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Regulation and control of energy coupling at the cellular level

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1. Introduction

One can look at a cell as a complex network of metabolic reactions, that are coupled via common intermediates and also via interactions that involve modulation of the activities of the participating enzymes. Key coupling intermediates in metabolism in the soluble phase are the adenine and nicotinamide nucleotides. For reactions occurring at or in the (intra)cellular membranes ionic gradients and potentials play a role in the coupling. The metabolic network is regulated so as to respond appropriately to variations in conditions and work load. This involves quantitative fine tuning of numerous reaction fluxes.

Consequently, to understand the metabolic functioning of intact cells, one must know quantitatively how the whole network depends on the activities of the enzymes and the concentrations of available substrates [1,2]. In this report, we describe how segments of metabolism can be analyzed in such a quantitative way. As a representative system, we chose one of the main pathways of metabolism: the uptake of glucose and its conversion via glycolysis, in Enterobacteriaceae and the yeast *Saccharomyces cerevisiae*. The mechanism and regulatory properties of this pathway have been relatively well established in Enterobacteriaceae. This knowledge serves as starting point for the studies in yeast.

2. Transport and signalling in enterobacteriaceae

In Enterobacteriaceae the PEP:glucose phosphotransferase system (PTS; in this communication I refer only to glucose, although there are many PTS sugars) catalyzes the uptake of its cognate substrate, glucose. Its components are also directly involved in the regulation of metabolism and transport of other carbon substrates (for review see Ref. [3]). The inhibition by glucose (or another rapidly metabolized sugar) of metabolism of other substrates has been called catabolite repression. The mechanism of this has been solved partly in Enterobacteriaceae [3].

The PTS consists of a number of proteins that transfer a phosphate group from PEP to the membrane-embedded component (Enzyme $IICB^{Glc}$), which ultimately transfers the phosphate group to glucose. The entry of glucose is coupled obligatorily to its phosphorylation.

It appears that the degree of phosphorylation of one of the phosphate-transferring components of the PTS, Enzyme IIA^{Glc}, has an important function in the regulation of metabolism: the non-phosphorylated form inhibits a number of other uptake systems, by binding directly to them. One example is the lactose permease. Whenever glucose is present in the medium, the active functioning of the PTS will lead to (partial) dephosphorylation of Enzyme IIA^{Glc} and, through its binding to the lactose permease, inhibition of lactose uptake. This explains why the cells use glucose in preference over lactose. Importantly, this mechanism allows for the exclusion of inducers of many operons encoding catabolism of substrates, other than glucose. This phenomenon has been called 'inducer exclusion'.

On the other hand, the phosphorylated form of Enzyme IIA^{Glc} is thought to activate adenylate cyclase, thereby indirectly increasing the transcription of a number of operons, that encode enzymes that metabolize other substrates than glucose [3]. Thus, when glucose is actively taken up, the change in degree of phosphorylation of Enzyme IIA^{Glc} will simultaneously

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inhibit uptake of other metabolites (inducers) and inhibit transcription of the operons that they induce. Conversely, when glucose is absent, Enzyme IIA^{Glc} will be phosphorylated and, via cAMP, stimulate the synthesis of metabolic operons for other substrates.

It is to be expected that catabolite repression and inducer exclusion depend critically on the relative amounts and activities of the proteins involved. This expectation has been verified in some cases [for instance, 4]. We have undertaken a study of the quantitative control by the different enzymes of the PTS on the rate of metabolism and its regulation. We found that the control by Enzyme $IICB^{Glc}$ on the process of uptake of glucose is significant, but is small for a more complex system, such as glucose oxidation, as expected [5]. During these studies, we discovered that the control in a group-transfer system may be higher than expected [6]. Thus, it has become important to determine the control by each of the enzymes of the PTS. Such a project is presently under way in our laboratory (Van Der Vlag, unpublished data).

3. Transport and signalling in *Saccharomyces cerevisiae*

The system of glucose uptake in *S. cerevisiae* has not been defined as clearly as in Enterobacteriaceae. Although in the early days it was postulated that also in yeast there is a PTS-like system, i.e., a mechanism of direct coupling between transport and phosphorylation of glucose [7], this idea has been abandoned by most investigators. Nevertheless, there appears to be a close connection between the glucose uptake system and the enzymes involved in glucose phosphorylation [8,9].

One of the problems in quantitative studies of metabolism of glucose in *S. cerevisiae* has resided in the assays for uptake activity. Already within a few seconds, which is the time scale used in most uptake assays, it had been found that most of the sugar that had been taken up was converted to sugar phosphates. Thus, the measured rate of label incorporation seemed to be determined both by the activity of the transport step and the activity of the sugar kinases. To be able to distinguish between the control by transport and phosphorylation activities on glucose metabolism, we improved the method for the measurement of sugar uptake.

We developed a method by which metabolism in yeast cells can be stopped within 10 ms, by quench-flow into methanol at −40°C [10]. This method does not disrupt the cell membrane and, therefore, the cells can be washed extensively in methanol to remove external substrates. After addition of chloroform, the intracellular metabolites can be released at neutral pH. This procedure allows for the measurement of intracellular labile metabolites on a very short time scale.

We compared the kinetics of glucose uptake in *S. cerevisiae* derived from the classical 5 s uptake assay and from 0.2 s rapid uptake measurements. We found that there was not much difference if cells were used that were taken directly from a growing culture. The affinity for glucose and the maximal rate of its uptake were the same with both methods.

However, if we compared the kinetics of glucose uptake by the two methods in cells that had been starved and incubated with cyanide, we found significant differences. It turned out that the rate of glucose uptake leveled off after a relatively short time (0.2 s). Thus, the 5 s measurement underestimated the rate of glucose uptake. A plausible explanation for this is the depletion of ATP, caused by the preincubation under starvation conditions. This illustrates how the activity of the kinases (through the ATP level) can influence the apparent activity of the glucose uptake, if measured over 5 s.

The phenomenon of catabolite repression, discussed above for Enterobacteriaceae, is also present in yeast [11,12]. Thus, in the presence of glucose, the synthesis of a number of enzymes (among which respiratory chain complexes and gluconeogenic enzymes) is inhibited, whereas synthesis of others is stimulated. It is tempting to take the Enterobacteriaceae as an example in studying the mechanism of this signalling effect.

A number of proteins have been suggested to play a role in the signalling by glucose in yeast. One candidate for the signalling molecule is the sugar kinase [8]. This idea is based on the observation that mutants in specific domains in the sugar kinases lack (some of) the glucose signalling phenomena.

Another candidate for a central glucose signalling molecule is the so-called General Glucose Sensor (GGS), described by Thevelein and co-workers [13]. Mutants in this protein lack (most of) the glucose signalling phenomena. Furthermore, a *ggs* deletion mutant can not grow on glucose in batch culture. Addition of glucose to such a mutant, grown on another substrate, leads to the accumulation of sugar phosphates in the cells to high concentrations. Thevelein [13] proposed that an imbalance between uptake and further metabolism is the cause of the growth defect in the *ggs* mutants: the transport and the kinase allow entry and phosphorylation of glucose at such a rate that all intracellular phosphate is scavenged, inhibiting further flux. This is reminiscent of the early experiments of Harden and Young, who found that in yeast extracts the rate of glucose conversion decreased after some time, due to the fact that all available phosphate was bound in the form of sugar phosphates [14].

Surprisingly, it turned out that the *ggs* gene codes for one of the subunits of trehalose phosphate synthase [15]. This observation led Thevelein to propose that the

synthesis and breakdown of trehalose is in some way involved in the homeostasis of inorganic phosphate in the yeast cells [13].

If the above model is correct, *ggs* mutants should grow if glucose entry were attenuated. This is precisely the condition that prevails in a chemostat. Indeed, we could maintain a *ggs* deletion mutant in a chemostat culture at a low growth rate for several weeks. The steady-state glucose concentration and the rapid kinetics of glucose uptake in these cells were very similar to those of a wild-type *S. cerevisiae*.

Thus, glucose uptake and phosphorylation appear to be 'normal' in a *ggs* deletion mutant. The difficulty in the mutant to cope with an excess of glucose indicates that in these cells an imbalance between uptake plus phosphorylation relative to further metabolism occurs, possibly as the result of lack of some feedback control on the uptake step. In this context it may be noted that Blazquez et al. recently reported that trehalose phosphate is an inhibitor of hexokinase [16].

Once the components, involved in the initial steps of glucose uptake and signalling in *S. cerevisiae* have been identified, their relative importance for control of glucose metabolism should be evaluated. This can be done by modulating their expression, as has been done for the components of the PTS in Enterobacteriaceae (Ref. [5]; Van Der Vlag, unpublished data).

4. Dynamics

The importance of a proper balance between uptake and further metabolism of glucose in *Saccharomyces cerevisiae* can be further illustrated in the phenomenon of glycolytic oscillations. If to a suspension of starved yeast cells glucose is added, followed by cyanide, a train of oscillations in intracellular NADH is observed [17,18]. This phenomenon has been studied extensively in yeast extracts [19]. In the case of intact yeast cells, we found that the duration of the train of oscillations depends critically on the stage of growth at which the cells are harvested [20]. Sustained oscillations are observed if the cells are harvested at the time of transition between growth on glucose and growth on ethanol. It was shown that this transition coincides with a transition in the kinetics of glucose uptake from a low- to a high-affinity state [21].

Based on the known properties of glycolytic oscillations in cell extracts, it is to be expected that a critical balance between the activities of the uptake system and other activities in the cells is required for sustained oscillations to occur. This could be further rationalized by mathematical models of glycolysis, which also show that oscillations can occur in certain regions of kinetics of ATP synthesis and breakdown [22,23].

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