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Elusive Control

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The concept of a single rate-limiting step was proven to be too simplistic for understanding control and regulation of metabolism. Consequently, searches have identified relatively few steps with high control. Here we review a number of such searches and indicate what mechanisms may be responsible for this elusiveness of control. It turns out that this elusiveness of control has itself led to increased understanding of the roles played in metabolic control and regulation of such diverse factors as distributiveness of control, condition dependence, enzyme elasticity, homeostasis, control hierarchies, the input into a pathway, coenzyme sequestration, and redundancy and diversity of control function.

KEY WORDS: Metabolic control; analysis; and regulation; control hierarchies; channelling; homeostasis; enzyme organization.

INTRODUCTION

In the early days of metabolic regulation, it was considered relatively simple to identify the major sites of control of metabolic fluxes: the first and irreversible step in a pathway was considered to be the rate-limiting step. This view is illustrated in the upper part of Fig. 1. Since then life has not become simpler for scientists interested in the regulation of cell function. From theoretical analyses it became clear that the first enzyme in the pathway need not be the rate-limiting step, indeed that there need not be a single rate-limiting step. It was shown that there is no unequivocal relationship between distance from equilibrium and rate limitingness, that rate limitation depends on conditions, and, even worse, that precise experimentation, possibly including molecular genetics and some mathematics, are needed to establish which step controls a flux and to what extent it does so.

In parallel, when extents to which important enzymes control important fluxes were determined experimentally, control appeared to be elusive. In argi-

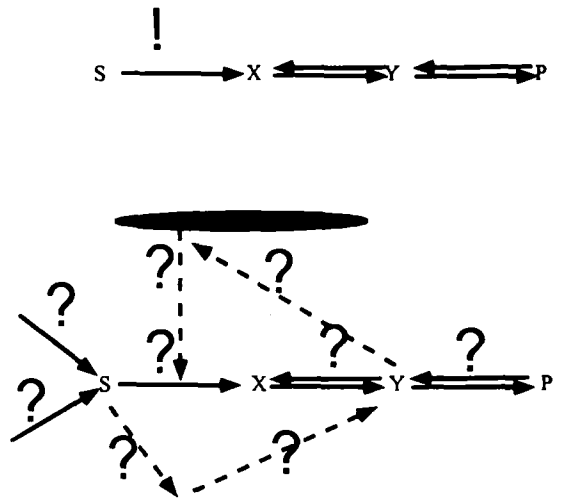

Fig. 1. Elusiveness of control. The upper part of the figure illustrates how one often looks at a metabolic pathway, attributing control to the first enzyme catalyzing an irreversible reaction. The bottom part of the figure illustrates the complexity that may be closer to reality. Control may be distributed over the enzymes in the pathway itself, reside in the substrate supply, reside in hierarchical control mechanisms adjusting the concentration or activity of the enzymes, or hide out in enzymes beyond the pathway such as in parallel pathways.

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nine synthesis in *Neurospora* none of the enzymes examined exerted much control (Barthelmess *et al.*, 1974). In isolated rat-liver mitochondria control of respiration hardly resided in the single irreversible step, i.e., cytochrome oxidase (Groen *et al.*, 1982; Hafner *et al.*, 1990). In yeast none of the glycolytic enzymes appeared to control glycolysis (Schaaff *et al.*, 1989; Brindle *et al.*, 1995), and control of tryptophan synthesis could only be ascertained up for up to 20% (Niederberger *et al.*, 1992). None of the components of the phosphotransferase system in Enterobacteriaceae appeared to control their growth rate (Ruijter *et al.*, 1991; Van der Vlag *et al.*, 1995) and neither did the proton translocating ATPase (Jensen *et al.*, 1993 a,b).

In this paper we shall review why in many cases control appeared elusive. Often the control does not reside in that first irreversible step of the pathway, but rather in any of the other steps directly or only indirectly connected to it (see the lower part of Fig. 1). We shall show how, by stimulating intensive and rational research, this elusiveness has led to increased insight in unsuspected aspects and mechanisms of control.

DISTRIBUTIVENESS AND VARIABILITY OF CONTROL

Explanation of elusiveness of control in classical genetics has in fact been a major driving force for the development of MCA (Kacser and Burns, 1973, 1981): The issue was to explain why most mutations are recessive. Recessiveness implies that effects on vital fluxes of 50% reductions in gene dosages are unnoticeable. Most probably, a 10% decrease in growth rate has remained unnoticed in the viability assays used. This means that the control coefficients of the enzymes encoded by the mutated genes must have been smaller than 0.15. Using a hyperbolic or power law approximation, the control coefficient of an enzyme on a flux can be estimated as the relative increase in flux in the wild-type compared to the heterozygote (cf. Savageau, 1972; Kholodenko *et al.*, 1981; Jensen *et al.*, 1995). Why is the control of most enzymes so small?

Kacser and Burns (1973) demonstrated that the total control on any flux by all enzymes must add up to 1 and that there is no general reason for control to reside in a single step (cf. Heinrich and Rapoport, 1974; Heinrich *et al.*, 1977). Consequently, the average flux control by an enzyme is 1 divided by the number of enzymes, which readily becomes smaller than 0.1. If control is distributed more or less at random, the flux control by most enzymes should not be far from this average.

More recent insights have adjusted this explanation of recessiveness somewhat, although its essence remains valid: Because some steps may exert negative control on a flux (Westerhoff and Arents, 1984), the average absolute control may exceed 1/number of enzymes. Because some enzymes may be involved in group-transfer reactions or metabolite channeling, the sum of the flux control coefficients may exceed 1 [the sum might increase to 2, or drop below 1 (Kholodenko *et al.*, 1995b; Van Dam *et al.*, 1993; Kholodenko and Westerhoff, 1993, 1995a, Brand *et al.*, 1994)].

One reason why control is not always found where it is expected may reside in the phenomenon that the distribution of control among the enzymes tends to depend on the conditions, e.g., on the work load imposed on the pathway, on substrate/product concentrations, on hormonal stimulation. Indeed, distribution of the control in all cellular pathways investigated so far displays substantial variability. Already in the study of the control of mitochondrial oxidative phosphorylation of ADP by Groen *et al.* (1982) it was demonstrated that the control varied significantly with the work load imposed on the mitochondria. In state 4 of isolated mitochondria, control of respiration was shared between the proton leak and the respiratory chain, at intermediate respiration rate (state 3.5) it was shared between ADP transport and the work load, whereas in state 3 the control resided again substantially in the substrate dehydrogenases and the respiratory chain (Groen *et al.*, 1982; Brown and Brand, 1986; Kholodenko *et al.*, 1987; Westerhoff *et al.*, 1987; Hafner *et al.*, 1990; Kholodenko *et al.*, 1991). Also, control of gluconeogenesis in rat liver cells (Groen *et al.*, 1986) and control of glucose utilization in perfused rat heart (Kashiwaya *et al.*, 1994) were found to vary significantly with added nutrients and hormones.

ELASTICITY RATHER THAN DISTANCE FROM EQUILIBRIUM IS WHAT MATTERS FOR CONTROL

If control is distributed, what then determines where most of the control resides? Initially the paradigm was that enzymes catalyzing reactions that are far from equilibrium should exert most of the flux control. The basis for this (Newsholme and Start, 1973) was that the rate of a near-equilibrium reaction is highly responsive to changes in the concentrations of

its substrates and products, such that an increase in the enzyme's activity is incapacitated by the subsequent decrease in substrate concentration and increase in product concentration. This paradigm is deficient in an aspect that is particularly important for many biological situations: it does not take into account that enzymes that are far from equilibrium may be also be strongly sensitive to changes in metabolite concentrations, e.g., due to allosteric regulation. Indeed the pertinent paradigm is that the control exerted by an enzyme is inversely related to the sensitivity of the enzyme to changes in the metabolites. The sensitivity of the enzyme toward changes in a metabolite has been given a new definition: the elasticity coefficient. In the absence of allosteric or other "biological" regulation, the elasticity coefficients are larger for the reactions that are closer to equilibrium (Westerhoff and Van Dam, 1987; Hofmeyr, this volume).

Indeed, the difference between (or rather ratio of) elasticities explained why in mitochondrial oxidative phosphorylation cytochrome oxidase has a smaller control than ADP transport, even though the former is much farther from equilibrium than the latter: the respiratory chain in rat liver mitochondria is highly elastic with respect to the electrochemical potential difference for protons (Westerhoff *et al.*, 1987).

It may be noted that the inverse relationship between elasticity and control has an important consequence for how one views control and regulation. An enzyme such as phosphofructokinase has often been considered to be important for control because it is regulated allosterically by many factors, including metabolites such as ATP, AMP, and citrate. Yet, importance for control in this sense is not or even inversely related to being a rate-limiting step, i.e., to having a high flux control coefficient. High elasticity tends to condemn an enzyme to exerting little control.

In quantitative terms, the regulatory capacity of an enzyme (the enzyme's involvement in the regulation) is determined by the arithmetic products of its control coefficient and the elasticities to external and internal effectors. These products have been called regulatory strengths (Kahn and Westerhoff, 1993) or partial response coefficients (Kholodenko, 1988, 1990/1991). Although due to high elasticities phosphofructokinase might have high response coefficients and high regulatory potential (Hofmeyr and Cornish-Bowden, 1991), its impact on the glycolytic flux, when its concentration is modulated, is low (see, e.g., Kholodenko *et al.* 1981; Schaaff *et al.*, 1989; Brindle *et al.*, 1995). The importance of the enzyme for regulation is not directly related to the magnitude of its control coefficients.

TOTAL CONTROL RESIDES IN ALL ENZYMES OF THE SYSTEM; TRANSFER OF CONTROL IN CASES OF HOMEOSTATIC MECHANISMS

A summation theorem of MCA states that the sum of the control exerted by all enzymes in the system on any flux must equal 1. An implication is that some of the control may reside in enzymes that are outside the pathway, yet in the system. More examples of this point will follow, but here we shall consider the case of a pathway which is homeostatically controlled by extra mechanisms. For long, and for various reasons, phosphofructokinase has been expected to be the rate-limiting step for glycolysis. The experiments by Schaaf *et al.* (1989) and Brindle *et al.* (1995) have, however, shown that overexpression of the gene encoding phosphofructokinase hardly affected glycolytic flux in yeast. One explanation of this finding was discussed in the previous section. Another factor may dominate, however, i.e., the regulatory system comprising fructose-2,6-bisphosphate. An altered concentration of phosphofructokinase leads to a change in concentration of fructose-2,6-bisphosphate which then again affects the phosphofructokinase activity to the extent that the total phosphofructokinase activity remains virtually constant [Davies and Brindle, 1992; Brindle *et al.*, 1995].

HIERARCHICAL CONTROL

Conceptually, metabolic control analysis has been dominated by the concept of a metabolic pathway of which the substrate, product (S and P in Fig. 1), and enzymes are present at fixed concentrations, whereas the concentrations of the pathway intermediates (X and Y in Fig. 1) adjust so as to attain steady state. In intact cells, the enzyme concentrations may adjust through regulated gene expression. Hierarchical control analysis takes the regulation beyond metabolism into account. In theoretical terms it was shown that in the case of democratic hierarchies, the control exerted by a pathway enzyme on a pathway flux (depending on its precise definition) tends to be diminished when there is regulated gene expression (Westerhoff and Van

Workum, 1990; Westerhoff *et al.*, 1990; Kahn and Westerhoff, 1991).

In *E. coli* growing on succinate the H^+-ATPase is essential for growth. Yet when asking the question whether it controls growth rate, it turned out that its control on growth rate was virtually zero (Jensen *et al.*, 1993a). As expected, decreasing the concentration of the H^+-ATPase decreased the ATP/ADP ratio and increased membrane potential (Jensen *et al.*, this volume). The concomitant increase in respiration rate was unexpected, however. Most probably, this inverse respiratory control was caused by an increased expression of genes encoding respiratory chain components such as the b-cytochromes (Jensen *et al.*, this volume). The present working hypothesis is that the absence of control of the H^+-ATPase on growth rate is due to a hierarchical control loop through gene expression, where the decrease in ATP/ADP ratio leads to an increased concentration of the b-cytochromes, thereby buffering cellular energetics. Control has shifted away from the H^+-ATPase toward gene expression.

CONTROL MAY RESIDE IN SYSTEM BOUNDARIES

When discussing the control of a metabolic phenomenon such as a flux, it is important to specify the context. Standard metabolic control analysis does this by delimiting the system under consideration by constant concentrations of substances that lead into and out of the pathway (S and P in Fig. 1). If in the real system, such a pathway substrate is not a constant but may vary along with modulation of the pathway (e.g., if the enzymes producing S in the lower half of Fig. 1 are modulated), this requires an additional analysis. In terms of modular metabolic control analysis, such an analysis is straightforward and the phenomenon that a pathway substrate is a variable is therefore not a true limitation to MCA.

An extreme case of such variability occurs in the chemostat, where the flux (in terms of the growth rate of the cells) is fixed and the substrate concentration is a dependent variable. Adapted metabolic control analysis has shown that many of the control properties deviate from those found when the substrate concentration is fixed. Indeed, control is strongly determined by the boundary conditions. An obvious example is the fact that in a chemostat all enzymes must have control of zero on the growth rate, even if they could control growth rate in batch cultures (i.e., during growth at essentially constant substrate concentration) (Snoep *et al.*, 1994).

When Postma and co-workers modulated the components of the bacterial phosphotransferase system by using plasmid directed overexpression, they observed that none of the four components (i.e., neither Enzyme I, HPr, Enzyme IIA, nor Enzyme IICB) exerted control on growth rate in batch culture, whereas only Enzyme IICB exerted a control of approximately 0.7 on transport (Ruijter *et al.*, 1991; Van der Vlag *et al.*, 1995). Because the summation theorem appeared to mandate a total control of 1, some of the control appeared elusive. The lack of demonstrable control became even more acute when it was shown that for the phosphotransferase system one should expect the sum of the control coefficients to exceed 1 (Van Dam *et al.*, 1993; Kholodenko and Westerhoff, 1995a,b).

That the lack of control was caused by hierarchical control is unlikely, because changes in the concentration of the unmodulated phosphotransferase enzymes were insignificant (Ruijter *et al.*, 1991). The phosphotransferase system catalyzes the transfer of a phosphoryl group from phosphoenolpyruvate to the sugar to be transported. The phosphoenolpyruvate derives from the intracellular stores and may therefore not be constant under the conditions of the experiment. Consequently, some of the control of the flux through the phosphotransferase system may reside elsewhere. That this possibility is realistic has been shown by Rohwer and colleagues (personal communication): Under carefully controlled conditions they showed that the uptake activity of the phosphotransferase system was affected by changes in the concentration of the H^+-ATPase and by changes in proton permeability of the plasma membrane. At this stage, it is unclear if the effect runs through alterations in the phosphoenolpyruvate or pyruvate concentrations, or if there are unknown additional regulatory influences on the phosphotransferase system.

Another case where control appeared absent, presumably as a consequence of control outside of the pathway, was that of the control of growth rate by the β-galactosidase and lactose permease. The control by these enzymes together was much smaller than 1 and this has been attributed to control on passage of the substrate through the outer membrane (Dean, 1990).

LOSS OF CONTROL DUE TO SEQUESTRATION

Occasionally the theorems of MCA have been challenged (e.g., Ottaway and McMinn 1980; Albe *et al.*, 1990). One of the instances where they may appear to fail is where metabolite concentrations do not vastly exceed enzyme concentrations. Indeed, when one uses total rather than free concentrations of metabolites in the relationships, MCA theorems appear to fail; as for any kinetic or thermodynamic approach, one needs to take the free concentrations or "activities" as effectors of rates and steady states. Because of this issue, however, a less trivial point has remained hidden for quite some time. When an enzyme is increased in concentration, it may bind to another enzyme, or it may reduce the total concentration of a coenzyme ("conserved moiety" (Hofmeyr *et al.*, 1986), e.g., NADH + NAD). This phenomenon is called "sequestration." When that second enzyme, or the coenzyme, exerts control, the control measured for the former enzyme will be decreased and the sum of all the control coefficients over all enzymes may drop below 1 (Fell and Sauro, 1990; Kholodenko *et al.*, 1992).

In the glycolysis of some organisms, the concentration of enzyme active sites may well exceed metabolite concentrations (Albe *et al.*, 1990). Theoretical estimation of the total control exerted by the enzymes of the lower part of glycolysis [i.e., downstream aldolase (EC 4.1.2.13)] showed that it can be as low as 0.1 (Kholodenko *et al.*, 1992). A leaky dynamic channel where a usual reaction pathway coexists with the direct transfer of an intermediate (Friedrich, 1974; Cornish-Bowden, 1991; Mendes *et al.*, 1992) provides an example of enzyme sequestration. It has been shown that the sum of the enzyme control coefficients over the pathway flux should peak at about 2 at intermediate enzyme concentrations whereas it should drop below 0.5 at high protein concentrations [Kholodenko *et al.*, 1995b].

There are three ways to define the control exerted by an enzyme on the flux. They all inspect the relative effect on flux of a certain relative modulation of the enzyme. They differ in what precisely is modulated. In one (Kacser and Burns, 1973; Westerhoff *et al.*, 1984) the concentration of the enzyme is modulated. In a second (Heinrich *et al.*, 1977; Schuster and Heinrich, 1992) the activity of the enzyme is affected by a modulator and the effect of the modulator on pathway flux is compared to the effect of the modulator on the enzyme's activity as measured with the enzyme isolated from the pathway but under pathway conditions. The second definition may define more directly the control by the activity of the enzyme, rather than the control by the (concentration of the) enzyme. The control by the enzyme concentration is operationalized by modulated gene expression (e.g., Ruijter *et al.*, 1991; Chao *et al.*, 1993; Jensen *et al.*, 1993a), and the control of the enzyme activity can be measured using certain types of specific inhibitor (Kholodenko and Westerhoff, 1993, 1995b,c). When defined according to the former definition, flux control is subject to sequestration effects. When defined according to the latter definition, some of the sequestration effects disappear.

Not even with the second definition, however, all segregation effects disappear and in what has been called "nonideal" metabolic pathways (i.e., pathways with direct metabolite transfer between enzymes, or with low concentrations of coenzymes with respect to enzymes), there may not be an unequivocal way of defining the flux control by a given enzyme. In such nonideal metabolic pathways there are various ways of defining the control by an enzyme, including its impact control, its concentration control, and its modulator-dependent control (Kholodenko and Westerhoff, 1995c; Kholodenko *et al.*, 1995a). These complications, which are absent from "ideal" metabolic pathways, are mere reflections of the fact that control in biological systems is a variegated affair and are therefore of great interest for further research.

Experimental demonstrations of reduced control due to segregation are lacking, partly because in "nonideal" systems there are other phenomena that may lead to extra rather than less control.

LACK OF CONTROL DUE TO PARALLEL FACTORS, REDUNDANCY

When a factor is highly important for cell function, it may be conceived that evolution has arranged for a back-up factor. This then may cause functional redundancy of factors in the physiological state. Alternatively, regulation should subtly depend on conditions, and this is achieved by having multiple factors regulate in parallel. At low ammonia concentrations, the ammonia assimilation in *E. coli* proceeds through the glutamine synthetase reaction. The activity of glutamine synthetase is regulated by a cascade of three proteins. One of these proteins, PII, appeared to lack

control on the transient time (see below) of changes in glutamine synthetase activity. Further analysis demonstrated that under conditions of low ammonia, there is a second factor, called PIII, which may take over the role of PII (Van Heeswijk, Kahn, Hoving, Molenaar, and Westerhoff, in preparation).

CONTROL OF OTHER THAN STEADY-STATE PHENOMENA

In some cases there are sophisticated mechanisms that affect the activity of an enzyme, but they do not affect steady-state flux. For ambiguous regulatory enzymes (i.e., enzymes which both add to and remove a modification from another enzyme, depending on the signal), the prediction is that they should exert no control. The functional explanation of these situations may be that although these ambiguous enzymes do not themselves exert control, they mediate control by other factors. In addition however, such enzymes may control the response time of the system to changes in signal, without controlling the extent of the effect the signal has on the steady state. We think that this situation may well apply to phosphofructokinase-2 where it is ambiguous, and to adenylyl transferase and uridylyl transferase, two enzyme components of the cascade that regulates glutamine synthetase activity in *E. coli.*

CONCLUDING REMARKS

This paper has reviewed a number of cases where control has been or may prove to be elusive. In some of these cases the further analysis has identified hitherto unknown principles of control or regulatory molecules. We expect that the same will happen with further analyses of the yet unresolved, or yet to be discovered, cases of elusive control. Control in biology is a subtle matter and it is the search for the apparently elusive that leads to the largest increases in understanding.

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REFERENCES

Albe, K. R., Butler, M. H., and Wright, B. E. (1990). *J. Theor. Biol.* **143**, 163–195.
Barthelmess, I. B., Curtis, C. F., and Kacser, H. (1974). *J. Mol. Biol.* **87**, 303–316.
Brand, M., Vallis, B. P. S., and Kesseler, A. (1994). *Eur. J. Biochem.* **226**, 819–824.
Brindle, K. M.. Fulton, S., Sheldon, J., and Williams, S. (1995). *Biochem. Soc. Trans.* **23**, 376–381.
Brown, G. C., and Brand, M. D. (1986). *Biochem. J.* **234**, 75–81.
Chao, Y-P., Patnaik, R., Roof, W. D., Young, R. F., and Liao, J. C. (1993). *J. Bacteriol.* **175**, 6939–6944.
Cornish-Bowden, A. (1991). *Eur. J. Biochem.* **195**, 103–108.
Dean, A. M. (1990). In *Control of Metabolic Processes* (Cornish-Bowden A., and Cárdenas, M. L., eds.), Plenum Press, New York, pp. 389–398.
Fell, D. A., and Sauro, H. M. (1990). *Eur. J. Biochem.* **192**, 183–187.
Friedrich, P. (1974). *Acta Biochim. Biophys. Acad. Sci. Hung.* **9**, 159–173.
Groen, A. K., Wanders, R. J. A., Westerhoff, H. V., Van der Meer, R., and Tager, J. M. (1982). *J. Biol. Chem.* **257**, 2754–2757.
Groen, A. K., Van Roermund, C. W. T., Vervoorn, R. C. and Tager, J. M. (1986). *Biochem. J.* **237**, 379–389.
Hafner, R. P., Brown, G. C., and Brand, M. D. (1990). *Eur. J. Biochem.* **188**, 313–319.
Heinrich, R., and Rapoport, T. A. (1974). *Eur. J. Biochem.* **42**, 89–95.
Heinrich, R., Rapoport, S. M., and Rapoport, T. A. (1977). *Prog. Biophys. Mol. Biol.* **32**, 1–83.
Hofmeyr, J.-H. S., and Cornish-Bowden, A. (1991). *Eur. J. Biochem.* **200**, 223–236.
Hofmeyr, J.-H. S., Kacser, H., and Van der Merwe, K. J. (1986). *Eur. J. Biochem.* **155**, 631–641.
Jensen, P. R., Michelsen, O., and Westerhoff, H. V. (1993a). *Proc. Natl. Acad. Sci. USA* **90**, 8068–8072.
Jensen, P. R., Westerhoff, H. V., and Michelsen, O. (1993b). *EMBO J.* **12**, 1277–1282.
Jensen, P. R., Snoep, J. L., Molenaar, D., Van Heeswijk, W. C., Kholodenko, B. N., Van der Gugten, A. A., and Westerhoff, H. V. (1995). *Biochem. Soc. Trans.* **23**, 364–368.
Kacser, H., and Burns, J. A. (1973). In *Rate Control of Biological Processes* (Davies, D. D., ed.), London, pp. 65–104.
Kacser, H., and Burns, J. A. (1981). *Genetics* **97**, 639–666.
Kahn, D., and Westerhoff, H. V. (1991). *J. Theor. Biol.* **153**, 255–285.
Kahn, D., and Westerhoff, H. V. (1993). *Biotheor. Acta* **41**, 75–83.
Kashiwaya, Y., Sato, K., Tsuchiya, N., Thomas, S., Fell, D. A., Veech, R. L., and Passonneau, J. V. (1994). *J. Biol. Chem.* **269**, 25502–25514.
Kholodenko, B. N. (1988). *FEBS Lett.* **232**, 383–386.
Kholodenko, B. N. (1990/1991). *J. Nonlinear Biol.* **1**, 107–126.
Kholodenko, B. N., and Westerhoff, H. V. (1993). *FEBS Lett.* **320**, 71–74.
Kholodenko, B. N., and Westerhoff, H. V. (1995a). *Biochim. Biophys. Acta* **1229**, 256–274.
Kholodenko, B. N., and Westerhoff, H. V. (1995b). *Biochim. Biophys. Acta* **1229**, 275–289.
Kholodenko, B. N., and Westerhoff, H. V. (1995c). *Trends Biochem. Sci.* **20**, 52–54.
Kholodenko, B. N., Dibrov, B. F., and Zhabotinsky, A. M. (1981). *Biophysics (USSR)* **26**, 501–595 (Engl. transl., pp. 510–516).
Kholodenko, B. N., Zilinskiene, V., Borutaite, V., Ivanoviene, L., Toleikis, A., and Praskevicius, A. (1987). *FEBS Lett.* **223**, 247–250.
Kholodenko, B. N., Mildaziene, V. Yu., Borutaite, V. J., Ivanoviene, L. J., Yuodkaite, R. A., Katiliute, Z. P., and Toleikis, A. J.

(1991). *Biochemistry (USSR)* **56**, 1420–1428 (Engl. transl., **56**, 992–998).
Kholodenko, B. N., Lyubarev, A. E., and Kurganov, B. I. (1992). *Eur. J. Biochem.* **210**, 147–153.
Kholodenko, B. N., Molenaar, D., Schuster, S., Heinrich, R., Westerhoff, H. V. (1995a). *Biophys. Chem.* **56**, 215–226.
Kholodenko, B. N., Westerhoff, H. V., Puigjaner, J., and Cascante, M. (1995b). *Biophys. Chem.* **53**, 247–258.
Mendes, P., Kell, D. B., and Westerhoff, H. V. (1992). *Eur. J. Biochem.* **204**, 257–266.
Newsholme, E. A., and Start, C. (1973). *Regulation in Metabolism*, Wiley, London.
Niederberger, P., Prasad, R., Miozzari, G., and Kacser, H. (1992). *Biochem. J.* **287**, 473–479.
Ottaway, J. H., and McMinn, C. L. (1980). *FEBS Symp.* **60**, 69–82.
Ruijter, G. J. G., Postma, P. W., and Van Dam, K. (1991). *J. Bacteriol.* **173**, 6184–6191.
Savageau, M. A. (1972). *Curr. Top. Cell. Regul.* **6**, 63–130.
Schaaff, I., Heinisch, J., and Zimmerman, F. K. (1989). *Yeast* **5**, 289–290.
Schuster, S., and Heinrich, R. (1992). *BioSystems* **27**, 1–15.
Snoep, J. L., Jensen, P. R., Groeneveld, P., Molenaar, D., Kholodenko, B. N., and Westerhoff, H. V. (1994). *Biochem. Mol. Biol. Int.* **33**, 1023–1032.
Van Dam, K., Van der Vlag, J., Kholodenko, B. N., and Westerhoff, H. V. (1993). *Eur. J. Biochem.* **212**, 791–799.
Van der Vlag, J., Van't Hof, R., Van Dam, K., and Postma, P. W. (1995). *Eur. J. Biochem.*, **230**, 170–182.
Westerhoff, H. V., and Arents, J. C. (1984). *Biosci. Rep.* **4**, 23–31.
Westerhoff, H. V., and Van Dam, K. (1987). *Thermodynamics and Control of Biological Free Energy Transduction*, Elsevier, Amsterdam.
Westerhoff, H. V., and Van Workum, M. (1990). *Biomed. Biochim. Acta* **49**, 839–853.
Westerhoff, H. V., Groen, A. K., and Wanders, R. J. A. (1984). *Biosci. Rep.* **4**, 1–22
Westerhoff, H. V., Plomp, P. J. A. M., Groen, A. K., Wanders, R. J. A., Bode, J. A., and Van Dam, K. (1987). *Arch. Biochem. Biophys.* **257**, 154–169.
Westerhoff, H. V., Koster, J. G., Van Workum, M., and Rudd, K. E. (1990). In *Control of Metabolic Processes* (Cornish-Bowden, A., and Cardenas, M. L., eds.), Plenum Press, New York, pp. 399–412.