	M. Johnston
YM2953	<i>grr1</i>	<i>MATα ura3-52 his 3-200 ade2-101_{oc} lys2-801 leu2-3,-112 grr1::LEU2</i>	M. Johnston
YM2169	Wild-type for YM2953	<i>MATα ura3-52 his 3-200 ade2-101_{oc} lys2-801</i>	M. Johnston

* Strains SMC-19A, SMC-1B, SMC-1B/3, FD11003, FD11001, JS.cat3-2A and HRX.42-13C are congenic. Strains YM1871, YM2061, YM2953 and YM2169 are also congenic.

Fig. 1. Glucose-induced cAMP signal in exponential-phase cells of the glucose-repression mutant *hex2* (○, ●) and the control strain (▲, △), grown either on glycerol (●, ▲) or on glucose (○, △).

Fig. 2. Viability during nitrogen starvation of the *hex2* strain (●, ○), the spontaneous suppressor mutant *hex2 shx* (▲, △), and the control strain (●, ▲), grown on glucose-containing medium (○, △) or glycerol-containing medium (●, ▲).

containing medium, viability dropped much faster in the *hex2* mutant than in the wild-type strain (Fig. 2). This rapid drop in viability was not related to the presence of mitochondrial respiration in the *hex2* mutant (as opposed to the wild-type, where it is repressed by glucose) since it was not prevented by addition of 10 p.p.m. antimycin A (results not shown). On glycerol medium, the decrease in viability was slower in the *hex2* mutant, but this was also true in the wild-type strain (Fig. 2). In the course of these experiments, a spontaneous suppressor mutation (*shx*, for suppressor of *hex2*) was isolated in the *hex2* strain which resulted in much better survival in particular during the first few days on glucose-containing nitrogen-starvation medium (Fig. 2). After a long starvation period this *hex2 shx* strain still showed somewhat lower survival than the wild-type (Fig. 2). Like the *hex2* strain, the *hex2 shx* strain

was unable to grow on medium containing maltose (not shown). Determination of starvation resistance in glycerol medium could not be performed with the *hex2 shx* strain because it was unable to grow on glycerol (see below).

Subsequently, we measured by flow cytometry the ability of the *hex2* mutant to arrest in the G_1 phase of the cell cycle upon starvation for nitrogen in a glucose-containing medium. Whereas the cells of the control and *hex2 shx* strain had shifted significantly in DNA content 4 h after the transfer and nearly completely 24 h after the transfer, the cells of the *hex2* mutant were clearly deficient in normal G_1 arrest (Fig. 3). Although the proportion of cells with a 1C DNA content had increased somewhat 24 h after transfer to nitrogen-starvation medium, the majority of the *hex2* cell population still showed a 2C DNA content. For another glucose-repression mutant, *grr1*, it has also been reported that it shows a greater loss of viability than the corresponding wild-type strain when starved for nitrogen on a glucose-containing medium (Flick & Johnston, 1991). Flow cytometry measurements with two *grr1* strains showed that both strains were also deficient in normal G_1 arrest under such conditions (results not shown). Previously, a mutant deficient in stationary-phase entry, *whi2*, was also found to be glucose-derepressed (Mountain & Sudbery, 1990).

The trehalose level in wild-type cells transferred to glucose-containing nitrogen-starvation medium showed a rapid and large increase during the initial days of starvation. Upon prolonged incubation in the same medium the trehalose was slowly mobilized again over a period of about 10–15 d (Fig. 4). In the *hex2* mutant initial trehalose accumulation was equally rapid and intensive but subsequent mobilization, on the other hand, was about as fast as the initial accumulation. Rather than taking 10–15 d as in the wild-type strain, all the trehalose was mobilized again in only 2–3 d (Fig. 4). The *hex2 shx* strain accumulated much less trehalose than the other two strains during the first few days of nitrogen starvation (Fig. 4). Subsequently, when trehalose was mobilized again, there was a good correlation between remaining trehalose level (Fig. 4) and survival percentage (Fig. 2) in the three strains.

The suppressor mutation, *shx*, which spontaneously originated in the *hex2* strain and resulted in much better survival during starvation, has been characterized further. The double mutant *hex2 shx* was unable to grow on the nonfermentable carbon sources glycerol, ethanol, pyruvate and raffinose, and it also did not grow on galactose (results not shown). While the *hex2* mutant displayed a glucose-induced cAMP signal both in cells growing on glucose and in cells harvested at the time of glucose exhaustion, in neither of the two conditions did the *hex2 shx* strain show a glucose-induced cAMP signal (Fig. 5). Pretreatment with 5 mM glucose before addition of 100 mM glucose did not change these results (not shown), indicating that the cAMP increase in the *hex2* cells harvested at the time of glucose exhaustion is not due to the combination of an initially low intracellular pH and a glucose-generated ATP increase (Argüelles *et al.*, 1990).

Fig. 3. Quantification of DNA content by flow cytometry during nitrogen starvation on glucose-containing medium of cells of the *hex2* strain, the *hex2 shx* strain and the control strain. The first peak represents cells with a 1C DNA content, the second peak cells with a 2C DNA content. Samples were taken at 0, 4 and 24 h after transfer to nitrogen-starvation medium. Upper row, exponential-phase glucose-grown cells. Middle row, 4 h, and lower row, 24 h after transfer to glucose-containing nitrogen-starvation medium.

Fig. 4. Trehalose content after transfer to glucose-containing nitrogen-starvation medium of cells of the glucose-repression mutant *hex2* (○), the spontaneous suppressor mutant *hex2 shx* (□) and the control strain (△).

Fig. 5. Glucose-induced cAMP signal in exponential-phase glucose-grown cells (■, □) and in cells harvested just after glucose exhaustion (○, □) of the glucose-repression mutant *hex2* (●, ○) and the spontaneous suppressor mutant *hex2 shx* (■, □).

Since these results indicated that *shx* might be a derepression mutation like *cat1/snf1* or *cat3/snf4*, we determined invertase activity in the *hex2 shx* mutant. In the control strain invertase activity was low in exponential phase on glucose and increased upon glucose exhaustion; in the *hex2* mutant it was high in both conditions, while in the *hex2 shx* strain it remained very low in both conditions (Table 2). These data confirmed that *shx* is a derepression mutation, epistatic to *hex2*. Complementation experiments with the *hex2 shx* strain and *cat1/snf1* and *cat3/snf4* strains showed that *shx* is a recessive mutation different from both these known derepression mutations (results not shown). Tetrad analysis of a diploid strain obtained by crossing the *hex2 shx* strain with a wild-type strain did not show a two-to-two segregation for the *shx*-conferred

inability to grow on nonfermentable carbon sources, indicating involvement of more than one mutation. Because of the difficulty in obtaining enough complete viable tetrads, further genetic analysis to determine the exact number of mutations involved was discontinued.

DISCUSSION

The absence of glucose-induced cAMP signalling in yeast cells growing on glucose could be explained in different ways: feedback-inhibition of cAMP synthesis, glucose receptor or glucose sensor desensitization, etc. The data presented in this paper provide new evidence for involvement of the glucose-repression pathway in down-

Table 2. Invertase activity in the control, *hex2* and *hex2 shx* strains

Growth phase	Invertase activity [nmol glucose liberated min ⁻¹ (mg protein) ⁻¹]		
	Control	<i>hex2</i>	<i>hex2 shx</i>
Mid-exponential phase on glucose medium (YDP)	83	753	95
Late exponential phase on glucose medium (YPD)	66	842	77
Glucose just exhausted in the same medium	454	239	131

regulating glucose-induced cAMP signalling in cells growing on glucose. The glucose-repression deficient mutant *hex2* shows constitutive glucose-induced cAMP signalling (Fig. 1) while the *shx* derepression mutation abolishes glucose-induced cAMP signalling, also in cells obtained under conditions in which wild-type cells have become derepressed (glucose just exhausted) (Fig. 5).

Downregulation of glucose-induced activation of cAMP synthesis might be important under conditions in which ample glucose is present but where growth is impaired or prevented due to shortage or deficiency, respectively, of one or more other essential nutrients. Such conditions are likely to occur in grape juice, a common natural habitat of yeast, where glucose concentrations in the range 1–2 M are typical (Bisson *et al.*, 1993). Reduction of the growth rate or entrance into the stationary-phase G_0 are well known to be associated with increases in the level of trehalose and glycogen, with enhanced expression of e.g. heat-shock proteins and catalase, and with a general increase in stress resistance (Werner-Washburne *et al.*, 1993). All these properties are counteracted by an elevated cAMP level or elevated activity of cAMP-dependent protein kinase. In addition, a high cAMP level or high activity of cAMP-dependent protein kinase also causes inability to arrest properly at the start point in the G_1 phase of the cell cycle (Matsumoto *et al.*, 1983; Toda *et al.*, 1985). Therefore, continuous glucose-induced stimulation of the Ras-adenylate cyclase pathway might be injurious to proper G_1 arrest and proper acquisition of G_0 characteristics.

Our results support this idea. Incubation of the *hex2* mutant on nitrogen-starvation medium containing ample glucose prevents proper G_1 arrest (Fig. 3) and proper maintenance of trehalose (Fig. 4), and results in rapid loss of viability (Fig. 2). A strong argument that the glucose-repression defect is responsible for the rapid loss of viability on glucose-containing nitrogen-starvation medium is the isolation of the spontaneous epistatic derepression mutation *shx* which caused much better survival (Fig. 2) without suppression of other characteristics of the pleiotropic *hex2* phenotype. Glucose-induced cAMP signalling (Fig. 5) and derepression of invertase (Table 2) were both abolished by the *shx*

mutation, supporting the connection between constitutive derepression, constitutive glucose-induced cAMP signalling and loss of viability. G_1 arrest (Fig. 3) and trehalose maintenance (Fig. 4) were also largely restored by the derepression mutation, supporting the idea that their deficiency in the *hex2* mutant was also due to the defect in glucose repression.

On glycerol medium the loss of viability was clearly reduced but this was also observed with the wild-type, where the difference between glucose and glycerol was at least as large as in the *hex2* mutant (Fig. 2). However, in addition to its glucose repression deficiency the *hex2* mutant has a pleiotropic phenotype. It also displays higher hexokinase activity. Hence, it cannot be excluded that in cells grown on a nonfermentable carbon source like glycerol the glucose-repressible protein might be present in a higher level in *hex2* cells compared to wild-type cells. Such overexpression would then abolish any relative improvement in viability the *hex2* mutant might have when grown on glycerol compared to glucose, because the higher level of the glucose-repressible protein would in this case cause overstimulation of cAMP synthesis.

In conclusion, our results suggest that the glucose-repressible character of glucose-triggered activation of cAMP synthesis serves to prevent overstimulation of the Ras-cAMP pathway under conditions in which ample glucose is present and another nutrient is restricting growth. In the natural environment of *S. cerevisiae* such conditions might be very common. Under such conditions the cells should be able to arrest at the start point of the cell cycle and to enter the G_0 phase. Arrest at start and acquisition of the physiological characteristics typical of G_0 is known to be counteracted by high cAMP levels. Glucose repression of the pathway involved in glucose-triggered activation of cAMP synthesis allows the cells to acquire all characteristics typical of cells with a low cAMP level in the presence of an excess of glucose. Our results therefore provide further support that the function of glucose-triggered activation of the Ras-cAMP pathway is limited to the transition between the gluconeogenic-respiratory and the fermentative growth mode and that

the 'high protein kinase A phenotype' of cells growing on glucose is not due to continuous glucose-triggered stimulation of the Ras-cAMP pathway. In cells growing exponentially on glucose, a novel pathway, not activated by glucose alone but rather by a complete glucose-containing growth medium, is in our view responsible for the 'high protein kinase A phenotype', presumably by direct activation of the free catalytic subunits of protein kinase A. Exhaustion of whatever essential nutrient in the medium and also exhaustion of the glucose in the medium shuts off this fermentable-growth-medium-induced pathway and at the same time results in loss of the 'high protein kinase A phenotype'. As a result both cells growing on non-fermentable carbon sources and stationary-phase cells, including those in a medium with ample glucose present but another nutrient lacking, display a 'low protein kinase A phenotype'.

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