ANAEROBIC ENERGY METABOLISM IN
YEAST AS A SUPPLY-DEMAND SYSTEM

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A century ago Eduard Buchner (1897) made the remarkable discovery that a cell-free soluble fraction of yeast could still convert glucose to ethanol, in other words, cell-free systems can still metabolize. Now, a hundred years later, we have a highly detailed picture of the glycolytic pathway, its enzymes and the mechanisms that regulate them. Can we claim a full understanding of what controls the carbon flux through glycolysis and the role of the mechanisms that regulate it? Reading any modern biochemistry textbook one gains the distinct impression that the answer is yes. In a nutshell, the story [as told in Stryer (1995), a popular representative of the genre] proceeds by identifying hexokinase, phosphofructokinase, and pyruvate kinase as potential sites of control, due to the fact that they catalyse “essentially irreversible reactions”; then all the “regulatory properties” of these enzymes are described in terms of allosteric effects, covalent modification by phosphorylation, and transcriptional control to cover regulation in the full time-scale from seconds to hours. Phosphofructokinase, being the true committing step of glycolysis, is identified as the most important “pacemaker” and regulatory enzyme, responding both to energy charge through the ATP/AMP ratio and to biosynthetic precursors. The other two kinases, hexokinase and pyruvate kinase, however, “also set the pace of glycolysis”.

This description sounds familiar and is deemed satisfactory by many because it conforms fully to what we shall call the “classical” view of metabolic regulation, where fluxes are controlled by “rate-limiting” steps which, in turn, are regulated by one or more of a number of molecular mechanisms. It usually seems taken for granted that increases in the activities of one or more of the rate limiting steps
of glycolysis would lead to an increase in flux to ethanol. Tests of this prediction have been made possible by the development of recombinant-DNA technologies that allow the quantitative manipulation of the *in vivo* enzyme concentration profile of cells.

Appropriately, one of the first of such tests was done on yeast glycolysis. Schaff *et al.* (1989) over-expressed eight different enzymes of the *Saccharomyces cerevisiae* glycolytic and fermentative pathway by placing their genes on multicopy vectors; in so doing they were able to increase the specific enzyme activities between 3.7 and 13.9-fold. They over-expressed these enzymes singly or in pairs (phosphofructokinase/pyruvate kinase or pyruvate decarboxylase/alcohol dehydrogenase) and measured the effect on the rate of ethanol production and the level of a number of metabolites in logarithmically growing cultures. In terms of the prevailing view that glycolytic flux-control resides in glycolysis itself the results were surprising: under no circumstances did increases in the activities of the different glycolytic enzymes significantly affect the rate of ethanol production ("and therefore the flux through glycolysis") or affect the concentrations of "key" metabolites in comparison to the wild type.

If flux-control does not reside in the "rate-limiting" enzymes, where does it reside? Schaff and colleagues conclude that their data support the view of metabolic control analysis (Kacser *et al.*, 1995; Heinrich and Rapoport, 1974) that the control of flux is shared among all enzymes of a metabolic system, a concept for which in general there is now ample evidence (Fell, 1996). What is not clear is what they regard as their "metabolic system". Those trained to take the classical view would in all probability regard only the glycolytic pathway as their system and argue that even if flux-control does not reside in one or even in a few glycolytic enzymes it must still be shared among all the glycolytic enzymes, the implication being that if one simultaneously over-expressed all the enzymes of glycolysis one would theoretically expect the glycolytic flux to increase proportionally. Transport of glucose into the cell should of course also be considered part of the glycolytic apparatus; in fact, it has been suggested that substantial control on glycolytic flux is exerted by the uptake systems (Galazzo and Bailey, 1990; Bisson *et al.*, 1993).

In the remainder of this chapter I shall argue for another possibility, namely that *steady-state glycolytic flux is controlled by reactions outside of what has traditionally been regarded as glycolysis, more specifically, it is controlled by those cellular processes that consume the key product of glycolysis, ATP*. Furthermore, I shall argue that this is fully
in accordance with the widely-accepted view that the properties of metabolic systems have evolved to fulfill one or more functions.

YEAST GLYCOLYSIS AS PART OF AN ATP SUPPLY-DEMAND SYSTEM

Where does a metabolic pathway begin and where does it end? We instinctively draw boundaries around pathways in order to simplify description of the metabolic tangle on the charts that adorn many a classroom and laboratory wall. The traditional textbook description of a metabolic pathway such as fermentative glycolysis in yeast is based on the net reaction

\[ \text{glucose} + 2\text{ADP} + 2\text{P}_i \rightarrow 2\text{ethanol} + 2\text{CO}_2 + 2\text{ATP} \]

There is, however, an important difference between glucose, ethanol, and \( \text{CO}_2 \) on the one hand, and ADP and ATP on the other. The first are “external” substrates and products in the sense that they are ingested and excreted; they form natural metabolic boundaries. The second are “internal” substrates and products; they form artificial metabolic boundaries at which the metabolic pathway connects with other cellular processes. So, what we call an “end-product” is often the substrate for other cellular processes, e.g. ATP for biosynthesis and growth, amino acids for protein synthesis, nucleotides for nucleic acid synthesis. In principle there is of course nothing wrong with compartmenting a complex network into separate modules for purposes of analysis, whether experimental or theoretical; a problem arises, however, when the internal boundaries are carried over into an analysis of control and regulation. The reason is that while one or more steps in any module may exercise a high degree of control of some steady-state variable in the module when it is studied in isolation, it is possible that they lose that control completely when the system is expanded to include other connecting modules; this will happen when the module as a whole has little control over that variable within the expanded system (that this is a fundamental result from metabolic control analysis has not exempted control analysts from sometimes making the same mistake). The surprise caused by the failure of over-expressed glycolytic enzymes to affect flux can be largely ascribed to an unfulfilled expectation that the control profile obtained for the glycolytic module in isolation can be extrapolated to the whole system of which glycolysis is part.
That the mistake of extrapolating an intramodular control profile is often made is strange in view of the seemingly general acceptance of the functional view of metabolism (to which I fully subscribe), i.e. the view that the kinetic and regulatory properties of the enzymes of intermediary metabolism have been moulded by evolution in such a way as to allow metabolic pathways to fulfil one or more “metabolic functions”. Taken seriously, this view should at least ensure that the properties of a pathway is always related to the greater cellular context. The functional view is, however, fraught with difficulties: it is one thing to accept that metabolic pathways have been “purposefully designed” by evolution; it is quite another to decide for which function(s) a particular pathway has been designed, a question that remains mostly a matter of informed guessing by reverse engineering. Nevertheless, there seems to be general consensus that the function of yeast glycolysis under anaerobic growth conditions is to maintain the cell’s energy charge at a high level (maintain the adenylate pool mostly in the form of ATP) while supplying carbon skeletons for biosynthesis mostly from pyruvate and phosphoenolpyruvate.

The analogy of the cell as a “chemical factory”, developed by Cascante and Marti in this book (pp. 119–214), leads naturally to an economic view that cellular processes consist of supply-demand or production-consumption systems. The acceptance of this analogy or terminology is not essential for the type of analysis to be described (one could just describe it as a modular analysis), but it does emphasize my own conviction that any analysis should take into account the perceived function. I shall thus analyse glycolysis as a feedback-regulated ATP-supply system for other cellular processes that consume ATP (collectively called the “demand”). Of course the introduction of such a terminology also has a persuasive element: one expects a well designed feedback-regulated supply-demand system with as main function the production of some important end-product to supply that product at a rate reflecting the demand; therefore, one expects most, probably all, of the flux-control to be associated with the collective demand for the end-product. Nevertheless, this expectation can be fully tested by experiment and need never be accepted unquestioningly.

Under anaerobic, fermentative conditions in yeast, glycolysis serves as the only net producer of ATP (it also, of course, consumes ATP in order to prime its substrates). Its key enzymes are sensitive to the composition of the adenylate pool consisting of ATP, ADP and AMP so that glycolysis is a feedback-regulated system. ATP is consumed by various demand processes: biosynthesis (if the yeast
grows), active transport, maintenance of intracellular ionic composition, etc. This ATP supply-demand system is depicted in Fig. 1. The steady-state fluxes through the different glycolytic enzymes that consume or produce ATP are not necessarily strictly coupled. Each of these four enzymes occurs in a unique glycolytic sequence delimited by branchpoints. Hexokinase and phosphofructokinase are separated by the branches from glucose 6-phosphate to trehalose and to pentose phosphates, phosphofructokinase and phosphoglycerate kinase by the branch from dihydroxyacetone phosphate to glycerol, phosphoglycerate kinase and pyruvate kinase by the branch from phosphoenol pyruvate to biosynthesis. Only under non-growing conditions are the fluxes through most of these branches from glycolysis small enough for there to exist a stoichiometrically coupled “glycolytic flux” that can be measured by either the rate of glucose consumption or the rate of ethanol production (which should be 1:2 on a concentration-time\(^{-1}\) basis). If these conditions are not satisfied, the individual steady-state fluxes through the different sections of glycolysis should be determined. For the purpose of the following analysis we assume the experimentally obtainable situation where a single glycolytic flux can be measured. The role of adenylate kinase is considered below, but

Fig. 1. The main reactions involved in ATP production and consumption in a fermenting yeast cell. Abbreviations: HK: hexokinase; PFK: phosphofructokinase; PGK: phosphoglycerate kinase; PK: pyruvate kinase; AK: adenylate kinase. The reaction catalysed by adenylate kinase is depicted with a dotted line to indicate that it is considered to be in equilibrium, therefore carrying no net flux. The number associated with the adenylate kinase reaction indicates reaction stoichiometry. The block designated “Demand” symbolizes the set of non-glycolytic ATP-consuming reactions.
DISTRIBUTION OF CONTROL IN ANAEROBIC ENERGY METABOLISM IN YEAST

The power of metabolic control analysis lies in its ability to relate systemic properties such as flux and concentration-control to the kinetic properties of the components of the system. It provides a clear answer to the question: which properties of the ATP-supply and demand blocks determine how control of the steady-state flux and concentrations of adenylates is distributed between these blocks? To begin, we seek the simplest representation of the system that retains all its essential features; then we identify the steady-state variables.

In the absence of adenylate kinase the system in Fig. 1 can be simplified for the purposes of analysis to a two-member moiety-conserved cycle that interconverts ATP and ADP [a moiety-conserved cycle interconverts different forms of a chemical moiety, the sum of which remains constant (Reich and Sel’kov, 1981; Hofmeyr et al., 1986)]. If the value of the sum ATP + ADP is known, one concentration can be calculated given the other — there is only one independent concentration. However, a more suitable choice of independent variable is either the mole fraction ATP/(ATP + ADP) or the ATP/ADP ratio (Atkinson, 1977; Reich and Sel’kov, 1981; Hofmeyr et al., 1986). In steady state the other variable is the flux, i.e. the steady-state rate through each block.

In yeast cytoplasm an active adenylate kinase maintains an equilibrium distribution of AMP, ADP and ATP within the conserved sum ATP + ADP + AMP. The ATP–ADP cycle is no longer moiety-conserved; the conserved sum of adenylates now includes AMP. Even so, there is still only one independent concentration variable (given the values of any one of the three concentrations, the sum, and the equilibrium constant of the adenylate kinase reaction, the other two concentrations can be calculated). Here suitable independent variables are either the energy charge ec, defined by Atkinson (1977) as

\[ ec = \frac{[\text{ATP}] + \frac{1}{2}[\text{ADP}]}{[\text{ATP}] + [\text{ADP}] + [\text{AMP}]} \]  

or, analogous to the ATP/ADP ratio for an ATP-ADP cycle, the ratio of
charged adenylates over uncharged adenylates:

\[
\frac{\text{Charged adenylates (c)}}{\text{Uncharged adenylates (u)}} = \frac{ec}{1-ec} = \frac{\text{[ATP]} + \frac{1}{2}[\text{ADP}]}{\text{[AMP]} + \frac{1}{2}[\text{ADP}]}
\]

(2)

This unfamiliar ratio can be interpreted as follows: due to the stoichiometry of the adenylate kinase reaction half of the ADP pool is available to (theoretically) donate a phospho group, and is therefore charged, while the other half is available to accept a phospho group, and is therefore uncharged.

The system in Fig. 1 is therefore in reality a simple cyclical supply-demand system for energy charge (Fig. 2A). If the charged/

![Diagram](image)

Fig. 2. Energy charge supply-demand system in a anaerobically fermenting yeast cell. As explained in the text, when the branches to glycolysis carry no flux, there is one glycolytic flux measured either as the rate of glucose consumption or the rate of ethanol or CO₂ production; this glycolytic flux can be regarded as the supply flux to ATP. In the presence of an active adenylate kinase the system becomes a conserved cycle for adenylate moiety; in such systems the energy charge \((ec)\) becomes the independent variable (Atkinson, 1977; Reich and Sel’kov, 1981). The scheme in A shows how the system shown in 1 simplifies under these conditions to a simple two-member moiety-conserved cycle around the charged adenylate fraction \((ec)\) and the uncharged adenylate fraction \((1-ec)\). Any two-membered moiety-conserved cycle can also be represented as a linear two-component system linked by the ratio of the two forms of moiety: this system is shown in B for the variable \(ec/(1-ec)\). In both A and B the block designated “Demand” symbolizes the set of non-glycolytic reactions that lower the energy charge by consuming ATP. In both schemes the negative feedback effect of components of the adenylate pool on the glycolytic enzymes is indicated.
uncharged ratio \((c/u)\) defined in eqn. 2 is considered to be the independent variable, the system simplifies even further to the linear supply-demand system in Fig. 2B (note that no information has been lost in this simplification). A full analysis of the control and regulation of this last linear type of system has been published (Hofmeyr and Cornish-Bowden, 1991; Hofmeyr, 1995). Here only those features of the analysis relevant to the present discussion will be repeated. For purposes of functional analysis, it may be preferable to analyse energy metabolism in terms of supply of and demand for energy charge, using Fig. 2A as basis, because we generally understand the function of energy metabolism to be the maintenance of a high energy charge. Such an analysis follows the same lines as the one given below, although it is slightly more complicated.

The systemic control properties of the supply-demand system are quantified in terms of control coefficients, defined as follows (with the flux-control coefficient of the supply block as an example):

\[
C^f_{\text{supply}} = - \frac{\partial \ln f}{\partial \ln v_{\text{supply}}} \tag{3}
\]

Because the changes are measured on a logarithmic scale they are equivalent to percentage changes. Operationally, \(C^f_{\text{supply}}\) is the percentage change in steady-state flux caused by a 1% change in the activity of the supply block, \(v_{\text{supply}}\), which could be brought about by a simultaneous 1% change in the activity of all the steps in the supply block.

The kinetic properties of the supply and demand blocks that are important for the present control analysis are the responses in supply and demand rates to changes in the linking variable \(c/u\). These responses are called elasticity coefficients defined (for, say, the supply block) as follows:

\[
\varepsilon^v_{\text{supply}} = \frac{\partial \ln v_{\text{supply}}}{\partial \ln (c/u)} \tag{4}
\]

The graph in Fig. 3 is called a rate characteristic, and clearly shows the physical meaning of the elasticity coefficients of supply and demand with respect to the \(c/u\)-ratio. That the steady-state control distribution depends solely on the values of the elasticity coefficients at that steady state is evident from the following results from metabolic
control analysis (Hofmeyr and Cornish-Bowden, 1991):

\[ C_{\text{supply}} = \frac{\varepsilon_{\text{demand}}}{\varepsilon_{\text{demand}} - \varepsilon_{\text{supply}}} \]  \hspace{1cm} (5)

\[ C_{\text{demand}} = \frac{-\varepsilon_{\text{supply}}}{\varepsilon_{\text{demand}} - \varepsilon_{\text{supply}}} \]  \hspace{1cm} (6)

\[ C_{\text{c}'/u}^{\text{supply}} = \frac{1}{\varepsilon_{\text{demand}} - \varepsilon_{\text{supply}}} \]  \hspace{1cm} (7)

\[ C_{\text{c}'/u}^{\text{demand}} = \frac{-1}{\varepsilon_{\text{demand}} - \varepsilon_{\text{supply}}} \]  \hspace{1cm} (8)

Fig. 3. Hypothetical rate characteristics for the supply and demand blocks of the system in Fig. 2B that ensure flux-control by demand. The logarithm of the fluxes local to the supply and demand blocks are plotted as a function of the logarithm of the \( c/u = ec/(1 - ec) \) ratio. The intersection of the two curves is the steady state. The slopes of the tangents to the two curves at steady state (magnified area) or at any other point are the elasticity coefficients of supply and demand with respect to the \( c/u \) ratio. Experimentally, the data for constructing the curves are obtained by co-response analysis (Hofmeyr and Cornish-Bowden, 1996) or top-down analysis (Brand, 1996): the supply curve is obtained by modulating the activities of one or more steps of the demand using inhibitors, variable expression, etc. (Fell, 1992, 1996), while measuring the steady-state flux and the steady state concentrations of ATP, ADP and AMP at each level of demand activity (the three dotted lines represent different possible supply responses at lower values of the \( c/u \) ratio). The demand curve is similarly obtained by titrating supply activity. The true form of the demand curve may of course be different in the region of low \( c/u \) — here simple saturation kinetics was assumed. A control-analytic interpretation of these rate characteristics is given in the text.
Remember that the supply elasticity is usually negative (compare the slope of the supply curve in Fig. 3) so that the $-e_{c/u}^{\text{supply}}$ terms in these equations can be replaced by $+\left|e_{c/u}^{\text{supply}}\right|$

It should be clear that the partitioning of flux-control between the supply and demand blocks depends on the relative values of the elasticities of supply and demand. An increase in the supply elasticity (or decrease in the demand elasticity) shifts flux-control to the demand, while the reverse shifts flux-control to the supply. In reality, effective flux control by demand (say, a flux-control coefficient of around 0.95 and higher) requires the demand elasticity to be at least 19-fold smaller than the supply elasticity. Realistic supply elasticities rarely exceed the value of 4 (the Hill coefficient of feedback), so that flux-control by demand requires demand elasticities of less than 0.2. Therefore, under conditions of flux-control by demand the sensitivity in the $c/u$-ratio, as measured by $C_{c/u}^{\text{supply}}$ and $C_{c/u}^{\text{demand}}$, is largely determined by the value of $e_{c/u}^{\text{supply}}$, i.e. by the kinetic properties of the supply block. The higher the supply elasticity at constant demand elasticity, the more effective the buffering of the $c/u$-ratio.

Compare these results to Fig. 3. The hypothetical rate characteristics have been constructed in such a way that, at the steady state where the rate curves intersect, the flux is controlled by demand because the slope of the demand curve approaches zero, while the slope of the supply curve is steep. Changes in the demand activity will move the steady-state point only in the direction of the rate axis, while changes in supply activity will have virtually no effect on the steady-state flux. The steep slope of the supply curve at steady state ensures that neither changes in demand nor changes in supply have much of an effect on the $c/u$-ratio. This result leads to an important generalization: if, in a coupled two-component system where the coupling variable affords the only means of communication between the blocks, the one component controls the flux, then the stabilization of the coupling variable becomes a function of the other component; the more sensitive this component is to changes in the coupling variable (the stronger the feedback), the better the buffering of the variable. From Fig. 3 it is also clear that demand controls the flux only within a certain activity range; if demand activity becomes too high it loses control.

It is not my intention to explore in full the vastly more complicated territory opened by the obvious question: which properties of a block ensure a low or a high elasticity? However, the first step in answering this question does resolve the matter of where the control
profile obtained for the glycolytic module in isolation fits into the supply-demand analysis developed above. The supply elasticity with respect to $c/u$ can be resolved into the following expression containing the control properties of those glycolytic enzymes that are directly affected by changes in $c/u$ (see Hofmeyr and Cornish-Bowden, 1991):

$$
\varepsilon_{\text{supply}}^{c/u} = C_{\text{PK}}^{\text{supply}} \varepsilon_{\text{cPK}}^{c/u} + C_{\text{PGK}}^{\text{supply}} \varepsilon_{\text{cPGK}}^{c/u} + C_{\text{HK}}^{\text{supply}} \varepsilon_{\text{cHK}}^{c/u} + C_{\text{PFK}}^{\text{supply}} \varepsilon_{\text{cPFK}}^{c/u}
$$

(9)

The names of the abbreviated enzymes are given in the legend of Fig. 1. Note again the difference between, for example, $C_{\text{PFK}}^d$, which quantifies the effect of a change in phosphofructokinase activity on the steady-state flux through the full supply-demand system, and $C_{\text{PFK}}^{\text{supply}}$, which quantifies the effect of a change in phosphofructokinase activity on the steady-state flux through the glycolytic supply system when studied in isolation. A numerical example explains this difference: In a system where demand controls the flux to a degree of 95%, i.e., where $C_{\text{demand}}^d = 0.95$, the collective control exerted by the glycolytic supply enzymes is 5%. Within the supply block itself, however, the collective control of these enzymes is 100%. It therefore follows that although, say, phosphofructokinase may exert substantial control within glycolysis itself (let us assume $C_{\text{PFK}}^{\text{supply}}$ to be 0.6), its effect on the flux through the whole system would be very small ($C_{\text{PFK}}^d = 0.05 \times 0.6 = 0.03$).

Each term in the sum in eqn 9 is a product of a control coefficient of a step and an elasticity of that step with respect to $c/u$. Such a term can only contribute significantly to the sum if neither of these coefficients is small — the enzyme must both exert substantial flux control and be sensitive to $c/u$. The next steps in the analysis would therefore ask (i) what determines the flux-control distribution within glycolysis (here, for instance, the internal feedback patterns would be important), and (ii) which enzyme properties determine sensitivity with respect to $c/u$ (the enzymes in question of course respond only indirectly changes in $c/u$ via changes in the ATP/ADP or ATP/AMP ratios, so it should first be calculated how these ratios change with $c/u$). The analysis given in Hofmeyr and Cornish-Bowden (1991) can be consulted as an example. The classical view of the regulation of glycolysis discussed in the introduction, with its emphasis on the rate-controlling nature of the three kinases and their allosteric interaction with the adenylates therefore conforms to a high value of the supply elasticity with respect to $c/u$.

Similarly, the demand elasticity can be broken down into a sum
of terms. Let us assume that there are two demand reactions with partial fluxes $J_1$ and $J_2$ so that in the steady state $J_1 + J_2 = J_{\text{supply}}$. It can be shown that

$$\varepsilon_{c/u}^{\text{demand}} = J_1 C_1 \varepsilon_{c/u}^{\text{h}} + J_2 C_2 \varepsilon_{c/u}^{\text{v}}$$  \hspace{1cm} (10)

For the demand to control flux, it was shown above that $\varepsilon_{c/u}^{\text{demand}}$ must be much smaller than $\varepsilon_{c/u}^{\text{supply}}$. The contribution of each term to the sum in eqn 10 is in the first place proportional to the flux carried by the corresponding demand block, but besides this either a low control coefficient or a low elasticity coefficient would minimize the term. For ATP-consuming reactions, it is most probably low sensitivity with regard to adenylates, due to saturation effects, that ensure small values of the elasticity coefficients in the right-hand terms of eqn 10.

With this background, the next section explores some implications for biotechnology, more specifically the question of how, assuming a demand-controlled yeast glycolytic system, the flux to ethanol can be manipulated.

**MANIPULATION OF ETHANOL PRODUCTION IN YEAST**

Ethanolic fermentation by the yeast *S. cerevisiae* underlies many biotechnological processes, many of which have as main aim the production of ethanol from some fermentable substrate. Before the advent of recombinant DNA technology, the only hope of improving this process was through mutation/selection, essentially a black box approach that does not rely on an understanding of the underlying processes. Now that it has become possible to selectively manipulate the expression of individual enzymes, it has also become clear that any view of regulation that concentrates on only the supply side of intermediary metabolism fails to provide a basis for the design of manipulation strategies. Can the understanding gained by supply-demand analysis of energy metabolism aid the design of rational strategies for improving this process?

In order to focus the discussion, consider a specific problem: how should we manipulate yeast cells so as to increase the specific rate of ethanol production (rate per gram of biomass)? In general, supply-demand analysis tells us that if flux is controlled by demand it can only be increased by increasing the demand activity in some way; flux can only be increased by pulling the system from the demand
side, not by pushing it from the supply side. This strategy that has been termed \textit{subversion} (Cornish-Bowden \textit{et al.}, 1995) to indicate that the natural regulatory mechanisms are positively utilized rather than opposed. In the case of yeast fermentation, the product of interest, ethanol, is produced by the supply block, but is not involved in the coupling of the supply and demand blocks (in contrast, many other metabolites of biotechnological importance, such as, for example, amino acids, couple their intracellular supply and demand blocks). Therefore, to increase the flux to ethanol, the internal demand for ATP has to be increased, either by increasing the activity of the existing demand reactions or by introducing a new ATP-utilizing reaction that partially uncouples ATP-supply from the existing internal demand (in Fig. 4 this extra demand step has been called a “leak”). The second option is probably the “cleanest” option, since the natural ATP-consuming reactions (mostly involved in biosynthesis/growth) are left undisturbed.

The added ATP “leak” has to be carefully controlled. The technology for accomplishing this in yeast and in a number of other organisms has been developed recently in a collaboration between the groups of Peter Jensen and Karen Hammer at the Technical University of Denmark and of Hans Westerhoff and Jacky Snoep of the Free University of Amsterdam.

![Diagram](image)

\textbf{Fig. 4.} Increasing the flux to ethanol in a demand-controlled system using the strategy of subversion (Cornish-Bowden \textit{et al.}, 1995). The demand for charged adenylates is increased by introducing an internal leak for ATP. As long as the collective demand for charged adenylates (including the leak) continues to control the flux, the flux to ethanol is increased by an amount stoichiometrically proportional to the internal leak (dotted lines).
University of Denmark and of Hans Westerhoff and Jacky Snoep of the Free University in Amsterdam. A specific ATP-hydrolysing enzyme uncoupled from any accompanying activity has been cloned, making it possible to introduce a clean internal ATP leak that does not interfere with other cellular processes. To control the level of expression of this ATPase a method has been developed to generate a set of promoters with varying strength, both weaker and stronger than the wild-type promotor. This allows the optimum amount of ATP leak to be hard-wired into the cell, thus overcoming the obvious problems that accompany the use of inducible promoters in industrial fermenters.

DISCUSSION

The central message of this analysis of anaerobic energy metabolism in yeast is that we cannot hope to achieve an understanding of control and regulation of a feedback-regulated metabolic pathway such as glycolysis if we do not take into account the demand processes for key products of glycolysis such as ATP. That the demand for metabolic “end products” is an important factor in metabolic behaviour is of course not a new idea. Nevertheless, the quantitative relationships between supply and demand as explained above and in Hofmeyr and Cornish-Bowden (1991) were not made clear in the classical view of metabolic regulation. For example, contrast these two statements from one of the most eloquent proponents of the classical view, Daniel Atkinson: “Kinetic regulation of the first reaction in a sequence controls the flux through the entire sequence” (Atkinson, 1977) and “It is evident that flux-control coefficients close to zero are essential if the flux through a sequence is to be regulated in response to metabolic need. As in any feedback-regulated system, flux must reflect demand, not the properties of the component enzymes” (Atkinson, 1990). Taken at face value, these two statements are incompatible and a potential source of confusion, although their author clearly understood that they referred to different levels of analysis: “There is no logical or semantic difficulty, however, in the perception that net demand and the first step both exert total control over the flux; we are merely dealing with different levels in the regulatory system” (Atkinson, 1990). In their now classical paper on the control of flux, Kacser and Burns (1973) also made this distinction clear. They showed that in a feedback system a high elasticity of supply with respect to a metabolic end-product tends to transfer flux-control out of the loop to the processes
that consume end-product. However, for this supply elasticity to be high it is also necessary that the step subject to end-product inhibition have a relatively high flux-control coefficient within the loop. So, it is perfectly feasible that the demand controls the flux in the complete supply-demand system (with no flux-control associated with any of the supply steps), while, simultaneously, the feedback-inhibited step controls the flux in the supply block — the two situations refer to what for the purposes of analysis are different systems. In their treatise, Westerhoff and van Dam (1987) also emphasize that in supply-demand systems flux is expected to reflect metabolic demand because of a high supply elasticity: “The control of the flux in such a pathway (for instance ATP production) by the final product should be strong, but control by the substrate concentration should be negligible”.

At present there is only partial experimental evidence that glycolytic flux in yeast is controlled by the collective ATP demand. In a number of reconstituted glycolytic systems (also that of yeast) the ATPase or ATP-consuming process that was included as part of the system exerted a substantial amount of control over the flux, but whether the ATP-consuming activity used was comparable to that of the natural ATP demand is not clear (Scopes, 1974; Algar and Scopes, 1985; Welch and Scopes, 1985; Scopes, 1997). These studies force an important distinction to be made with regard to the ATP-demand. On the one hand it is clear that without the presence of ATP demand a glycolytic steady state cannot exist; as Scopes puts it in this book (p. 152), “glycolysis makes ATP, and without simultaneous breakdown of ATP it would soon stop”. On the other hand, as shown by the analysis in this paper, the mere existence of the ATP-demand does not ensure that it controls the flux; it is the sensitivity of the supply and demand blocks with respect to the energy charge that determines the flux-control distribution between supply and demand. Another study found that glycolytic flux is increased by increasing the ATP-demand via dissipation through a futile cycle (Navas et al., 1993), which would only be possible if the collective ATP-demand already has substantial control over the flux. A recent kinetic model of yeast glycolysis (Galazzo and Bailey, 1990), based on $^{31}$P NMR measurements of intracellular metabolite concentrations of suspended and immobilized cells, predicts a value of 0.35 for the flux-control coefficient associated with ATP-consumption. However, it also predicts flux-control coefficients of 0.45 for the glucose permease and 0.2 for phosphofructokinase, which would seem partially to contradict the results of (Schaaff et al., 1989) which were discussed in the Introduction.

A more definitive study of how the control of flux is partitioned
between ATP-supply and a hierarchy of ATP-consuming processes was conducted with oxidative phosphorylation in mammalian cells and not, unfortunately, with yeast glycolysis (Buttgereit and Brand, 1995). It was shown that under the specific conditions used in the study 88% of the control over the flux through cytoplasmic ATP resided in the ATP-consuming reactions, while the remaining 12% was associated with ATP supply (a combination of oxidative phosphorylation and proton leak). Unfortunately the ATP and ADP concentrations were not measured, which precluded the construction of the rate characteristics for this ATP supply-demand system. Nevertheless this important study should serve as a paradigm for future studies of other ATP or energy charge supply-demand systems, such as, for instance, that in yeast.

Informed readers may feel that this chapter has erred on the side of belabouring the obvious. Nonetheless, modern biochemistry textbooks and a host of papers in the biotechnology literature make it abundantly clear that its central message has either not been heard or not been taken to heart. With few exceptions, discussions of issues like flux-control and its biotechnological manipulation continue to focus solely on the supply side, ignoring the demand. I hope to have shown here that this is only half of the story: if we are to understand the regulation of metabolism, especially of key metabolic processes such as glycolysis, and if we are to design successful strategies for manipulating these processes, our analyses must be performed in the context of the full supply-demand system. Furthermore, I propose that such analyses could form a solid foundation of an integrated view of metabolic regulation that merges the classical, the functional, and the control analytic views. An important insight afforded by this integrated view is the realization that when demand is the dominant flux-controller, as can be expected in functional feedback systems, the role of those supply enzymes traditionally described as “regulatory” is that of steady-state concentration control, not steady-state flux-control.

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