25 Reprogramming of metabolic systems by altering gene expression

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Introduction

With the increasing power of genetic analysis and DNA microarray techniques a new kind of biological knowledge is available: it is possible to measure changes in gene expression levels on the same time scale as changes of metabolite concentrations. From this kind of investigations it became obvious, that the action of the genetic apparatus can be well adjusted to the necessities of the cell metabolism [1,2].

In this contribution a theoretical approach is developed to explain changes in gene expression according to the requirements of the metabolic apparatus. These requirements result from the fact that the metabolic system has to provide the cell with a large number of different substances as well as with energy, for example in the form of ATP. In particular, metabolism has to ensure the survival of the organism under varying external conditions. Since biological organisms are an outcome of mutation and selection processes, one can expect that their functions have been optimized during evolution. Hence, we try to explain certain features of these systems by applying optimisation principles. In extension to previous investigations for systems in steady state [3,4] we allow for time dependent changes in the levels of enzyme concentrations. The model is based on the assumption that the gene expression apparatus can adjust to the demand of the metabolic system and can increase or decrease the amount of certain enzymes by a change in the expression levels of the respective genes. The limited protein storing and producing capacity [4,5] is included via a fixed total amount of enzyme. In particular we treat the following question: which time-dependent distribution of enzyme concentrations allows the system to fulfil a given performance function in an optimal

way?

The choice of such a performance function is a matter of debate [3,4,6]. In the following we consider: (i) unbranched metabolic chains using as performance function the 'transition time' for the conversion of an initial substrate to the end product, and (ii) a skeleton model of the energy metabolism in yeast using as performance function the 'time of survival' during the 'diauxic shift' of metabolism.

Results and discussion

Optimal expression of enzymes in an unbranched chain under time dependent conditions

Let us consider the system with a metabolic chain with enzymes E_i which are coded by the genes G_i (Scheme 1).

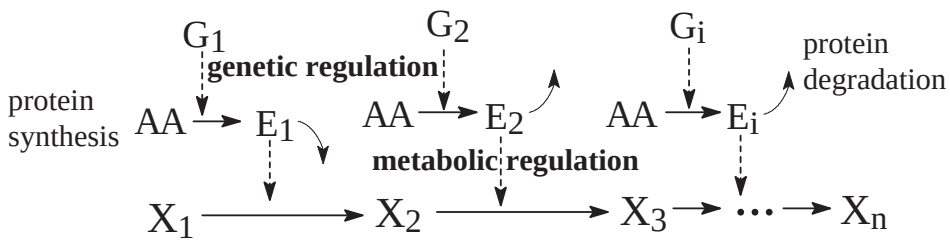

Fig. 25.1 Scheme 1

AA symbolises the amino acid pool from which the enzymes are synthesised. We assume that the protein synthesis is rather fast, such that the enzyme concentrations may be quickly adapted to the metabolic demands. In detail we consider the case where the system depicted in Fig. 25.1 contains only two reaction steps. Starting from $X_1(0) = C$, $X_2(0) = X_3(0) = 0$ we are looking for such a genetic programme of expressing the enzymes E_1 and E_2 that leads to a time optimal conversion of the initial substrate into the end product X_3. In mathematical terms we are interested to determine those functions $E_1(t)$ and $E_2(t)$ minimizing the transition time, τ, that is

$$\tau = \frac{1}{C}\int_0^\infty (C - X_3)dt = \frac{1}{C}\int_0^\infty (X_1 + X_2)dt \to \min \tag{25.1}$$

under the constraint $E_1(t) + E_2(t) = E_{tot} = \text{const.}$ (for general definitions of transition times, see [7,8]). Furthermore, we assume that the rates of the two reactions may be described by the bilinear functions $V_1 = k \cdot E_1 X_1$ and $V_2 = k \cdot E_2 X_2$. A detailed treatment of this mathematical problem leads to the interesting result that

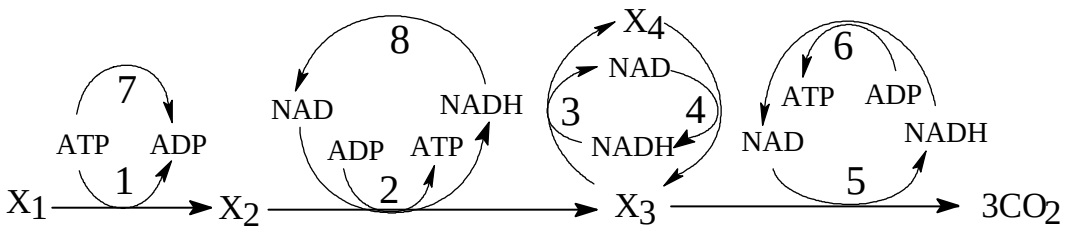

Fig. 25.2 Simplified model of the central metabolism: reaction 1: upper part of glycolysis with consumption of 2 molecules ATP; reaction 2: lower part of glycolysis with formation of 2 molecules ATP and one molecule NADH; reaction 3: pyruvate decarboxylase/alcohol dehydrogenase 1; reaction 4: alcohol dehydrogenase 2 and subsequent reactions leading to the formation of Acetyl-CoA; reaction 5: lumped reactions of the TCA cycle leading to formation of 4 molecules NADH; reaction 6: oxidative phosphorylation; reaction 7: external ATP consumption; reaction 8: external NADH consumption. X_1: glucose; X_2: pool of triose phosphates; X_3: pool of pyruvate and Acetyl-CoA; X_4: ethanol. Systems equations: $V_1 = E_1X_1ATP$, $V_2 = E_2X_2NAD \cdot ADP$, $V_3 = E_3X_3NADH$, $V_4 = E_4X_4NAD$, $V_5 = E_5X_3NAD$, $V_6 = E_6NADHADP$, $V_7 = k_7ATP$, $V_8 = k_8, NADH$ $dNADH/dt = -dNAD/dt = V_2 - V_3 + V_4 + 4V_5 - V_6 - V_8$, $dADP/dt = -dATP/dt = 2V_1 - 2V_2 - 3V_6 + V_7$, $dX_1/dt = -V_1$, $dX_2/dt = 2V_1 - V_2$, $dX_3/dt = V_2 - V_3 + V_4 - V_5$, $dX_4/dt = V_3 - V_4$

the optimal $E_1(t)$ and $E_2(t)$ are not continuous functions. Instead, the enzyme concentrations change in a discontinuous manner between two constant values at a switching time $t = T$. In particular one gets

$$0 \le t < T: \qquad E_1 = E_{tot}, E_2 = 0 \tag{25.2}$$

$$T < t < \infty: \qquad \frac{E_1}{E_{tot}} = \frac{(3-\sqrt{5})}{2} = 0.38196\ldots,$$

$$\frac{E_2}{E_{tot}} = \frac{(-1+\sqrt{5})}{2} = 0.61803\ldots, \tag{25.3}$$

with

$$T = \frac{1}{k \cdot E_{tot}} \ln\left(\frac{2}{3-\sqrt{5}}\right) = \frac{0.96242...}{k \cdot E_{tot}} \tag{25.4}$$

This solution implies that in the first phase ($t < T$): $X_1 + X_2 = C$ and in the second phase ($t > T$): $X_2/X_1 = \text{const}$. It is interesting to note that during the second phase E_{tot} is distributed among the two enzymes according to the 'Golden ratio'. The basic characteristics of the optimal solutions given in eqs. 25.2–25.4 were confirmed by applying numerical methods, in particular by using a genetic algorithm. To this end, the time axis is divided into a finite number of time intervals ΔT. Each 'species' is characterised by a randomly selected distribution of enzyme concentrations along the chain within the different time intervals and the optimum time dependent profiles $E_i(T)$ are determined by 'mutation' and 'selection'.

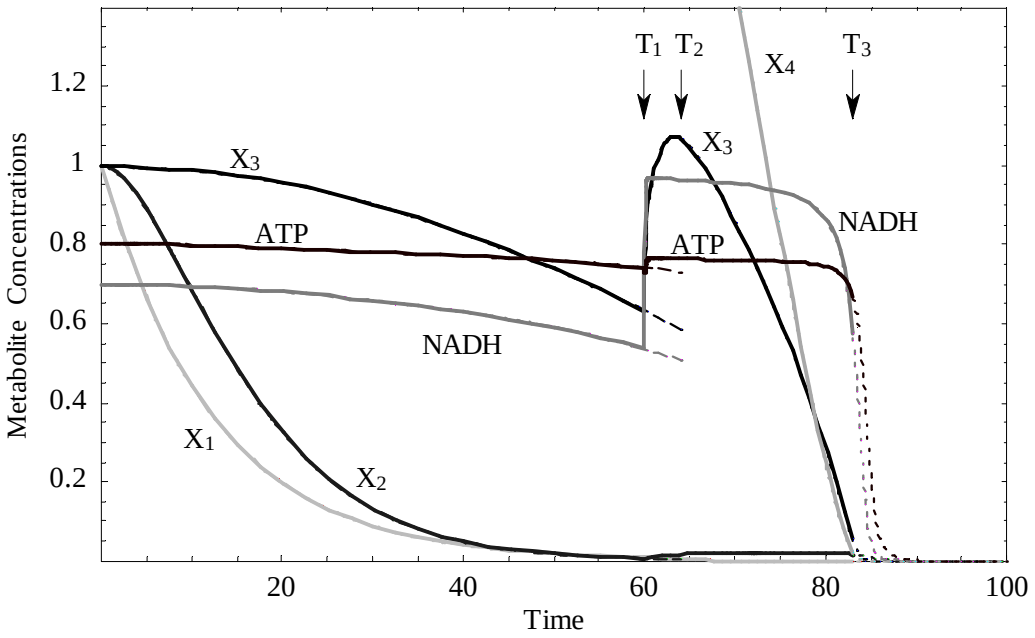

Fig. 25.3 Time course of the metabolite concentrations starting from a steady state at $t = 0$. The first interval lasts from zero till $t = T_1 = 60$, here the enzyme concentrations $E_i^{(1)}$ are valid. The second interval with $E_i^{(2)}$ ends at $t = T_3 = 83.02$, where the ATP concentration droped below the critical value. $T_2 = 64.82$ is the survival time in case that the system is not allowed to switch the enzyme concentrations but has to work the whole time with $E_i^{(1)}$ (dashed lines). Dotted lines indicate the time courses after reaching the critical values (at T_3). Initial values and parameters: $X_1(0) = X_2(0) = X_3(0) = 1$, $X_4(0) = 10$, $NADH(0) = 1 - NAD(0) = 0.7$, $ATP(0) = 1 - ADP(0) = 0.8$, $ATP_{\text{crit}} = 0.7$, $NADH_{\text{crit}} = 0.5$, $k_7 = 3$, $k_8 = 0.1$ (time, concentrations, and rate constants in arbitrary units).

Optimal expression of enzymes of the central metabolism of *Saccharomyces cerevisiae* during diauxic shift

The presented approach is applied to a minimal model of the central metabolism of *S. cerevisiae* to explain the relative changes of the gene expression levels during the diauxic shift as described in [1]. In these experiments it has been observed that the gene expression (RNA levels) for the enzymes of glycolysis and ethanol formation are down regulated during the depletion of glucose whereas the gene expression for enzymes of the TCA cycle, and of some steps of the biosynthetic pathways (gluconeogenesis, glycogen formation) is increased. Similar results have been obtained by cultivating yeast cells under glucose limitation conditions over 250 generations [9]. To account for this behaviour as result of evolutionary optimization we considered a simplified reaction scheme depicted in Fig. 25.2 The considered performance function is the time T the cells may survive given a lim-

Table 25.1 Enzyme concentrations. In the first phase, the $E_i^{(1)}$ are fixed to the steady-state values, in the second phase the $E_i^{(2)}$ may adjust to allow maximal survival time.

Reaction number	$E_i^{(1)}$	$E_i^{(2)}$	$E_i^{(2)}/E_i^{(1)}$
1 ('upper glycolysis')	0.10	0.33	3.27
2 ('lower glycolysis')	2.71	0.78	0.29
3 ('ethanol formation')	4.28	0.23	0.05
4 ('ethanol degradation')	1.00	4.75	4.75
5 ('TCA cycle')	0.54	4.54	8.34
6 ('respiratory chain')	5.32	3.35	0.63

ited initial concentration of glucose. The cells are considered to be alive as long as the concentrations of ATP and NADH are above critical values ATP_{crit} and $NADH_{crit}$. Optimal time courses for the enzyme concentrations $E_i(t)$ of reactions 1 through 6 were determined for the criterion $T \rightarrow \max$.

Starting from a steady state the system is allowed to evolve according to the systems equations. In a first phase, the enzyme concentrations must be the same as in the steady state, in the second phase they can be adjusted (at fixed total enzyme concentration) to yield a longer survival time. Optimal solutions are determined using the genetic algorithm described above. For a certain set of parameters the respective enzyme concentrations are given in Table 25.1 and the time courses of the metabolite concentrations are depicted in Fig. 25.3.

From Fig. 25.3 it becomes obvious that the cell can survive remarkably longer if it is allowed to change the concentrations of the individual enzymes. In the presented case available enzyme is shifted from reactions 2 and 3 ('lower glycolysis' and 'ethanol formation') to reactions 4 and 5 ('ethanol degradation' and 'TCA cycle'), while reactions 1 and 6 are also influenced, but to a lower extend. The storage compound, ethanol, is used in different time regimes depending on whether enzyme concentration switches are allowed or not. The tendency of changes corresponds to the experimental results described in [1,9].

The presented approach is new since it creates for the first time a bridge between two kinds of well elaborated theories: genetic networks for the explanation of genetic activity patterns and metabolic modelling under evolutionary optimization assumptions [3,4,6].

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