EFFECTS OF TEMPERATURE ON NUCLEO-MITOCHONDRIAL INTERACTIONS IN THE YEAST SACCHAROMYCES CEREVISIAE

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Received February 27,1976

Summary: The temperature, as well as several antibacterial antibiotics could be used to differentiate mitochondrial protein synthesis (MPS) from the cytoplasmic (CPS) one in the yeast Saccharomyces cerevisiae. In fact MPS and CPS have respectively the optimum at 30°C and 36°C. A series of cellular processes, as the mitotic reproduction in presence of non-fermentable carbon sources, the synthesis of galactose pathway enzymes and the meiotic process have the same optimal temperature (30°C), whereas the growth of the wild type in presence of fermentable carbon sources and of a galactose repressor constitutive mutant (i-) have the optimal temperature of 36°C, in agreement with our previous hypothesis in which the expression of some sections of the nuclear genetic complement is dependent on regulatory functions controlled by MPS.

INTRODUCTION

It is well established that in the yeast Saccharomyces cerevisiae mitochondrial system for protein synthesis (MPS) differs in several aspects from the cytoplasmic one (CPS) (I,2,3).

We have therefore analyzed in vivo the dependence on the temperature of the above functions as well as of some biological processes which other kinds of evidence seem indicate to depend on the activity of MPS (4,5).

Our results show that the optimal temperature for MPS in vivo is 30°C and for CPS is 36°C .

MATERIAL AND METHODS

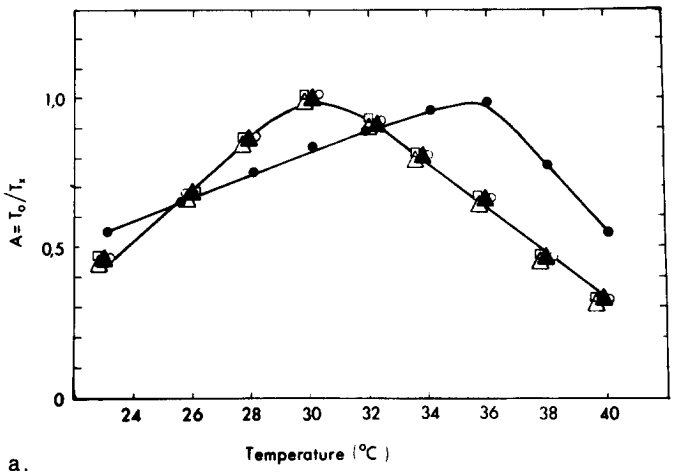
Yeast strains . S. cerevisiae 5300 mat I-A/B prototroph, and 5352/7d mat I-A, ura_3, ilv_1, i^- (galactose constitutive).

Culture Media. The following culture media have been used:

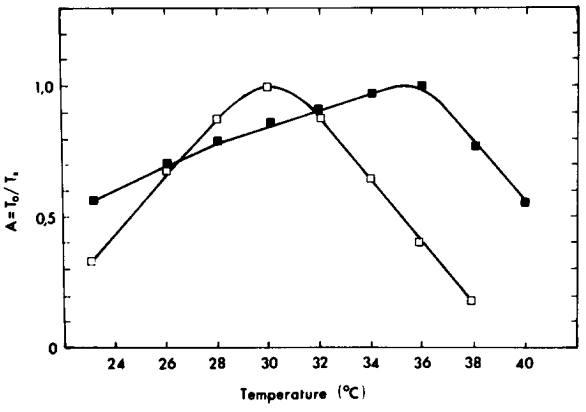
-Complete medium: M (6) with glucose 0,6% (W/V), or glycerol 2% (W/V) or ethanol 2% (V/V) or galactose 2% (W/V) as carbon sources.

- Sporulation medium: 23DHA (6).

For the description of the strains we have used the nomenclature indicated by M.E. Plischke, R.C. von Borstel, R.K. Mortimer and W.E. Cohn in "Genetic markers and associated gene products in Saccharomyces cerevisiae."



a.



b.

Fig. 1a - Optimal temperature for the growth of S. cerevisiae strain 5300 in presence of different carbon sources.
(●—●) glucose; (○—○) galactose; (□—□) glycerol; (▲—▲) lactate; (△—△) ethanol.
Growth rate A = To/Tx is indicated as the ratio between the division time at the optimal temperature (To) and the division time at the different temperatures indicated (Tx).

Fig. 1b - Optimal temperature for the growth with galactose as carbon source of : (□—□), S. cerevisiae 5352/7d; (■—■) S. cerevisiae 5352/7d; galactose constitutive (i-).

Division time. The cultures were grown overnight at 28°C on alternating shaker in the complete medium with 0,2% glucose as carbon source.

The samples, collected by centrifugation (3500xg for 10'at room temperature) at the beginning of the stationary phase, were resuspended in fresh medium supplemented with the carbon sources indicated in Fig. 1a and 1b and kept at the scheduled temperatures for the determination of the division time.

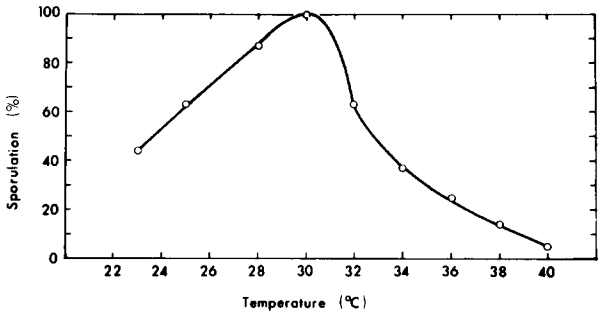


Fig. 2 - Optimal temperature for sporulation of S. cerevisiae strain 5300.

Sporulation. Cells of the strain 5300, grown as previously described until to a final concentration of 4.10^7 cells/ml were collected by centrifugation (3500xg for 10' at room temperature), resuspended at the concentration of 2.10^7 cells/ml in the sporulation medium and incubated at the different temperatures indicated in Fig. 2.

The effect of the temperature on the degree of sporulation was estimated and expressed as the percentage of the sporulated cells at the optimal temperature.

Determination of cytoplasmic and mitochondrial protein synthesis in vivo. The cells, grown as previously described, were assayed for cytoplasmic protein synthesis at different temperatures (see Fig. 3a,b) by adding 0.5μCi/ml of $1^-(^{14}C)$ aminoacids mixture (specific activity 52mCi/mA). The radioactive material incorporated in the cytoplasmic proteins was determined by Millipore filter tecnique as decribed by Bilinski and Jachymczky (7) and by Kennel (8).

Mitochondrial protein synthesis was estimated as described by Schweyen and Kaudewitz (6) at the temperature indicated in Fig. 3a, b.

The mitochondrial radioactive materials, adsorbed to Millipore filters (0.25μ), were solubilized by keeping overnight the samples in 1 ml of soluene 350^R.

Each filter was dissolved in 10 ml of Toluene based scintillating mixture (0,5% of 2.5 - Diphenyloxazole and 0.05% of p-bis (O-Methylstryryl - Benzene) in Toluene and the radioactivity was determined in a Beckman spectrometer LS 100.

RESULTS AND DISCUSSION

I - Optimal temperature for MPS and CPS in vivo.

As shown in Fig.3a and 3b the optimal temperature for MPS and CPS in vivo are significantly different.

CPS: the incorporation of ^{14}C - labeled aminoacids in Erythromycin inhibited cells shows a two-fold increase between 25° and 30°C, achieving its maximal value at 36°C (optimal temperature) and decreases at negligible value at 45°C.

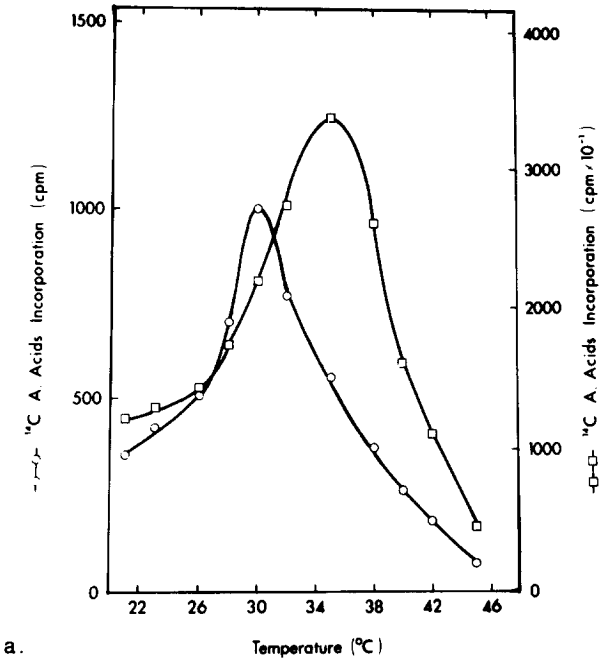


Fig. 3a - Optimal temperature for CPS (-□-□-) and MPS (-○-○-) in the diploid S. cerevisiae strain 5300

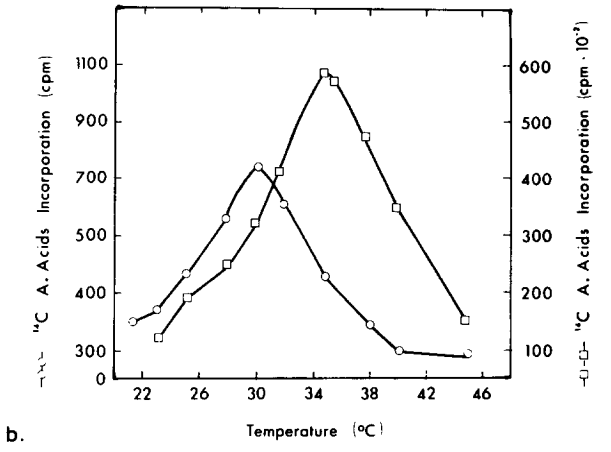


Fig. 3b - Optimal temperature for CPS (-□-□-) and MPS (-○-○-) inhaploid S. cerevisiae strain 5352/7d.

MPS: the incorporation of ^{14}C - labeled aminoacids in the Erythromycin sensitive fraction increases rapidly from 20°C to 30°C (optimal temperature), decreasing at negligible value at 45°C.

Therefore, the optimal temperature seems to be another parameter that differentiates MPS from CPS *in vivo*, in addition to the sensitivity to some antibiotics such as Erythromycin, Cloramphenicol and Tetracyclines (10,11,12).

2 - Optimal temperature for reproduction of S. cerevisiae

a) Fermentable carbon sources

As shown in Fig. 1a the optimal temperature for the mitotic reproduction of *S. cerevisiae* with glucose as sole carbon source is 36°C, whereas the optimal temperature for the growth with galactose is 30°C.

Since glucose and galactose are both fermentable carbon sources, this difference in the optimal temperature could be explained on the basis of one of the following hypothesis:

I- The optimal temperature for the activity *in vivo* of one of the enzymes belonging to the Leloir pathway for the galactose catabolism is 30°C.

2- The optimal temperature for the synthesis or the activity of a regulative function of the inducible system for the synthesis of the "galactose enzymes" is 30°C.

3- The optimal temperature for the synthesis or the activity of a function not belonging to the *gal* system but whose presence is needed for its expression, as in the case of CPR for catabolite repression sensitive system (13,14), is 30°C.

We have then determined the optimal temperature for the adaptation and growth with galactose of a mutant constitutive for *gal* system, wich carries a mutation in the gene coding for the repressor (i^-) (5).

As shown in Fig. 1b, the optimal temperature for the growth of the constitutive strain is 36°C, coincident with the optimal for glucose utilization.

This observation discharges the hypothesis 1) and 3) and agree with the hypothesis 2).

b) Non fermentable carbon sources

As shown in Fig.1a, the optimal temperature for the growth in presence of non-fermentable carbon sources as glycerol, lactate and ethanol, is the same (30°C) and coincides with the optimal temperature for the growth of an inducible strain with galactose as carbon source.

The fact that the galactose constitutive (i^-) strain, wich grows with galactose as carbon source in respiratory deficient condition and in presence of an inhibitor of MPS as Erythromycin has the optimal growth temperature at 36°C, as well the observation that the induction of galactose system is blocked in respiratory deficient condition and is sensitive to the above antibiotic (5), could be explained assuming that the inducible strain grows at 30°C insofar as enzyme induction dependes on MPS, whose optimum lies at 30°C, whereas the constitutive one could grow at 36°C, since the mutation in the repressor (15) make the regulatory system indipendent from the activity of MPS.

Sporulation . In a previous paper we have attributed the failure of heterozygous mat IA/B yeast to sporulate in the respiratory deficient conditions or in presence of Erytromycin, to the dependence at the commitment to sporulation on regulatory functions controlled by the mitochondrial protein synthesis (4).

We have then determined the optimal temperature for sporulation, which, as shown in fig. 2, is 30°C, which coincides with the optimal temperatures for MPS in the diploid strain utilized (Fig.3a).

In conclusion the fact that the optimal temperature for (i) the utilization of the galactose in an inducible strain and ii) the sporulation in the heterozygous mat IA/B diploids coincides with the optimal temperature for MPS, whereas a constitutive mutant of the gal system has an optimal temperature for the growth in presence of galactose coincident with the optimal temperature for CPS, is in agreement with our previous hypothesis (5) in which the induction of the gal system, as well as the sporulation process, are mediated by regulatory factors whose activity and/or synthesis depends on the mitochondrial protein synthesis.

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