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ROLE OF THE MITOCHONDRIAL PROTEIN SYNTHESIS IN THE CATABOLITE REPRESSION OF THE PETITE-NEGATIVE YEAST K.LACTIS

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SUMMARY: The effect of glucose in two different strains of the petite-negative yeast K.lactis is studied. The results obtained show that one strain (K.lactis CBS 2359) is glucose repressible for Glutamate Dehydrogenase and β-Galactosidase,whereas the other one(CBS 2360) is almost completely insensitive. The effect of Erythromycin on expression of catabolite repression in CBS 2359 is also analyzed. The results show that the dependence of catabolite repression on mitochondrial protein synthesis reflect the degree of interaction between the nuclear and mitochondrial compartments.

INTRODUCTION

It is known that in prokaryotes (1) as well as in simple eukaryotes,many enzymatic activities are influenced by the nature and concentration of the carbon source in the growth medium (2). In Saccharomyces cerevisiae,a petite-positive yeast,some mitochondrial (3,4) as well as cytoplasmic functions are repressed when the cells are grown at high glucose concentration (5,6). On the contrary most of the petite-negative species of yeasts are insensitive to catabolite repression of the respiratory enzymes (7,8).
Nevertheless among the petite-negative yeasts,exceptional strains are known which are repressible by glucose in respect to mitochondrial functions (9,10). It appears therefore of interest to study whether a relationship exists between the glucose effect and the petite-positive and the petite-negative property,since these appear to reflect different states of interaction between the mitochondrial and nuclear compartments (11).

In this report we describe,in particular,the effect of high concentrations

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of glucose on the respiratory activity and of some cytoplasmic enzymes of two strains of Kluyveromyces lactis, both petite-negative (12) ,which differ with respect to the effect of Erythromycin and Rifamycin (13) on the cell growth.While the ERY and RIF inhibit total protein and RNA synthesis in K.lactis strain 2360,they do not have this effect on another strain(CBS 2359). Thus these strains differ with respect to the interaction of the nuclear and mitochondrial compartments.

It will be shown that the two strains,which are both petite-negative and insensitive to glucose catabolite repression of the respiratory enzymes, exhibit different behaviour as concerns the dependence of some cytoplasmic enzymes on glucose concentration. In particular in K.lactis 2359,that ressembles S.cerevisiae in terms of the response to ERY,is glucose sensitive, whereas the other strain is virtually insensitive to the effect of glucose on cytoplasmic enzymes.

Thus it appears that both the extent of catabolite repression and the degree of sensitivity of global protein and RNA synthesis to mitochondrial inhibitors(i.e.ERY and RIF)reflect the state of the cell with respect to the interplay of the nuclear and mitochondrial compartments. Furthermore,the relationship between the compartments is subject to genic control as strains of K.lactis differ from one another in this regard.

MATERIAL AND METHODS

Strains and Culture Media. The haploid prototroph strains K.lactis CBS 2359 and 2360 were used. The cells were grown at 28°C in M medium (14) buffered at pH 6.2 with 0.06 M citrate-phosphate buffer containing glucose at different concentration as reported in results.

Assay of β-Galactosidase and Glutamate Dehydrogenase. β-D-Galactoside galactohydrolase (E.C.3.2.1.22.) is assayed as described by Cohn and Monod (15) and the unit of enzymatic activity is expressed as the amount of enzyme that releases 1 μmole of O-nitrophenol/min/mg protein at 30°C. L Glutamate:NAD oxidoreductase (E.C.1.4.1.3.) is assayed as described by Grisolia et al.(16) and the unit of enzymatic activity is expressed as the amount of enzyme that causes a change in absorbancy of 0.1 per min under the standard conditions of assay. The protein content is determined by the biuret reaction using crystallizzed egg albumin as standard (17).

TABLE 1: Effect of glucose concentration on respiratory rates

% glucose(w/v)in the growth medium	Respiration rates	
	K.lactis 2359	K.lactis 2360
0.1	0.15	0.16
0.6	0.16	0.15
1	0.15	0.15
3	0.17	0.16
6	0.15	0.18
10	0.18	0.16

1 unit= μl O_2 x min^{-1} x $(10^7$ cells$)^{-1}$

Glucose uptake. The cells,grown on buffered M medium with 0.1% glucose(w/v) with or without Erythromycin,were harvested and resuspended at the concentration of $1 \cdot 10^7$ cells/ml in the same medium(with or without ERY)at the different concentrations of glucose with a constant ratio of ^{14}C-labeled sugar. At the scheduled times 0.2 samples were collected and poured with 5 ml ice cold distilled water,filtered on cellulose millipore filter,washed twice with 10 ml of ice cold distilled water. The filters were dried and radioactivity determined in a Beckman liquid scintillation counter.
Cell respiration. Cells collected at the early stationary phase were suspended in 0.06 M phosphate buffer pH 6.2 at the concentration of $2 \cdot 10^7$ cells/ml. The respiration of cells was tested at 28°C in the presence of 0.2% glucose (w/v) in a YSI Model 53 Biological Oxygen Monitor. Respiration rates are expressed as μlitres of O_2 consumed per minute for $1 \cdot 10^7$ cells.
Determination of the Minimal Inhibitory Concentration. $5 \cdot 10^5$ cells/ml were inoculated in buffered minimal medium (pH 6.2) containing 1%(w/v)glucose or glycerol and the scheduled Erythromycin concentration. Cell counting was carried out after 48 h of aerobic growth at 28°C.

RESULTS

The respiratory rates of K.lactis CBS 2359 and K.lactis CBS 2360,measured as QO_2 of intact cells,does not change in cultures grown in media containing 0.1%, 0.6%, 1%, 3% and 10% of glucose,in agreement with the data reported by DeDeken (7-8) on the insensitivity to the catabolite repression of several petite-negative species of yeasts (Table 1).
The situation is quite different in the two strains as concerned with the glucose effect on Glutamate Dehydrogenase and β-Galactosidase,cytoplasmic

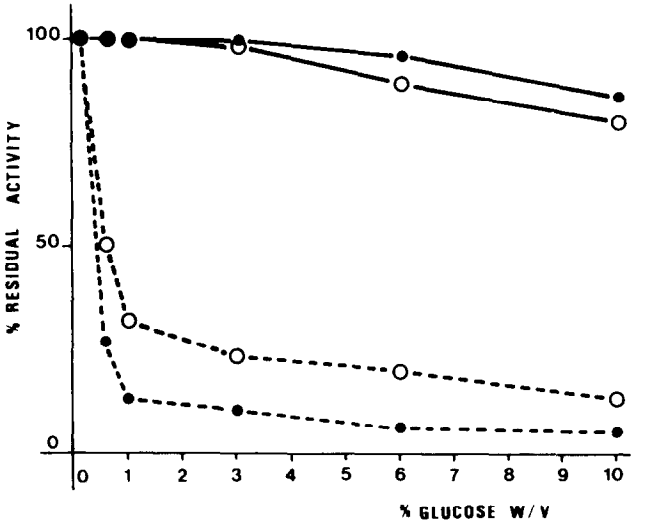

Figure 1. Effect of glucose concentration on Glutamate Dehydrogenase(●) and β-Galactosidase(O). Glutamate Dehydrogenase of K.lactis 2359(-----); Glutamate Dehydrogenase of K.lactis 2360(———);β-Galactosidase of K.lactis 2359(-----);β-Galactosidase of K.lactis 2360(———).

enzymes,as shown in Fig.1.In fact,in K.lactis CBS 2359,the activity of Glutamate Dehydrogenase of cells grown in the presence of 1% glucose is lowered to 25% with respect to the activity of the cells grown with 0.1% of glucose,and the β-Galactosidase activity of cells grown in the presence of 1% glucose is 10% of the one of cells grown with 0.1% glucose.

On the contrary,in K.lactis CBS 2360 we do not observe any variation of the activity of the two enzymes in the range between 0.1% and 3% of glucose, while the residual activity of the two enzymes is 70% when the cells are grown in the presence of 10% glucose,concentration at which the activity of the enzymes of K.lactis CBS 2359 is lowered to 5%.

In conclusion,it seems that the two yeasts behave in the same way as concerned with the catabolite repression on mitochondrial enzymes,whereas they behave in the opposite way as concerned the repression of cytoplasmic activities. Since the results obtained,reported in Fig.2,show that the glucose uptake is the same in the two strains analyzed,we can exclude that the dif-

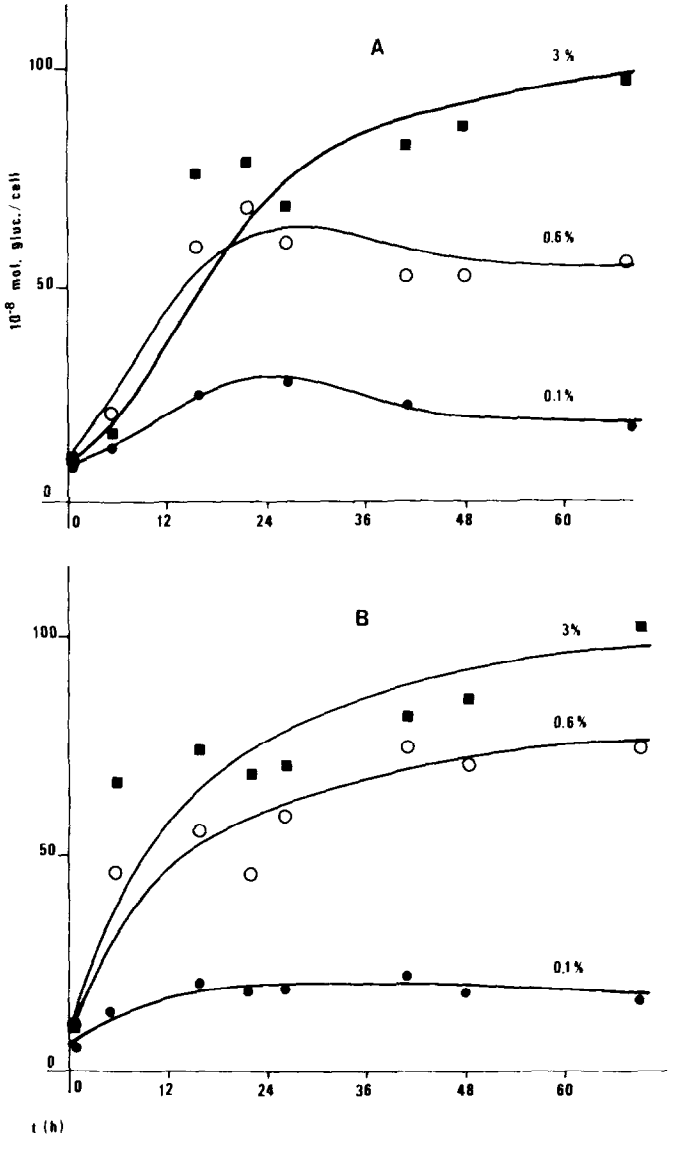

Figure 2. Uptake of ^{14}C glucose in K.lactis 2359 (A) and K.lactis 2360 (B). The concentration of glucose (w/v) are indicated in the figures.

ferent behaviour towards catabolite repression rests on a different uptake of the sugar, hence on different intracellular concentration of glucose derived metabolites.

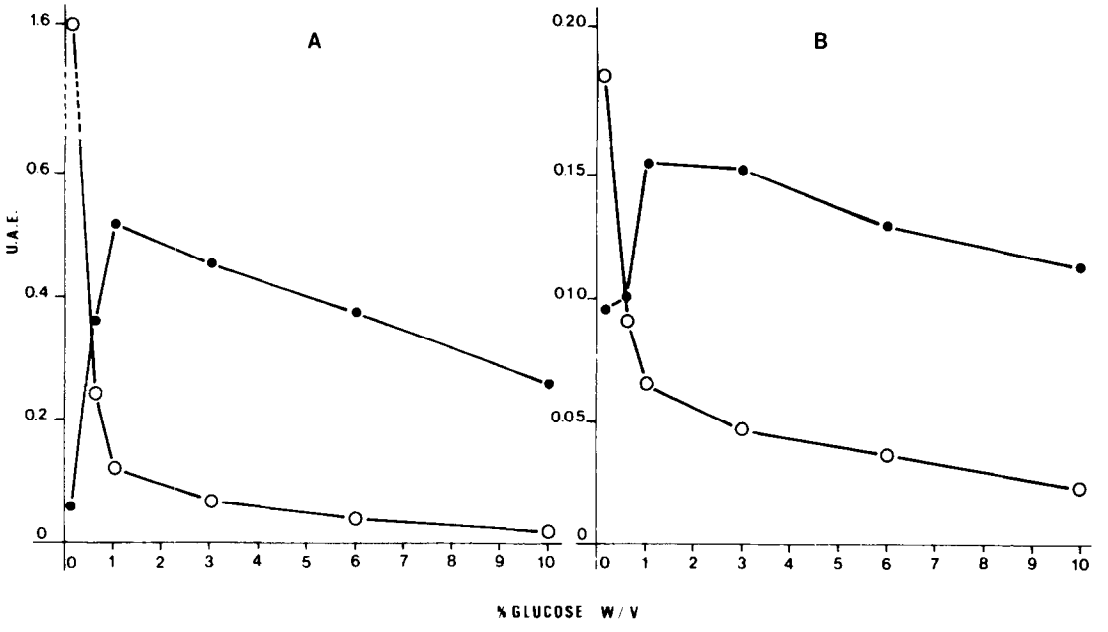

Figure 3. Effect of different concentration of glucose on Glutamate Dehydrogenase and β-Galactosidase in K.lactis 2359 grown with or without Erythromycin. A.Activity of Glutamate Dehydrogenase in cell free extracts of cultures grown in absence (O) or in presence (●) of Erythromycin (1500 g/ml). B.Activity of β-Galactosidase in cell free extracts of cultures grown in absence (O) or in presence (●) of Erythromycin(1500 g/ml).

Since in the two strains CBS 2359 and CBS 2360 mitochondrial protein synthesis plays a different role with respect to several cellular activities,we have investigated whether this parameter,i.e.catabolite repression,was influenced in some way by mitochondrial protein synthesis. The data obtained from the analysis of the effect of MPS on catabolite repression in the repressible strain are reported in Fig.3.

At low concentration of glucose the cells grown in the presence of ERY posses both Glutamate Dehydrogenase and β-Galactosidase activities lower than that of the cells grown without ERY. At higher concentrations of glucose however,the enzymatic activities are definitely higher in the presence of ERY than in its absence. We can exclude that this difference rests on different intracellular glucose concentration,since the results obtained, re-

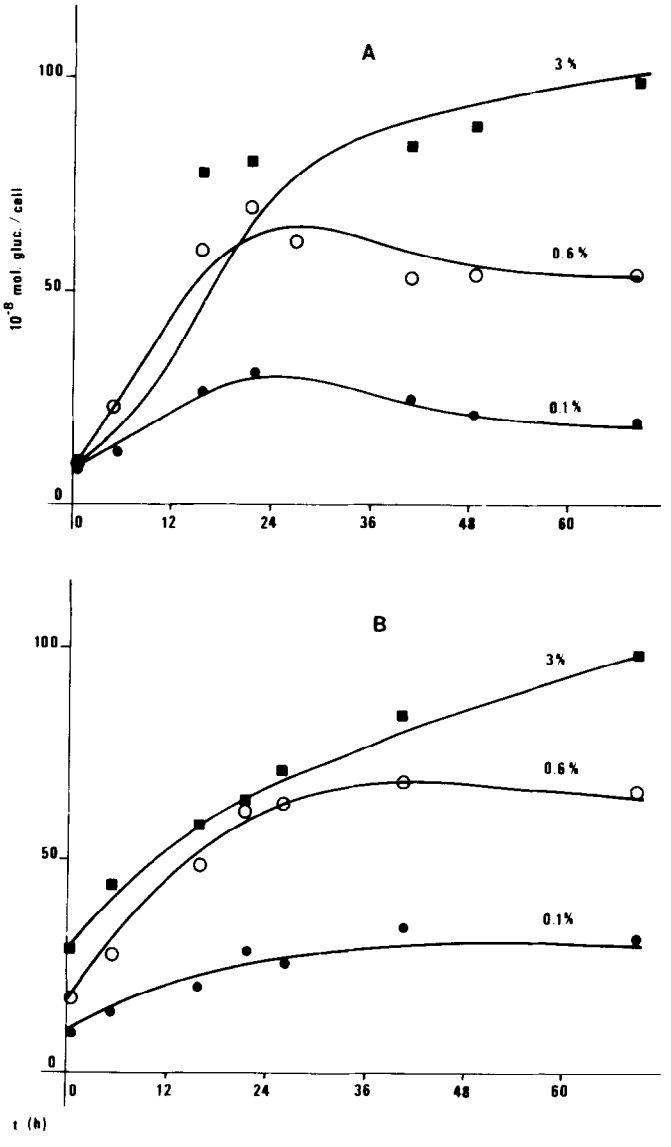

Figure 4. Uptake of ^{14}C glucose in K.lactis 2359 grown in absence (A) or in presence (B) of Erythromycin (1500 g/ml). The concentrations of glucose (w/v) are indicated in the figures.

ported in Fig.4, show that the glucose uptake is not lowered by ERY. Hence we can conclude that the establishement of catabolite repression in K.lactis CBS 2359 needs the intervention of mitochondrial protein synthesis.

DISCUSSION

In the two strains of K.lactis the weight of mitochondrial intervention is different. In one of them it conditions the cell division but to a lesser extent in the other. The degree of mitochondrial intervention thus was studied at the level of catabolite repression of cytoplasmic enzymes. In the strain in which the mitochondrial protein synthesis (MPS) conditions the cytoplasmic one,indicating hence the existence of a stronger nucleo-mitochondrial interaction,one sees that the strain is insensitive to the catabolite repression. On the contrary,in the strain that does not give rise to petite (rho^-) mutants,but in which the nucleo-mitochondrial connection is not so stringent,catabolite repression of the cytoplasmic enzymes exists and is mediated by MPS.

Thus the relationship between catabolite repression and MPS is under genetic control and reflects the different levels at which nuclear and mitochondrial compartments interact in the physiology of the cell.

The data reported above as well as previously published results indicate the existence of at least 3 possible states with respect to the interaction of the nuclear and mitochondrial compartments: 1.petite-positive and sensitive to catabolite repression (e.g.S.cerevisiae); 2.petite-negative and insensitive to catabolite repression (e.g.Schizosaccharomyces pombe and K. lactis CBS 2360); 3.petite-negative and MPS mediated catabolite repression (e.g.K.lactis CBS 2359).

In confirmation of an enormous flexibility of the system that links the nuclear and mitochondrial compartments with respect to the management of the cellular processes,the case we have reported in K.lactis seems paradigmatic.

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