REGULATION OF GLYCOLYTIC ENZYME BY PHOSPHORYLATION IN *SACCHAROMYCES CEREVISIAE*, PFK2 AND TREHALASE CASE

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Introduction. Fructose 2,6-bisphosphate (F26BP) plays a key role in the regulation of carbohydrate metabolism in yeast. In this context the properties and the control of enzymes synthesizing and degrading F26BP are of great interest. F26BP is specific produced from fructose-6-phosphate (F6P) and ATP by phosphofructo kinase 2 (PFK2) and degraded in F6P and P_i by F26BPase and alkaline phosphatase. cAMP-dependent protein kinase A (PKA) activates PFK2 which is deactivated by a protein phosphatase (PP2A)[1,2]. Similarly, PKA and PP2A act on Trehalase (TRE) which transforms trehalose into two glucose molecules. The reversible covalent modification of proteins by protein kinases and phosphoprotein phosphatases is recognized as a major regulatory process in several microorganisms. Unlike protein kinases, phosphoprotein phosphatases appear to dephos-phorylate a broad spectrum of enzymes. Their regulation can occur by competition between various phosphoprotein subs-trates for the same enzyme, non-competitive interaction of a protein modifier and the specific interaction of ligands with the phosphoprotein substrates of the enzyme[3]. The aim of this work is to attain an improved understanding of this regulatory mechanism through mathematical modeling of the activation via phosphorylation and dephosphorylation of PFK2 and TRE.

Methodology. Intracellular concentrations of cAMP, F26BP, ATP and PFK2 activity were measured by Vaseghi[4]. TRE activity was measure by Guerrero-Martín as proposed[1]. Both activities were measured during a glucose perturbation in a glucose-starved cell sample of culture in steady state of *S. cerevisiae*. The enzyme concentration in cell was determinated using 2D-electrophoresis[5]. The mathematical model was integrated using ACSL version 11. Parameter estimation was performed using OpdesX2.0.4 through simulated.

Results and discusion. The production of active PKA and PP2A through cAMP was balanced considering mass conservation laws. This is the first step to activate PFK2 and TRE. The maximal rates of PFK2* and TRE* producing reaction were a function of the concentration of the active form from PKA and PP2A,

$$r_{enz} = f(C_{PKA^*}, C_{PP2A^*}) \cdot \frac{C_{enz}}{K_{m,enz} + C_{enz}} \cdot \frac{C_{ATP}}{K_{m,ATP} + C_{ATP}}$$

The degrading rate was considered to follow Hill-type kinetics with respect to PFK2* and TRE*, respectively. Competitive inhibition of PP2A and PKA was observed to affect the maximal rate in both cases. This indicates that PKA and PP2A compete for the same P_i site. PFK2 phosphorylation can be partially explained by the cAMP signal as shown in Fig. 1A. However, the dephosphorylation is probably regulated by an additional mechanism different from the cAMP signal. TRE* dynamical behavior could be better approximated than that of PFK2P (Fig. 1B). The cAMP concentration during the pulse experiments is drawn in Fig. 1C. Our results agree with the findings of other authors[1,2].

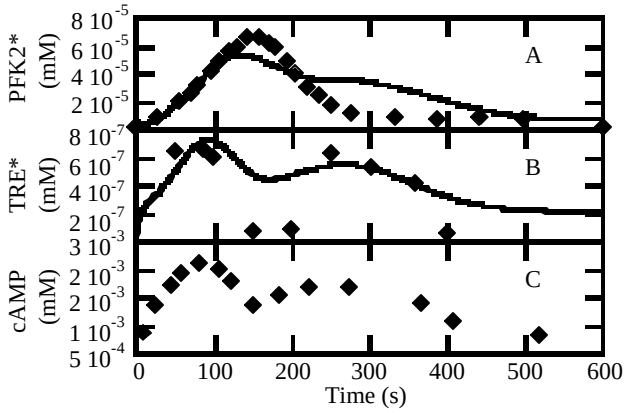

Fig. 1. Simulation of PFK2P (A) and TREP (B) concentration. cAMP concentration (C). (?) Measured data, (-) simulate.

Conclusions. Our results show that the PFK2* and TRE* dynamics during a glucose pulse can be adequately described by accounting for the role of PKA and PP2A in controlling the activation state of them via phosphorylation-dephosphorylation. Evidence has been obtained that a further regulatory mechanism may act upon the dephosphorylation of PFK2.

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