Exposing a complex metabolic system: glycolysis in Saccharomyces cerevisiae

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CHAPTER 6

THE DANGER OF METABOLIC PATHWAYS WITH TURBO DESIGN

6.1 Summary

Many catabolic pathways begin with an ATP-requiring activation step, after which further metabolism yields a surplus of ATP. Such a 'turbo' principle is not only useful but also contains an inherent risk. This is illustrated by a detailed kinetic analysis of a paradoxical S. cerevisiae mutant that fails to grow on glucose due to an over-active initial part of glycolysis, and is defective in an apparently unrelated enzyme, trehalose 6-phosphate synthase. The ubiquity of activated pathways in living systems suggests that there may be many more genes which cause rather paradoxical regulation phenotypes when deleted, i.e. growth defects caused by enhanced utilization of growth substrate.

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6.2 Introduction

A strategy followed in many catabolic pathways is that the substrate is first ‘activated’ in a reaction that requires ATP. In glycolysis, ATP is invested at the hexokinase and phosphofructokinase steps; in (α and β) fatty acid oxidation, ATP is invested for the formation of fatty-acyl-coenzyme A; and in active transport systems, ATP is invested to get the substrate inside the cell. This investment is subsequently recovered by the generation of a surplus of ATP further downstream in the pathway (Fig. 1A). A feasible reason for these activation steps is that it helps to make these and the subsequent steps in the pathway thermodynamically downhill [26]. The principle of the turbo engine is similar: exhaust gases are fed back to enhance the fuel input step (Fig. 1B). Such a ‘turbo’ design of catabolic pathways has been suggested to be the result of evolutionary optimisation with respect to flux, at least for glycolysis [142, 241].

Biologically one may expect the turbo principle to be most useful when there is a continuous supply of substrate, as is the case in multicellular organisms that maintain internal homeostasis. In many other (micro-)organisms, however, where the availability of substrate can be highly variable, such a turbo design may be a mixed blessing. In this paper we suggest that the striking phenomenon of substrate accelerated death, which has been observed in both prokaryotes and in eukaryotes such as Saccharomyces cerevisiae, may be the consequence of such a turbo design. Substrate-accelerated death has been observed when cells were subjected to excess of the substrate that previously limited growth in continuous culture [60, 278]. In (wild-type) yeast, substrate-accelerated death has been observed for maltose [279], but not for glucose (see, e.g. [306]), suggesting that there are specific regulatory mechanisms preventing lethal effects of sudden availability of glucose.

Genetic studies in S. cerevisiae have generated highly paradoxical mutants (fdp1 [370], cif1 [252], byp1 [51, 166]), which were unable to grow on high concentrations of glucose, even though the first steps in glycolysis seemed to be activated, rather than impaired. These mutants
therefore appear to be defective in a regulatory mechanism that should prevent glucose-accelerated death in yeast. Using a mathematical model of yeast glycolysis, a likely explanation of the phenotype of these particular mutants in *S. cerevisiae* is presented. Unexpectedly, the turbo design of yeast glycolysis was central to the metabolic problems of these mutants. Our analysis shows that metabolic pathways with turbo design require special types of regulation in environments with rapidly changing substrate availability.

6.3 The phenotype of the *tps1Δ* mutant

The *fdp1/cif1/byp1* mutants were unable to grow on glucose. It was a surprise that the mutations were allelic [367] and that the primary lesion was in the gene *TPS1*, which encodes trehalose 6-phosphate synthase [22, 363, 380]. Until that moment, trehalose synthesis was conceived of as a branch of glycolysis with no function other than the formation of storage carbohydrates and the acquisition of stress tolerance [273]. Very little trehalose is made during exponential growth on glucose [103]. Consequently, it seems puzzling that trehalose metabolism is needed for growth on glucose.

A *tps1* disruptant accumulates hexose phosphates, whereas ATP and inorganic phosphate are consumed rapidly [165, 167, 252, 367, 368, 370]. No steady state is attained, and the accumulation of hexose phosphates continues until all phosphate has been incorporated into sugar phosphates. It appears that the first steps of glycolysis are too fast for the rest of metabolism to cope with [363].

It is now clear that the metabolic action of Tps1 is to inhibit (one of) the first steps of glycolysis, and thereby to restrict the glucose influx into glycolysis [363]. Growth on glucose can be restored in a *tps1* mutant by reduction of the hexokinase activity [167] or reduction of the glucose transport activity [227]. The finding that trehalose 6-phosphate (Tre6P), the metabolic product of Tps1, inhibits hexokinase *in vitro*, may offer a direct mechanism [38], but it cannot be excluded that Tps1 interacts directly with a glucose transporter and hexokinase [165, 361, 363]. In this paper we do not want to discuss the exact molecular mechanism, but rather address the question of why and when the design of the

![Figure 2. Schematic representation of the core model of glycolysis. See Appendix I for details on the kinetics of the steps.](image)
glycolytic pathway demands a ‘guard at the gate of glycolysis’ [363] for instance in the form of Tps1, in order to function properly.

6.4 Simulation of glycolysis without feedback inhibition of hexokinase

First we examined the behavior of a core model of yeast glycolysis without any special regulation on the first ATP-consuming step. To focus on the essentials, this model consists of only four steps, i.e., hexokinase (HK), phosphofructokinase (PFK), the lower part of glycolysis, and a general ATPase to remove the excess ATP produced by glycolysis (see Fig. 2; for details of the stoichiometry and enzyme kinetics, we refer to Appendix I). In figure 3A-B, a time course is shown for the model lacking a guard at the gate of glycolysis at a high concentration of glucose (i.e. high relative to the affinity of the hexokinase block for glucose). Without the feedback inhibition on HK, the hexose monophosphates (HMP) and fructose 1,6-bisphosphate (F16bP) started and continued to accumulate, and ATP barely recovered from its initial drop to below 0.3 mM (Fig. 3A). No steady state was reached for the hexose phosphates; they continued to accumulate. However, the rates of the reactions became almost constant (Fig. 3B), and so did the ATP level.

The ATP level remains constant, whenever the rate of ATP consumption \( v_{\text{consumption}} \) is equal to the rate of ATP production \( v_{\text{production}} \). In the model, this means:

\[
\begin{align*}
\v_{\text{consumption}} &= \v_{\text{HK}} + \v_{\text{PFK}} + \v_{\text{ATPase}} \\
\v_{\text{production}} &= 4 \v_{\text{lower glycolysis}}
\end{align*}
\]  \( (6.1) \)

Fig. 3A confirms that this was the case: after some ten minutes, the rates of the reactions fulfilled the above relationship, and therefore, ATP became constant (Fig. 3A).

However, the system will only reach a true steady state when (i) the rate of the HK reaction, that of the PFK reaction and that of the lower part of glycolysis are the same, and (ii) the rate of the ATPase is twice the rate of these other steps:

\[
\v_{\text{HK}} = \v_{\text{PFK}} = \v_{\text{lower glycolysis}} = 2 \v_{\text{ATPase}}
\]  \( (6.2) \)

This second steady state condition was not met in the simulation of unguarded glycolysis, i.e. the rate of the lower part of glycolysis did not become equal to that of the hexokinase and PFK module. Rather the kinase fluxes greatly exceeded the flux through lower glycolysis: \( \v_{\text{HK}} > \v_{\text{PFK}} > \v_{\text{lower glycolysis}} \) (Fig. 3B), causing the accumulation of both HMP (\( \v_{\text{HK}} > \v_{\text{PFK}} \)) and F16bP (\( \v_{\text{PFK}} > \v_{\text{lower glycolysis}} \)) (Fig. 3A). These results reproduced the phenotype that is observed experimentally when a yeast \( \text{tps1} \Delta \) mutant is given high amounts of glucose, especially the accumulation of hexose phosphates at constant ATP concentration (see [165, 227] for clear examples of this phenotype).
Time simulation of the core model for three different cases: unguarded glycolysis without feedback on hexokinase (A-B), glycolysis with product inhibition of hexokinase (C-D), and an unguarded glycolysis with reduced hexokinase activity (E-F).
6.5 A guard at the gate of glycolysis helps

In Figure 3C-D, guarded glycolysis is simulated. The guard is modelled as an inhibition of the hexokinase module by HMP (see Appendix I for details). For \textit{S. cerevisiae}, this regulation would involve Tps1. The introduction of such feedback inhibition was effective in restoring a steady state at the high glucose concentration. The hexose monophosphates, F16bP and ATP concentration reached constant levels, and the ATP level was considerably higher than that in the unguarded glycolysis (Fig. 3A). The rates of HK, PFK and the lower part of glycolysis did become equal, and that of the ATPase became twice that rate. The model with regulation of the hexokinase module was therefore able to reach a complete steady state at high glucose levels, as does a wild-type yeast cell.

It has been shown that deletion of hexokinase PII can suppress the \textit{tpslA} phenotype in \textit{S. cerevisiae} [167], and that reduction of glucose transport can do the same in \textit{Kluveromyces lactis} [227]. In figure 3E-F, rather than introducing feedback inhibition, the activity of the hexokinase module was reduced to about 20% of its wild-type level. This reduction of the hexokinase-module activity led to restoration of a complete steady state (i.e., both the concentration of ATP and of the hexose phosphates being constant in time), as did the introduction of feedback inhibition.

We conclude that the calculations confirm that feedback regulation on the gate of glycolysis is necessary at high glucose concentrations to achieve a complete and viable steady state.

6.6 Why does glycolysis go awry?

What exactly goes wrong in unguarded glycolysis? In the absence of feedback inhibition from hexose monophosphates to the hexokinase module, the rest of metabolism can only communicate with the HK module via the ATP concentration. A detailed analysis of the ATP-dependency of the model's four modules is carried out in Appendix II. In summary, it shows that the hexose phosphates are not at steady state because of the absence of a regulatory feedback between upper and lower of glycolysis other than ATP. Due to the turbo design of glycolysis, there are two requirements for a steady state to be achieved: the ATP production and consumption should be equal (see Eq. 6.1) and the rates of the enzymes of glycolysis should be the same (and twice that of the ATPase, see Eq. 6.2). The latter condition implies the former, but not vice versa. It is apparent that in the absence of feedback regulation on HK (as in the case of the \textit{tpsl} mutant), the ATP concentration is tuned as to fulfill the former condition. ATP as sole feedback regulator is ineffective because it becomes trapped at a concentration that tunes ATP production to ATP consumption (see Fig. 4B). Being trapped in that 'ATP-steady state', it can no
longer serve to tune hexose phosphate synthesis to hexose phosphate degradation, as to fulfill the second condition. A wild-type yeast cell does have the required extra feedback mechanism in the form of Tps1. The case of reduced HK activity (Fig. 3E-F) demonstrates that such a feedback mechanism is only required when the capacity of the first (ATP-driven) reaction exceeds that of the later ATP producing reaction.

6.7 Why did yeast evolve feedback of the HK module by Tps1, and not by direct product inhibition of hexokinase?

The action of Tps1 is to inhibit the hexokinase module, either by trehalose 6-phosphate inhibition or some interaction of Tps1 with the HK module. In the model, inhibition by trehalose 6-phosphate was used and a quadratic relationship between the concentration of HMP and that of Tre6P was employed (see Appendix I). One may wonder whether the feedback now indirectly carried out by Tps1 via Tre6P might also be achieved by direct product inhibition of hexokinase by HMP, which is common in many mammalian systems. One important difference between mammalian cells and yeast cells is the relatively constant glucose concentration encountered by the former as compared to the latter. It may be that regulation by Tps1 has specifically evolved to cope with sudden and large variations in the extracellular glucose concentration. This possibility is illustrated in Table I, where the behaviour of four versions of the model was calculated at a 100 times lower extracellular glucose concentration than used in Figs 3 and 4. Because of the low glucose concentration, all models reached a steady state, irrespective of the presence of feedback inhibition on the HK module. The steady-state concentrations of ATP, HMP and F16bP, and the steady-state flux through the modules were calculated for unguarded glycolysis, glycolysis with reduced HK activity and glycolysis with two types of feedback on the HK module: via Tre6P or directly via HMP.

The model representing the tps1Δ mutant (unguarded glycolysis) showed a higher steady-state glycolytic flux and a higher steady-state ATP level compared with the other models. In the model with reduced HK activity, the rate of ATP consumption was always higher than that of the ATP production (due to the activity of the ATPase), and ATP rapidly decreased to zero. The fluxes were therefore zero as well. In the model with Tre6P-mediated feedback inhibition of hexokinase, which ensured a steady state at high glucose concentrations (Fig. 3B-C), the flux and steady-state ATP concentration were about half those of the model of the tps1 deletant. If, however, direct feedback inhibition by HMP was used rather than inhibition via Tre6P, (in such a way that the same steady state would have been reached at the high extracellular glucose concentration), the model performed much worse than the Tre6P inhibition model: the ATP level and the flux through glycolysis were now less than a tenth of those of the tps1 deletant (Table I). This is explained as follows: to achieve the same inhibition by Tre6P or HMP at high glucose
concentrations, the inhibitory constant of HMP needed to be much smaller than that of Tre6P (see the legend of Table I), and this stronger inhibition would then be a liability at the low glucose concentration.

<table>
<thead>
<tr>
<th>Model</th>
<th>[HMP] (mM)</th>
<th>[F16bP] (mM)</th>
<th>ATP (mM)</th>
<th>$v_{HK}$ (mM min$^{-1}$)</th>
<th>$v_{PFK}$ (mM min$^{-1}$)</th>
<th>$v_{lower}$ (mM min$^{-1}$)</th>
<th>$V_{ATPase}$ (mM min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$tps1_\Delta$</td>
<td>2.1</td>
<td>0.3</td>
<td>0.5</td>
<td>4.7</td>
<td>4.7</td>
<td>4.7</td>
<td>9.4</td>
</tr>
<tr>
<td>reduced HK</td>
<td>0.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tre6P inhibition</td>
<td>1.4</td>
<td>0.2</td>
<td>0.3</td>
<td>2.75</td>
<td>2.75</td>
<td>2.75</td>
<td>5.5</td>
</tr>
<tr>
<td>HMP inhibition</td>
<td>0.8</td>
<td>0.02</td>
<td>0.04</td>
<td>0.45</td>
<td>0.45</td>
<td>0.45</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Table I. Steady-state concentrations and rates for different models at an extracellular glucose concentration of 0.1 mM, rather than 10 mM. The direct feedback inhibition of HK by HMP is set as to reach the same steady state as for Tre6P inhibition at the high glucose concentration of 10 mM. The $K_i$ of HK for Tre6P was therefore 11.06 times larger than for HMP (as, rather than $[\text{Tre6P}] / K_i,\text{Tre6P} = [\text{HMP}]^2 / K_i,\text{HMP}$, one has the term $[\text{HMP}] / K_i,\text{HMP}$. The concentration of HMP was 11.06 mM in the steady state of Fig. 2C).

The inhibition via an external effector introduces an extra layer of regulation (i.e. regulation of the regulator Tre6P by the HMP concentration, and possibly by other factors), thereby increasing the dynamic range of the inhibition under highly variable environmental conditions. As a consequence, a regulatory mechanism is obtained which limits the turbo effect at high but not at low glucose concentrations. This then may be the reason why indirect feedback through Tps1 is preferred over feedback through direct product inhibition. It may be noted that expression of $TPS1$ is glucose repressed [103, 403] and its activity controlled by post-translational modification [272]. The dynamic range of Tps1 is therefore not only enlarged by the specific kinetics of the enzyme, but also on the level of gene expression and specific activity.

It is interesting that the conditions under which trehalose metabolism is important coincides with the conditions under which the danger of the turbo design of glycolysis is potent, i.e. in glucose-derepressed cells with an uninduced lower part of glycolysis. It appears that Tps1 has evolved to fulfil a dual function, i.e. trehalose formation under glucose limitation and feedback regulation at subsequent sudden exposure to glucose excess.
6.8 The danger of pathways with turbo design

It is important to note that the problems that we have analyzed in this paper are quite specific for pathways with what we have called a turbo design. If, in transitions where substrate becomes suddenly available in excess, there are bottlenecks further down in the pathway, then turbo-pathways are especially vulnerable. As the coupling to ATP hydrolysis renders the activating step thermodynamically irreversible, regulation of the rate of that step cannot occur at the level of mass action [159]. Some regulation is needed on the level of enzyme activity. Whereas adaptation to the new environment via regulation of gene expression and protein turnover will take minutes to hours, accumulation of intermediates will occur in seconds to minutes. Therefore, it is expected that kinetic signals, and not adaptive ones, are required to signal dangerous bottlenecks upon a sudden transition to substrate availability. These fast kinetic signals allow the slower adaptive responses (which could also remove existing bottlenecks) time to take effect. In S. cerevisiae, therefore, glucose induction of the lower part of glycolysis goes hand in hand with glucose repression of the Tps1.

One might think that in the short term the ATP level should be a good and sufficient kinetic signal, as the first step uses it as a substrate. A step that is driven by ATP hydrolysis should be considered an ATPase, and if it proceeds too fast, the resultant decrease in ATP should automatically slow down the step. No additional regulation is needed, it is just a matter of sufficient sensitivity of the first step for ATP. However, increasing the sensitivity of HK for ATP in the model by decreasing its affinity for ATP from 0.15 mM to 5 mM did not prevent the accumulation of hexose phosphates whilst the ATP level became constant (at 2.7 mM; results not shown).

It is here that our analysis has pointed to the specific danger that pathways with turbo design face: the fact that the steps after the activated one produce a surplus of ATP, can lead to a state in which ATP consumption and ATP production are equal, even when the flux through the carbon skeleton is not in steady state (cf. Eq. 6.1 and 6.2). Thus, accumulation of intermediates may continue at a constant level of ATP, and the ‘trapped’ ATP concentration can therefore not be used to signal the failure of the rest of the pathway. This phenomenon has been observed in the tps1Δ mutant of S. cerevisiae, but is a general danger for activated pathways when the activity of the activated (ATP-consuming) step is in excess over that of the ATP generating steps. Indeed, the substrate accelerated death of wild-type S. cerevisiae grown under maltose limitation and subsequently pulsed with excess maltose, was attributed to an unrestricted uptake of maltose, while the ATP level remained relatively constant [279]. In yeast maltose is actively taken up by a proton-symporter [335] and can therefore be considered an ATPase, with glycolysis (considered as one module) producing at least four ATP molecules per maltose transported. Furthermore, substrate-accelerated death in bacteria is associated with a repression-derepression mechanism [61, 62], also pointing to poor (or too slow) adaptation to a
suddenly changed environment. Another good example is 'lactose-killing', which occurs when *E. coli* cells grown in a lactose-limited continuous chemostat are plated on lactose plates: 85-98% of the cells die, due to excessive uptake of lactose [90]. In *Trypanosoma brucei*, compartmentalisation of glycolysis into the glycosome overcomes the problem caused by the turbo design of glycolysis: inside the glycosome glycolysis proceeds until 3-phosphoglycerate, which is then transported into the cytosol, where it yields ATP via the pyruvate kinase reaction. There are therefore two pools of ATP, and the part of glycolysis that occurs in the glycosome invests as much (glycosomal) ATP in the hexokinase and phosphofructokinase step as it produces in the phosphoglycerate kinase reaction. Indeed, no feedback regulation of hexokinase has been found [16]. A kinetic model of the Trypanosomes' glycolysis [14], in which the compartment boundaries were removed and hence the turbo design of glycolysis restored, showed accumulation of hexose phosphates to irrealistically high levels (B.M. Bakker, personal communication).

The danger of the accumulation of intermediates can only effectively be cleared by some feedback inhibition of that ATP-activated step by another means than the ATP concentration. Direct product inhibition may work for cells in organisms with extracellular homeostasis or constant substrate supply, but may not have the dynamic range that may be required for others that live (or have evolved) under highly variable environmental conditions (Cf. Table 1). Substrate-accelerated death may therefore be related to the absence of specific regulatory mechanisms to prevent the turbo design of activated pathways to demand its toll before proper adaptation to the new environment has been achieved. The ubiquity of activated pathways suggests that there may be quite a few genes around that will lead to paradoxical phenotypes when deleted, *i.e.* growth defects at improved metabolic rates, due to (sudden) substrate availability.

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### 6.9 Appendix I: Description of the kinetic model

We have constructed a core model of glycolysis, consisting of hexokinase (HK), phosphofructokinase (PFK), the lower part of glycolysis and a general ATPase consuming the produced ATP (Fig. 2). The independent metabolites in this model are ATP, the hexose monophosphates (HMP, *i.e.* glucose 6-phosphate + fructose 6-phosphate) and fructose 1,6-
bisphosphate (F16bP). The model therefore comprised a set of three ordinary differential
equations (cf. Fig. 2):
\[
\frac{d[HMP]}{dt} = v_{HK} - v_{PFK}
\]
\[
\frac{d[F16bP]}{dt} = v_{PFK} - v_{\text{lower glycolysis}}
\]
\[
\frac{d[ATP]}{dt} = 4v_{\text{lower glycolysis}} - v_{HK} - v_{PFK} - v_{\text{ATPase}}
\]

Square brackets indicate the concentration of the metabolite, \( v \) is the activity of the enzyme.

HK represents both the transport step and the hexokinase. It exhibits irreversible
Michaelis Menten kinetics with two substrates, glucose (Glc) and ATP:

\[
v_{HK} = \frac{[\text{Glc}] \cdot [\text{ATP}]}{K_{Glc} \cdot K_{ATP}} \left( 1 + \frac{[\text{Glc}]}{K_{Glc}} \left( \frac{[\text{Tre6P}]}{K_{l,\text{Tre6P}}} \right) \left( 1 + \frac{[\text{ATP}]}{K_{ATP}} \right) \right)
\]

Where the term in square brackets was introduced only for the wild type model with Tre6P-
dependent feedback inhibition on the HK module (see below). The parameter values where:

\( V_{\text{HK}} = 68 \text{ mM min}^{-1} \)
\( K_{Glc} = 1 \text{ mM} \)
\( K_{ATP} = 0.15 \text{ mM} \)
\( K_{l,\text{Tre6P}} = 4.422 \text{ mM} \)

The kinetics of this module [332] are supposed to be dominated by HK, i.e. it is assumed
that the glucose transporter does not exert a significant control on the flux through this module
at the high glucose levels employed [357] (chapters 4 and 5). The HK is taken to be insensitive to
its products (product inhibition by G6P is very poor [378]).

Complete irreversibility and product insensitivity are simplifications made in the model to
enable a rigorous analysis in terms of rate characteristics of the participating enzymes (see
Appendix II). Including reversibility and weak product sensitivity in the model of unguarded
glycolysis leads to some high constant HMP level at which mass action and the weak inhibition
becomes significant (see chapter 6; cf. Fig. 3A), but it would not alter the essence of the
metabolic problem, i.e. accumulation of hexose phosphates at constant ATP level. For sake of
analytical power, we have not considered these kinetic details in our core model.

Although the precise molecular mechanism for the action of Tps1 is unclear, it is clear
that Tps1 negatively affects the activity of the HK module, either via competitive inhibition of
HK with respect to glucose [38], or via the formation of a complex involving both the transporter
and HK [363]. We have here used the competitive inhibition model, because the latter model is difficult to describe in terms of a rate equation. G6P is one of the substrates and F6P is an activator of Tps1 [223, 380]. Rather than introducing a new variable in the model, we have used a simple quadratic relationship between the Tre6P concentration and that of HMP: 

\[ [\text{Tre6P}] = [\text{HMP}]^2 \]

PFK is modelled as a two-substrate Monod-Wyman-Changeux enzyme [245], using the rate equation from [155] (also used in [108]) with HMP and ATP as its substrates. It has been modified to describe ATP inhibition rather than AMP activation, as AMP is not considered in the model. Other regulatory interactions of PFK have also not been included for sake of simplicity. The rate equation for PFK was:

\[ v_{\text{PFK}} = V_{\text{PFK}} \frac{g_r \cdot \lambda_1 \cdot \lambda_2 \cdot R}{R^2 + L \cdot T^2} \]  

(A6.3)

with:

\[ \lambda_1 = [\text{HMP}] / K_{R, \text{HMP}} \]  
\[ \lambda_2 = [\text{ATP}] / K_{R, \text{ATP}} \]  
\[ \lambda_3 = [\text{ATP}] / K_{i, \text{ATP}} \]  

R = 1 + \lambda_1 + \lambda_2 + g_r \lambda_1 \lambda_2 \]  
T = 1 + c_1 \lambda_1 + c_2 \lambda_2 + g_r c_1 \lambda_1 \lambda_2 \]  
L = L_0 \cdot \left( \frac{1 + c_i \lambda_3}{1 + \lambda_3} \right) \]

and parameter values:

\[ K_{R, \text{HMP}} = 1 \text{ mM} \]  
\[ K_{R, \text{ATP}} = 0.06 \text{ mM} \]  
\[ K_{i, \text{ATP}} = 10 \text{ mM} \]  
\[ g_r = 10 \]  
\[ g_t = 1 \]  
\[ c_i = 0.0005 \]  
\[ c_1 = 0.0005 \]  
\[ c_2 = 1 \]  
\[ L_0 = 1000 \]

The lower part of glycolysis, from aldolase to alcohol dehydrogenase, is also grouped into one module. The expression of most enzymes in this module, especially PDC, is glucose-induced [66, 326], suggesting that adaptation in this module is required to switch from gluconeogenesis to glycolysis. The important assumption in this model is that, at the moment of glucose addition, the capacity of the lower part of glycolysis is smaller than that of HK and PFK. Therefore, the lower part of glycolysis is modelled to follow Michaelis Menten kinetics with F16bP and ADP as substrates, and its \( V_{\text{max}} \) is set to be lower than that of PFK and HK:
\[ v_{\text{lower glycolysis}} = V_{\text{lower}} \cdot \frac{[\text{F16bP}] [5-\text{ATP}]}{K_{\text{F16bP}} K_{\text{ADP}}} \left( \frac{1}{1 + \frac{[\text{F16bP}]}{K_{\text{F16bP}}}} \right) \left( \frac{1}{1 + \frac{[5-\text{ATP}]}{K_{\text{ADP}}}} \right) \] (A6.4)

with \( V_{\text{lower}} = 20 \text{ mM min}^{-1}, K_{\text{F16bP}} = 1 \text{ mM} \) and \( K_{\text{ADP}} = 0.1 \text{ mM} \). The ADP concentration was taken as a function of the ATP concentration, with \([\text{ADP}] + [\text{ATP}] = \text{constant} = 5 \text{ mM} \).

The general ATPase is also described by Michaelis-Menten kinetics, with ATP as the substrate. The ATP-dependency of the general ATPase in our model is justified by experimental results of Buttgeriet et al., who showed that the rates of ATP-consuming processes depend on the availability of ATP [57]. Although the kinetics of such a general ATPase are necessarily oversimplified, its details do not affect the conclusions of this paper (results not shown). The rate equation for the ATPase module was:

\[ v_{\text{ATPase}} = V_{\text{ATPase}} \cdot \frac{[\text{ATP}]}{K_{\text{ATP}} + [\text{ATP}]} \] (A6.5)

with \( V_{\text{ATPase}} = 68 \text{ mM min}^{-1} \) and \( K_{\text{ATP}} = 3 \text{ mM} \).

The models were run on a personal computer, using the metabolic modelling software SCAMP [315].

6.10 Appendix II: How can ATP be at steady state whereas the hexose phosphates are not?

Fig. 3 shows that feedback inhibition on the HK-module or reduction of its \( V_{\text{max}} \) allowed glycolysis to achieve a steady state. How can this be understood? In Fig. 4A the rate characteristics of the model's four modules with respect to ATP are shown, at saturating concentrations of HMP and F16bP (as they are very high in the \text{tps}1\Delta \text{ mutant}). HK and ATPase show Michaelis-Menten hyperbola with a \( K_{\text{m}} \) for ATP of 0.15 mM and 3 mM, respectively (see Appendix I). PFK is only very slightly inhibited by ATP at the saturating F6P concentration, whereas the lower part of glycolysis is indirectly sensitive to ATP, as the sum of ATP and ADP is set constant (to 5 mM) in the models (see Appendix I). The lower part of glycolysis is taken to follow the rate of PFK, up to the \( V_{\text{max}} \) of the former (20 mM min\(^{-1}\)). From Fig. 4A it should be concluded that the only complete steady state where the rate of HK, PFK and the lower part of
glycolysis were equal, would be at an ATP concentration of zero. This is not the state that is reached in the model (unless the initial state was very close to this state, see also [13]).

The energetic implications of Fig. 4A are shown in Fig. 4B, where the rates of HK, PFK and the ATPase of Fig. 4A are summed into the total rate of ATP consumption, and the total rate of ATP production was equated to four times the rate of the lower part of glycolysis (cf. Eq. 6.2). There is an intersection point of the two curves at [ATP] > 0, where ATP production equals ATP consumption and therefore, ATP remains constant. (This ATP-'steady state' is stable, as an increase of ATP leads to a higher consumption than production of ATP, with a decrease of ATP as a result.) At this 'ATP-steady state', however, the rates of the HK, PFK and the lower part of glycolysis are not the same (Fig. 4A), and therefore no true steady state is achieved (see Eq. 6.2). This explains the observed patterns in metabolites: the only way for the lower part of glycolysis to slow down either PFK or HK is via the ATP level, but the ATP level is trapped in an ATP-'steady state', and accumulation of sugar phosphate cannot but continue; ATP cannot vary so as to cause the rates of HK, PFK the lower part of glycolysis and the ATPase to achieve a 'carbon-steady state' as well.

Figures 4C and D, show a similar analysis for the wild-type situation, i.e., with feedback inhibition of HK. The ATP dependence of the rates is now analysed at the steady-state level of HMP and F16bP (Fig. 4C). The rate of the lower part of glycolysis again follows the rate of PFK up to the \( V_{\text{max}} \) of the former. A flux control analysis [98, 144, 178, 180, 396] of the steady state of Fig. 3C-D showed that HK controlled the flux by 40%, PFK by 34%, and the ATPase by 25%. (A flux control of 50% by some enzyme means that an increase of the activity of that enzyme by 1% would lead to an increase of the steady-state flux by 0.5%.) The lower part of glycolysis exerted no control on the flux, which justifies its dependence on the rate of PFK. One may note that the total flux control is 100%.

As can be seen in Fig. 4D, as a consequence of the lowered rates of hexokinase and phosphofructokinase, there is a steady state for ATP at a higher ATP level. Importantly, at this ATP level (and of course at the corresponding HMP and F16bP levels) the rates of HK, PFK and the lower part of glycolysis are the same, and each is half the rate of the ATPase, compatible with a complete steady state (Eq. 6.2). Inhibition of PFK by ATP is crucial in this case, as it decreases the PFK activity to a level with which the lower part of glycolysis can cope.

In Fig. 4E and F, finally, the analysis is done for the case where HK activity was reduced to 22% of the wild-type level. HK completely controls the flux (its flux control amounted to 114%, that of the ATPase to -14%; the flux control coefficients of PFK and the lower part of glycolysis were 0%). Therefore, the rates of PFK and the lower part of glycolysis follow the rate of HK, until ATP becomes inhibitory at [ATP] > 1.7 mM. The reduced activity of HK causes the flux through hexokinase to be (just) sufficiently small to allow the lower part of glycolysis to follow, and a true steady state is reached. In this case, therefore, the need to regulate the HK activity is relieved by removing its excess capacity.
Figure 4. Rate characteristics of the steps of the core model with respect to the ATP concentration. See the text for explanation.
The energetic requirements of Fig. 4A are stated. The ATPase of the F$_1$-F$_0$ complex of ATP synthase is increased to four times the rate of the hydrolysis rate of the F$_1$-F$_0$ complex of ATP synthase. ATP production was equal to four times the rate of the hydrolysis rate of the F$_1$-F$_0$ complex of ATP synthase. There is an intersection point of the two curves. At this point, ATP synthesis is increased. At this point, ATP production leads to a higher concentration of ATP, with a decrease of ATP as a result. At this point, the steady state is achieved, the rates of the F$_1$, F$_0$, and the lower part of glycolysis are not the same as in Fig. 4A, and therefore the true steady state is achieved (see Eq. 4.2).

This explains the observed differences in metabolic states. The only way for the lower part of glycolysis to stay sharp after F$_1$ and F$_0$ are activated, but the ATP level is trapped as an ATP pool that is not used in ATP synthesis. ATP cannot vary as long as the rate of F$_1$, F$_0$, and the lower part of glycolysis are not the same. Therefore, the ATP pool becomes unstable in the absence of F$_0$. The ATP levels are not stable and the ATP levels do not oscillate as a result.

In Fig. 4B, the dynamics of glycolysis are shown. The ATP level (and of course the ADP level) is increased to four times the rate of glycolysis. The lower part of glycolysis with a constant ATP pool is shown. The glycogen is broken down for the synthesis of ATP through F$_1$. The ATP level is not affected in this case.