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A theory of optimal differential gene expression

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Abstract

We investigate a model of optimal regulation, intended to describe large-scale differential gene expression. Relations between the optimal expression patterns and the function of genes are deduced from an optimality principle: the regulators have to maximise a fitness function which they influence directly via a cost term, and indirectly via their control on important cell variables, such as metabolic fluxes. According to the model, the optimal linear response to small perturbations reflects the regulators' functions, namely their linear influences on the cell variables. The optimal behaviour can be realised by a linear feedback mechanism. Known or assumed properties of response coefficients lead to predictions about regulation patterns. A symmetry relation predicted for deletion experiments is verified with gene expression data. Where the optimality assumption is valid, our results justify the use of expression data for functional annotation and for pathway reconstruction and suggest the use of linear factor models for the analysis of gene expression data.

Keywords: Gene expression; Gene function; Genetic regulation; Linear model; Metabolic control analysis; Optimality principle

1. Introduction

Differential gene expression can provide information about the function of genes. Coregulation of genes has been related to shared function (Brown et al., 2000), interacting proteins (Grigoriev, 2001), or protein complexes (Jansen et al., 2002). Expression data have been used to reconstruct metabolic pathways (Zien et al., 2000) and to annotate genes (Zhou et al., 2002). Expression profiles can be decomposed into linear basis profiles (Alter et al., 2000; MacKay and Miskin, 2001; Fellenberg et al., 2001; Lazzeroni and Owen, 2002; Liebermeister, 2002; Moloshok et al., 2002), some of which are related to the regulation of distinct cellular processes. Although relations between gene function and differential expression (or the regulatory machinery behind it) have often been stated, their theoretical justification is usually not an issue. We show that such relations can be deduced from a principle of optimal regulation.

Regulation of cellular processes can be studied with respect to both its mechanism and its function. A particular gene expression pattern, for instance, can be attributed to a *causa efficiens*, such as a signalling pathway, which physically influences the transcript levels. Accordingly, expression data have been used to identify regulatory motifs in the genome (Brazma et al., 1998; Bussemaker et al., 2001). On the other hand, expression may be explained by a *causa finalis*, namely the fact that the gene products are needed by the cell under the given conditions. Biologists often

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tacitly presume a form of teleonaturalism (Allen, 1999) where "needed by the cell" translates to "increasing the cell's biological fitness, and thus selected for during evolution". The present analysis of optimal regulation is based on the assumption that living organisms do not only have certain optimality properties in their basic (healthy) state but also respond to perturbations in an optimal way. They are assumed to reach a state that is optimal under the new conditions, thus partly compensating for the impairment due to the perturbation. This assumption is corroborated by a huge number of observations, many of which are well-known. For example, blind people develop an improved perception of sounds, and the decrease in blood glucose due to starvation is partly compensated for by the degradation of glycogen.

To assume optimality is certainly an idealisation, but often used as an approximation of biological reality (Edwards et al., 2002; Heinrich et al., 1987, 1996; Klipp and Heinrich, 1999; Segrè et al., 2002). Optimality of flux distributions has been studied (Edwards et al., 2002; Segrè et al., 2002), and theoretical predictions based on optimisation could be validated by experiment (Ibarra et al., 2002). A relation between the optimal regulation of enzymes and their control on fluxes has been derived in (Klipp and Heinrich, 1999). Optimal control of time-dependent processes (Pontryagin et al., 1962) has been studied intensely, and also been applied to control of metabolic systems (Klipp et al., 2002). We propose a quantitative analysis of optimal differential expression (ANODE) in order to formalise intuition about "sensible" expression patterns: a system of regulatory variables x (for instance, gene transcript levels) affects a system of output variables y (see Cornish-Bowden and Cárdenas, 1993), such as metabolic fluxes. The states of both systems are evaluated by a common fitness function $F(x, y)$. We study the behaviour of ideal regulators which always adapt their values such as to maximise the local fitness. Among the output variables y, we shall consider only the "relevant" ones, namely those which actually play a role for the fitness function.

The text is organised as follows: in Section 2, the mathematical model is presented and optimal regulation patterns for different types of external perturbations are derived. A symmetry prediction for gene deletion experiments is tested with experimental data. In Section 3, we shall study the coregulation of regulators which control metabolism or which act in modules. Besides this, predictions for expression data are summarised. Section 4 is concerned with a linear feedback model that realises optimal regulation, with the evolutionary advantage of regulation, and with a relation between expression and growth in the presence of deletions. The results of this work are discussed in Section 5. The appendix contains a mathematical proof and a list of mathematical symbols.

2. Optimal linear regulation of stationary states

To illustrate our approach, let us consider how metabolic systems are controlled by differential expression of enzymes. Metabolic fluxes depend on cellular processes that produce or consume metabolites, on environmental parameters like nutrient supply, and on parameters influencing the enzymatic activities, such as temperature. In addition, metabolism is actively controlled by regulatory processes on different time scales: while fast responses are realised by activation and inhibition of enzymes, slow adaptation can be achieved by adjusting their expression. The linear influence of enzyme concentrations E_k on stationary fluxes J_i is quantified by the response coefficients matrix R^J_E. Metabolic control theory (Heinrich et al., 1996; Kahn and Westerhoff, 1991) describes how fluxes respond to changes of enzymes, which may be caused by changes in gene expression. One may also ask the inverse question: which enzyme changes are necessary to achieve a desired metabolic behaviour, such as homoeostasis or constrained maximisation of fluxes?The answer to this question depends on (1) the control of enzyme activities on metabolism, as studied by metabolic control analysis, and (2) assumptions about the objectives of the cell, described by a fitness function.

The performance of cellular subsystems can be rated by their contribution to the evolutionary fitness of the organism, that is, the expected long-term reproduction of the organism. In a particular environment, a few fluxes may effectively determine biomass production. For a metabolic system, we may consider a simple fitness function $V(J)$ scoring only those important fluxes, and assume that there is an evolutionary tendency to maximise this function. Such an objective function was studied previously (Heinrich et al.,

1987), notably the (mathematical) product of the two independent fluxes in a reaction system representing glucose metabolism. In the whole cell, many processes depend on common resources, so an optimal compromise must be chosen. The enzyme levels can adapt the metabolic system to external fluctuations and will thereby effectively increase the fitness, but enzyme production itself consumes cellular resources, which can be described by a negative contribution $U(E)$ to the total fitness $F(E, J) = U(E) + V(J)$. The optimal behaviour with respect to F represents a compromise between benefit and costs (Reich, 1983).

As a simple example (shown in Fig. 1), let us consider a chain of two chemical reactions $S_0 \leftrightarrow S_1 \leftrightarrow S_2$ with mass-action kinetics

$$\begin{aligned} v_1 &= k_1 E_1 S_0 - k_{-1} E_1 S_1 \\ v_2 &= k_2 E_2 S_1 - k_{-2} E_2 S_2 \end{aligned} \tag{1}$$

where E_1 and E_2 denote the enzyme concentrations. At fixed concentrations S_0 and S_2, the stationary flux $J = v_1 = v_2$ reads

$$J = \frac{E_1 E_2 (S_0 k_1 k_2 - S_2 k_{-1} k_{-2})}{E_1 k_{-1} + E_2 k_2} \tag{2}$$

A reasonable and frequently used ansatz for the fitness function is to use the flux itself $V(J) = J$ (Heinrich et al., 1987, 1996; Savinell and Palsson, 1992), while the enzyme levels are rated by a negative function

$$U(E) = -\gamma_1 (E_1 + E_2) - \gamma_2 (E_1 + E_2)^2 \tag{3}$$

The linear term describes costs per protein molecule, e.g., for the consumption of amino acids. High rates of protein synthesis require additional efforts, for instance, an increased production of ribosomes, which is punished by the quadratic term.

Maximising the effective fitness

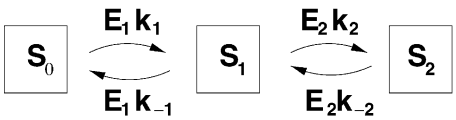
$$\begin{aligned} G(E) \equiv F(E, J(E)) &= -\gamma_1 (E_1 + E_2) \\ &\quad - \gamma_2 (E_1 + E_2)^2 + J(E_1, E_2) \end{aligned} \tag{4}$$

with respect to E_1 and E_2 yields unique optimal enzyme levels $(\bar{E}_1, \bar{E}_2)$. A small perturbation of the parameters, such as the concentrations of external metabolites or the rate constants, changes the fitness landscape $G(E)$. The optimum is shifted (see Fig. 1, right), but the enzyme levels can be adapted to reach new optimal values.

We shall now determine a linear approximation for the optimal response, written in the differential notation $\mathrm{d}\bar{E}$. At the local optimum, the gradient of the fitness function with respect to the enzyme concentration vanishes

$$0 = G_E \equiv \nabla_E G = \begin{pmatrix} \dfrac{\partial G}{\partial E_1} \\ \dfrac{\partial G}{\partial E_2} \end{pmatrix} \tag{5}$$

The total differential of G_E can be written as $\mathrm{d}G_E = \mathrm{d}\hat{G}_E + G_{EE}\,\mathrm{d}E$, where the first term $\mathrm{d}\hat{G}_E$ describes a change due to the parameter perturbation. The second



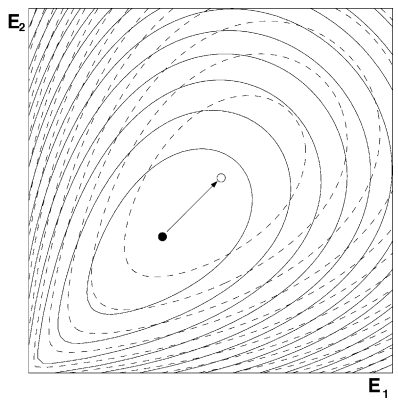


Fig. 1. Adaptation of enzyme levels. A linear chain of two reactions (left) is controlled by two enzymes E_1 and E_2. Their performance is evaluated by a fitness function $G(E_1, E_2)$. In this example, the fitness is given by the stationary flux J minus the costs $U(E_1, E_2)$ of protein production. The right diagram shows the fitness landscape $G(E_1, E_2)$, for two values of the external substrate S_0 (solid and dashed contour lines, respectively). The perturbation of S_0 causes a shift of the optimum, indicated by the arrow.

term $G_{EE}\,\mathrm{d}E$ reflects an adaptation $\mathrm{d}E = (\mathrm{d}E_1 \mathrm{d}E_2)^T$ of the enzymes, with

$$G_{EE} \equiv \begin{pmatrix} \dfrac{\partial^2 G}{\partial E_1^2} & \dfrac{\partial^2 G}{\partial E_1 \partial E_2} \\ \dfrac{\partial^2 G}{\partial E_2 \partial E_1} & \dfrac{\partial^2 G}{\partial E_2^2} \end{pmatrix} \tag{6}$$

The optimal adaptation $\mathrm{d}\bar{E}$ must ensure that Eq. (5) holds after the perturbation as well, so the respective differential $\mathrm{d}\bar{G}_E$ has to vanish, and therefore

$$0 = G_{EE}\,\mathrm{d}\bar{E} + \mathrm{d}\hat{G}_E \tag{7}$$

$$\rightarrow \mathrm{d}\bar{E} = -G_{EE}^{-1}\,\mathrm{d}\hat{G}_E \tag{8}$$

This example illustrates what we shall now tackle in a general way. A long-term objective is to explain correlations in genome-wide differential expression data. Calculating the optimal expression values would in principle require a model of the whole cell. However, the optimal response to small perturbations can be predicted from local properties of the model (namely derivatives) at the initial optimal state, so knowledge about metabolic response coefficients can be used to predict the coregulation of genes.

2.1. The mathematical model

The model of optimal regulation proposed in this section describes biological regulators which control stationary states. The cell state is described by a set of output variables y that depend on regulatory variables x and on environmental parameters α. The symbols x, y, and α denote vectors. Small changes of y are expanded as

$$\Delta y(x,\alpha) \approx (R_y^x R_\alpha^y) \begin{pmatrix} \Delta x \\ \Delta\alpha \end{pmatrix} + \frac{1}{2} \begin{pmatrix} \Delta x \\ \Delta\alpha \end{pmatrix}^{\mathrm{T}} \times \begin{pmatrix} R_{xx}^y & R_{x\alpha}^y \\ R_{\alpha x}^y & R_{\alpha\alpha}^y \end{pmatrix} \begin{pmatrix} \Delta x \\ \Delta\alpha \end{pmatrix} \tag{9}$$

The linear influences of the regulators and the environment on y are described by the response coefficients R_x^y and R_α^y (Heinrich et al., 1996). The second-order response coefficients R_{xx}^y and $R_{x\alpha}^y$ describe the quadratic effects of x and α (Höfer and Heinrich, 1993). Both x and y are rated by a fitness function $F(x, y)$ which, for simplicity's sake, is assumed to have the additive form (see also Reich, 1983; Savinell and Palsson, 1992)

$$F(x, y) = U(x) + V(y) \tag{10}$$

The gradient $F_y = \nabla_y F(x, y)$ will be called the marginal fitness of y, in analogy to the marginal utility defined in economics (Henderson and Quandt, 1980). The marginal fitness $F_x = \nabla_x F(x, y)$ of x is defined accordingly. The matrices F_{xx} and F_{yy} of second-order derivatives contain the curvatures of the fitness function. If U is a sum of terms depending on the individual regulators, then F_{xx} is diagonal. Sometimes an "isotropic" case will be considered where F_{xx} is a scalar times the identity matrix I. The effective fitness $G(x, \alpha) \equiv F(x, y(x, \alpha))$ is a function of x and α alone, with derivatives

$$\begin{aligned} G_x(x,\alpha) &= \nabla_x F(x, y(x,\alpha)) = F_x + R_x^{y^T} F_y \\ G_{xx}(x,\alpha) &= \nabla_x \nabla_x^T F(x, y(x,\alpha)) \\ &= F_{xx} + R_x^{y^T} F_{yy} R_x^y + T_{xx} \end{aligned} \tag{11}$$

as $F_{xy} = 0$ (see Eq. (10)). T_{xx} represents the tensor product[1] $(T_{xx})_{ik} \equiv (F_y)_l (R_{xx}^y)_{ik}^l$. It describes an effective fitness curvature due to the cooperation of regulators, for instance gene products acting in a complex, such as in metabolic channelling (Cornish-Bowden and Cárdenas, 1993). Instead of assuming the cost term $U(x)$, one could describe the costly side-effects of gene expression by additional output variables y. The x-dependent fitness term F_{xx} would then reappear as a part of T_{xx}.

The optimality principle postulates that, for any given α, the regulators assume a value $\bar{x}(\alpha)$ to reach a local fitness maximum (see Fig. 2, right). Optimality at $\bar{x}(\alpha)$ implies that G_x vanishes, so F_x and F_y are balanced according to

$$F_x = -R_x^{y^T} F_y \tag{12}$$

To ensure a unique local maximum, the effective fitness curvature matrix G_{xx} must have negative eigenvalues, so G_{xx} is invertible. If the number of regulators exceeds the number of output variables,

[1] Superscripts and subscripts represent variables and derivatives, respectively. According to the sum convention, terms are summed over all indices which occur both as superscript and as subscript.

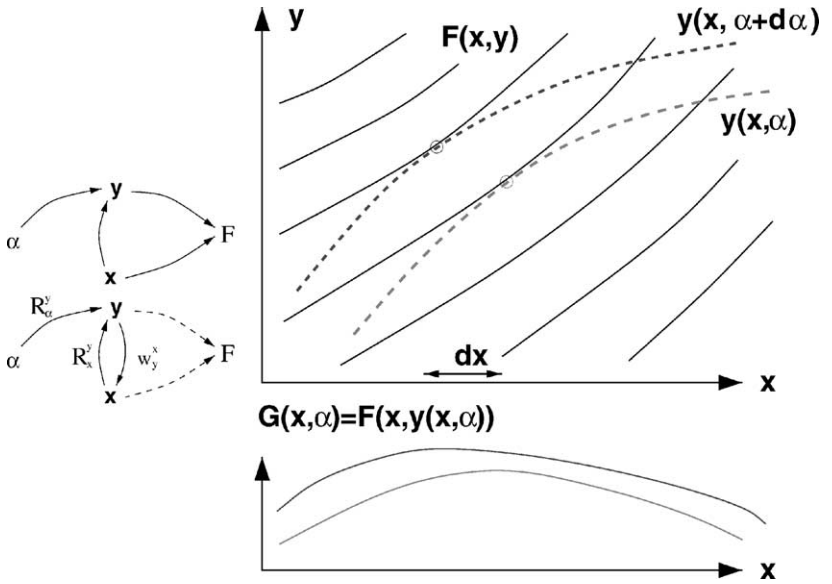


Fig. 2. Model of optimal regulation. Top left: The system variables y depend on the environment α and on the regulators x. The fitness function F scores both x and y. Bottom left: The optimal behaviour can be implemented by feedback signals between y to x (see Section 4.1). Optimality (dotted arrows) is ensured by an appropriate choice of the feedback coefficients R_y. Right: Optimal response to a perturbation of y. A one-dimensional case is shown while in general, x, α and y are multidimensional. For fixed environment α, y is a function $y(x, \alpha)$ of x (shown by dashed lines, for two values of α). The slope of this line is called the response coefficient R^y_x. The fitness function $F(x, y)$ (shown by solid contour lines), evaluated on the constraint line, yields the effective fitness $G(x, \alpha)$ (shown below). After a change of α, x has to adapt itself to reach again an optimal state (dots) maximising G.

then $R^{y^T}_x F_{yy} R^y_x$ in Eq. (11) has some vanishing eigenvalues, but by an appropriate choice of $U(x)$, a maximum can be ensured.

In the following, we shall study regulators in an optimal state which encounter a perturbation: two scenarios are studied, namely perturbations of y by perturbation of α, and perturbations of individual regulators x_i. In both cases, the optimal response $\mathrm{d}\bar{x}$ to maximise $\mathrm{d}F$ will be calculated in a local approximation. Concerning the initial optimal state, some simplifying assumptions are made: locally, all values of y can be reached by an appropriate choice of x, that is, R^y_x has full row rank. This implies that the dimension of y does not exceed the dimension of x and that $R^y_x F^{-1}_{xx} R^{y^T}_x$ is invertible. In general, the fitness function may depend on additional parameters, and the output variables may not be controlled independently. Formulae for these cases, additional model properties, and proofs for the formulae in the following sections can be found in Liebermeister, 2004.

2.2. Adaptation to a perturbation of output variables

Let us consider the optimal response to external perturbations of y, where α changes by a small amount $\mathrm{d}\alpha$. If the regulators remained constant ($\mathrm{d}x = 0$), y, F_y, and R^y_x would change by $\mathrm{d}\hat{y} \equiv R^y_\alpha \mathrm{d}\alpha$, $\mathrm{d}\hat{F}_y \equiv F_{yy} R^y_\alpha \mathrm{d}\alpha$, and $\mathrm{d}\hat{R}^y_x$, respectively, where the latter is defined by the tensor product $(\mathrm{d}\hat{R}^y_x)^l_i \equiv (R^y_{x\alpha})^l_{ik} \mathrm{d}\alpha^k$. In this text, two sorts of differentials will be distinguished: those with a circumflex (e.g., $\mathrm{d}\hat{y}$) denote changes due to an external perturbation for fixed x, while those with a bar (e.g., $\mathrm{d}\bar{y}$) contain the additional effect of an optimal response $\mathrm{d}\bar{x}$. Our objective is to determine $\mathrm{d}\bar{x}$ to maximise the fitness $G(x + \mathrm{d}\bar{x}, \alpha + \mathrm{d}\alpha)$. This requires that $\mathrm{d}\bar{G}_x$ must vanish, leading to (proof: Appendix A).

$$\mathrm{d}\bar{x} = -G^{-1}_{xx} \mathrm{d}\hat{G}_x \tag{13}$$

where

$$\mathrm{d}\hat{G}_x = R^{y^T}_x \mathrm{d}\hat{F}_y + \mathrm{d}\hat{R}^{y^T}_x F_y$$

The terms contributing to $\mathrm{d}\hat{G}_x$ describe two effects of the perturbation: $\hat{G}_x$ may change the marginal fitness of y, and it may also change the regulatory properties expressed by R_x^y. The latter happens, for instance, as enzymatic parameters are changed in a metabolic network.

While Eqs. (13) and (14) are a very general result, simple consequences can be drawn if the second effect is negligible because F_y or $R_{x\alpha}^y$ is sufficiently small. With this simplification, the optimal response reads

$$\mathrm{d}\bar{x} = -(F_{xx} + T_{xx} + R_x^{y^T} F_{yy} R_x^y)^{-1} R_x^{y^T} F_{yy} R_\alpha^y \,\mathrm{d}\alpha \tag{14}$$

The symmetric matrix T_{xx} can be formally incorporated into an effective fitness curvature $F_{xx}^* = F_{xx} + T_{xx}$. Note that only the second derivatives of the fitness appear in the formula, because the first derivatives are initially balanced (see Eq. (12)).

Instead of being neglected, the second term in Eq. (14) can also be incorporated into the first one. This is possible if the normalised response coefficients $x_k/y_i(R_x^y)_{ik}$, which describe relative influences, remain constant, because then

$$\frac{(\mathrm{d}R_x^y)_{ik}}{(R_x^y)_{ik}} = \frac{\mathrm{d}y_i}{y_i} \rightarrow \mathrm{d}R_x^y = \mathrm{dg}(\mathrm{d}y)\,\mathrm{dg}(y)^{-1} R_x^y \tag{15}$$

The symbol $\mathrm{dg}(y)$ denotes a diagonal matrix with the elements of the vector y in its diagonal.

The last term of Eq. (14) can be rewritten as

$$R_x^{y^T} \mathrm{dg}(F_y)\,\mathrm{dg}(y)^{-1}\,\mathrm{d}\hat{y} \tag{16}$$

and be incorporated into the first term: bearing in mind that $\hat{F}_y = F_{yy}\,\mathrm{d}\hat{y}$, we obtain

$$\mathrm{d}\hat{G}_x = R_x^{y^T}(F_{yy}) + \mathrm{dg}(F_y)\,\mathrm{dg}(y)^{-1}\mathrm{d}\hat{y} = R_x^{y^T} F_{yy}^*\,\mathrm{d}\hat{y} \tag{17}$$

So effectively the second term has disappeared, while F_{yy}^* contains an additional contribution $\mathrm{dg}(F_y)\mathrm{dg}(y)^{-1}$. Is it a reasonable assumption that normalised response coefficients are not affected by perturbations? For a linear reaction chain with linear kinetics, the normalised response coefficients do indeed not depend on the substrate concentration (Heinrich et al., 1996), while they do depend on the enzyme parameters. Thus, the assumption holds for a perturbation of the substrate, but not for a perturbation of enzyme parameters.

2.3. Obtaining a change of the output variables

Let us now consider a different scenario where a fixed change $\mathrm{d}y$ must be obtained by the regulators. Under the constraint that $\mathrm{d}y = R_x^y \mathrm{d}\bar{x}$, the fitness is maximised by[2]

$$\mathrm{d}\bar{x} = F_{xx}^{-1} R_x^{y^T} (R_x^y F_{xx}^{-1} R_x^{y^T})^{-1} \mathrm{d}y \tag{18}$$

In the isotropic case, this reduces to $\mathrm{d}\bar{x} = R_x^{y^+}\mathrm{d}y$, with the pseudoinverse[3] of R_x^y. If the fitness term U rates the regulators separately, F_{xx} is diagonal, and the diagonal elements $(F_{xx})_{ii}$ appear as weights in the formula: a large negative curvature leads to a weak response of the respective regulator $\mathrm{d}\bar{x}_i$. For reasons of consistence, Eq. (18) must also hold for any optimal response $\mathrm{d}y = \mathrm{d}\bar{y} - \mathrm{d}\hat{y}$ after a perturbations $\mathrm{d}\alpha$. Thus, we obtain the important result that for isotropic F_{xx}, any optimal expression profile is a linear combination of regulatory profiles, that is, the rows of R_x^y. On the other hand, if y must keep its original value despite a perturbation $\mathrm{d}\alpha$, the actual change $R_x^y \mathrm{d}\bar{x} + R_\alpha^y \mathrm{d}\alpha$ has to vanish, so we set $\mathrm{d}y = -R_\alpha^y \mathrm{d}\alpha$.

2.4. Adaptation to a perturbation of individual regulators

Besides perturbations of the output variables y, we can study perturbations of individual regulators x. In the case of gene expression, such perturbations may be realised by gene deletions (Hughes et al., 2000) or RNA interference (Fire, 1999), or may result from hereditary enzyme deficiencies. In the model, one regulator is moved away from the local optimum of the fitness landscape $G(x, \alpha)$, and the others can compensate for the loss. Let us assume that regulator x_i is changed[4] by a fixed value $\mathrm{d}\hat{x}_i$, that is,

[2] Proof: The optimal $\mathrm{d}\bar{x}$ is determined by the condition $F(x + \mathrm{d}\bar{x}, y + \mathrm{d}y) = \max$ with the constraint $R_x^y \mathrm{d}\bar{x} = \mathrm{d}y$, which can be solved using Lagrangian multipliers.

[3] The pseudoinverse of a matrix A is defined as $A^+ \equiv (A^{\mathrm{T}}A)^{-1}A^{\mathrm{T}}$.

[4] Alternatively, the perturbation can be modelled as a marginal fitness change $\mathrm{d}\hat{G}_x = F_{x\beta}\,\mathrm{d}\hat{\beta}$ due to an additional parameter β in the fitness $G(x, \alpha, \beta)$. The optimal response then reads $\mathrm{d}\bar{x} = -G_{xx}\,\mathrm{d}\hat{G}_x$.

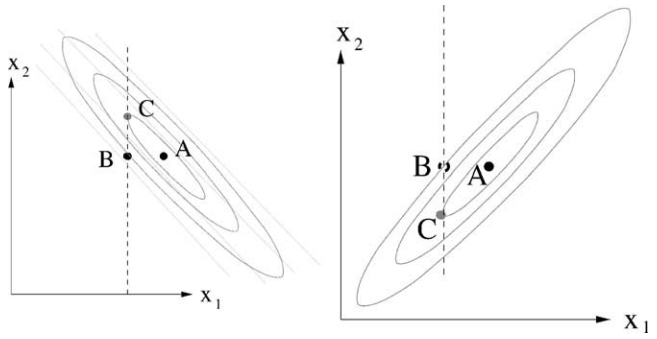


Fig. 3. Interplay between regulators in the fitness landscape $G(x, \alpha)$ for fixed environment α. Left: The fitness G with respect to two genes x_1 and x_2 has elliptic contour lines, with the optimum in the centre (A). Constraining x_1 to a smaller value (dashed line) would decrease the fitness (B). An activation of x_2 damps the fitness loss (C). The fitness landscape shown may result from a gene duplication: if both genes exert the same influence on y and if $F_{xx} = 0$, then the maximum of the fitness (diagonal contour lines) is non-unique. A finite term F_{xx} regularises the effective fitness function G (i.e., it causes all eigenvalues of G_{xx} to be nonzero), which leads to the elliptic contour lines. The term F_{xx} can be caused by a nonlinear effect in the cost term $U(x)$: for example, it might be more costly to synthesise two isoenzymes than to synthesise one enzyme with a given function. Right: Cooperation can be induced by second-order response terms R^y_{xx} contributing to F^*_{xx}. If genes x_1 and x_2 are both necessary for the same process, they tend to be coregulated.

$\mathrm{d}\hat{x} \equiv (0 \cdots 0\, \mathrm{d}\hat{x}_i\, 0 \cdots 0)^T$. The systemic response of the other regulators reads[5]

$$\mathrm{d}\bar{x} = G_{xx}^{-1} \frac{1}{(G_{xx}^{-1})_{ii}} \mathrm{d}\hat{x} \tag{19}$$

The small perturbation of a single gene leads to a fitness loss

$$\mathrm{d}^2 G = \frac{1}{2} \mathrm{d}\bar{x}^T G_{xx} \mathrm{d}\bar{x} = \frac{1}{2} \frac{(\mathrm{d}\hat{x}_i)^2}{(G_{xx}^{-1})_{ii}} \tag{20}$$

Small diagonal elements of G_{xx}^{-1} imply large fitness losses and may indicate essential genes.

Depending on the curvatures of the effective fitness landscape, gene pairs will either show coregulation or anti-coregulation as one of the genes is deleted (see Fig. 3). Both kinds of behaviour are possible even for genes exerting the same first-order control, described by R^y_x. Cooperating genes may also be coregulated on an evolutionary time-scale, by mutations: if one gene is deleted, a deletion of the second one may become an advantage. Thus, pairs of cooperating genes may become visible in phylogenetic profiles (Pellegrini et al., 1999), while pairs of genes compensating for each other should show phylogenetic anti-correlation (Morett et al., 2003).

2.5. Symmetric compensation of deletions

Let us consider a deletion experiment in which, in the ith sample, gene x_i (logarithmic expression value) is downregulated by $\mathrm{d}\hat{x}_i$. According to Eq. (19), the expression matrix X with the experiments in the rows should be decomposable into

$$X = G_{xx}^{-1} D \tag{21}$$

where D is diagonal. The symmetry of G_{xx}^{-1} implies a symmetric relation between the genes: if the loss of gene A leads to an activation of gene B, gene A should also be activated after the loss of gene B. Matrices derived from experimental data according to Eq. (21) were tested for their symmetry (see Fig. 4): Ideker et al. (2001) studied deletions of enzymes in the galactose pathway. The estimate[6] of G_{xx}^{-1} ac-

[5] Proof: The optimal regulatory profile $\mathrm{d}\bar{x}$ has to fulfil $G_x(x + \mathrm{d}\bar{x}, \alpha) - \lambda\, \mathrm{d}\hat{x} = 0$, where λ is a Lagrangian multiplier. We expand $G_x(x + \mathrm{d}\bar{x}, \alpha) \approx G_x(x, \alpha) + G_{xx}\, \mathrm{d}\bar{x}$. As $G_x(x, \alpha) = 0$ for the unperturbed state, $\mathrm{d}\bar{x} = \lambda G_{xx}^{-1}\, \mathrm{d}\hat{x}$. From $\mathrm{d}\bar{x}_i = \mathrm{d}\hat{x}_i$ follows $\lambda = 1/(G_{xx}^{-1})_{ii}$.

[6] We adjusted the column and row means of the whole data set (log 10 expression ratios) to zero and chose all available data to construct a matrix related to the genes GAL1, GAL2, GAL3, GAL4, GAL7, and GAL10 and the respective knock-out mutants. We then calculated the difference matrix X between the respective "+gal" and "-gal" samples and determined a diagonal matrix D such that the mean squares for the rows of XD^{-1} were similar to those of the columns. To do so, we iteratively normalised the matrix rows by the ratio between the sum of squares within columns and within rows.

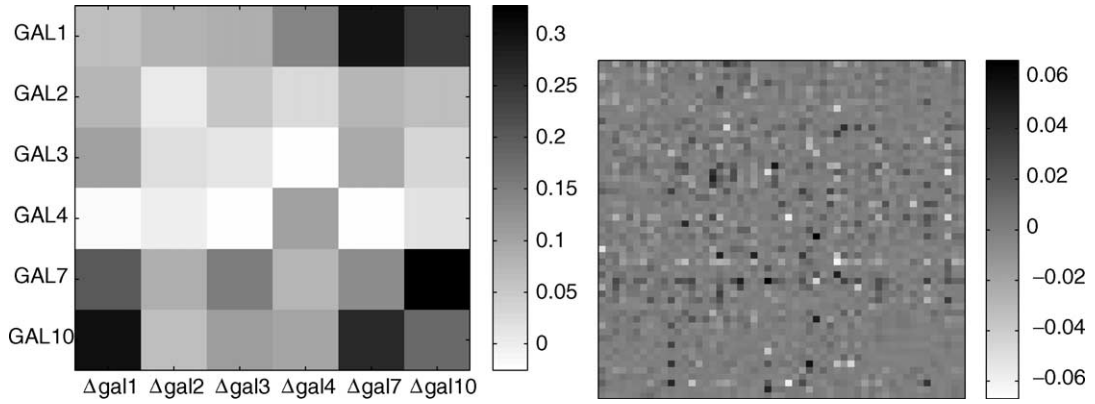


Fig. 4. Symmetric response to deletions. We studied expression matrices from deletion experiments: the columns correspond to deleted genes, while the rows correspond to the measurement of the same genes. According to Eq. (21), matrices were decomposed into a diagonal matrix and an estimate of the inverse fitness curvature matrix G_{xx}^{-1}. Symmetry of the reconstructed G_{xx}^{-1} was tested for two data sets. Left: The matrix extracted from Ideker et al. (2001) shows a strong symmetric part. Right: Matrix extracted from Hughes et al. (2000). The symmetric part is weak, but significant (see text).

cording to Eq. (21) shows a strong symmetric part. Hughes et al. (2000) deleted 248 genes[7] of various functions: here G_{xx}^{-1} shows only weak symmetry. The reason may be that many genes knocked out were transcription factors of various functions, so we can expect weak off-diagonal elements in G_{xx}. However, for metabolic genes, the matrix still contains a significant symmetric part[8]. Thus reciprocal compensation is found within the galactose pathway, but much less between different functional subsystems of the cell. It is questionable whether a gene deletion can be treated as a small perturbation. In some cases, this may indeed be the case, notably if the effects of the deletion are sufficiently buffered by the adaptation of other genes.

[7] Some genes were represented by more than one ORF.

[8] Only the 53 genes annotated with an EC number, according to KEGG (Kanehisa et al., 2002), were chosen. Values for which the estimated error of log ratios exceeded 2 or two times the absolute value were neglected, and variance stabilisation (Huber et al., 2002) was applied to the remaining values. For determining D, the neglected values were formally set to 0. The symmetry of the resulting matrix is weak. To decide whether the symmetric part was still significant, the standard deviations of the symmetric and antisymmetric parts (for the "reliable" off-diagonal elements) were calculated. The ratio of about 1.7 has a P-value of about 0.01, according to a permutation test in which the order of the matrix rows was randomised 500 times.

2.6. A cascade of responses distributes the perturbation

Near a fitness maximum, a regulatory system x buffers fitness fluctuations, in analogy to le Châtelier's principle, and this buffering can be described by a cascade of responses. Let us recall Eq. (13): if the marginal effective fitness of the regulators is perturbed by an amount $\mathrm{d}\hat{G}_x$, the matrix G_{xx}^{-1} describes how this perturbation becomes distributed over the whole system. If the fitness curvature with respect to x is high, that is, if $F_{xx}^{-1} R_x^{y^T} F_{yy} R_x^y$ has small absolute eigenvalues, then G_{xx}^{-1} can be expanded into a power series (compare Heinrich, 1985)

$$G_{xx}^{-1} = (1 + F_{xx}^{-1} R_x^{y^T} F_{yy} R_x^y)^{-1} F_{xx}^{-1} = \sum_{n=0}^{\infty} (-F_{xx}^{-1} R_x^{y^T} F_{yy} R_x^y)^n F_{xx}^{-1} \quad (22)$$

The series describes superposed responses of different order: an immediate response to the perturbation, which may have unfavourable side-effects, a response to these effects, and so on. The complete response represents a systemic compromise between all effects of the regulators. It has to be stressed that the cascade does not describe time-dependent behaviour. On the other hand, if $F_{xx} + T_{xx}$ is small ($F_{xx} + T_{xx} \to 0$),

Eq. (14) yields

$$\mathrm{d}\bar{x} \approx -R_x^{y^+} F_{yy}^{-1} \mathrm{d}\hat{F}_y \tag{23}$$

as it would result from optimising first $\mathrm{d}y$, and then $\mathrm{d}x$.

3. Control of structured systems

3.1. Coregulation

Let us consider a large number of perturbations applied in separate experiments. Coregulation of genes can be quantified by the linear correlation, that is, the covariance between gene profiles, normalised by the square roots of their variances. Let us assume that the second term in Eq. (14), describing a perturbation of the response coefficients, can be neglected. In this case, a simple relation holds between the optimal regulation pattern $\mathrm{d}\bar{x}$ and the marginal fitness change $\mathrm{d}\bar{F}_y$

$$\mathrm{d}\bar{x} = -F_{xx}^{-1} R_x^{y^T} \mathrm{d}\bar{F}_y \tag{24}$$

Given the covariance matrix cov $\mathrm{d}\hat{F}_y$ between the marginal fitness perturbations of y, the covariance matrix between the responses $\mathrm{d}\bar{x}_i$ reads

$$\begin{aligned}\mathrm{cov}(\mathrm{d}\bar{x}) &= G_{xx}^{-1} R_x^{y^T} \mathrm{cov}(\mathrm{d}\hat{F}_y) R_x^y G_{xx}^{-1} \\ &= F_{xx}^{-1} R_x^{y^T} \mathrm{cov}(\mathrm{d}\bar{F}_y) R_x^y F_{xx}^{-1}\end{aligned} \tag{25}$$

For strong isotropic fitness curvature ($F_{xx} \to -\infty$), this becomes, in first order

$$\mathrm{cov}(\mathrm{d}\bar{x}) \propto R_x^{y^T} \mathrm{cov}(\mathrm{d}\hat{F}_y) R_x^y \tag{26}$$

In this approximation, two genes are coregulated if they have strong effects on the same variables, or on variables with large common marginal fitness fluctuations. Accordingly, cooperating enzymes are likely to be coregulated, as it was empirically shown for interacting proteins (Grigoriev, 2001), permanent protein complexes (Jansen et al., 2002), and subsets of cooperating enzymes (Schuster et al., 2002).

3.2. Metabolic systems

We shall now consider the control of a metabolic system where the variables y represent stationary fluxes J or concentrations S, while the regulators x represent enzyme activities E. Here the summation and connectivity theorems of metabolic control theory (Heinrich et al., 1996) imply relations between the optimal regulation patterns and the structure and kinetics of the metabolic network.

A metabolic system (Heinrich et al., 1996; Heinrich and Schuster, 1998) can be characterised by the following quantities: the stoichiometric matrix N contains the stoichiometric coefficients, each column describing one of the reactions. K is a maximal kernel matrix of stationary fluxes, fulfilling $NK = 0$. The link matrix L (Reder, 1988) is defined by $N = LN^0$, where N^0 contains a maximal set of linearly independent rows of N. By relating the concentrations of all metabolites to those of the independent ones, the link matrix describes the conservation relations. The elasticities $\epsilon_k^i \equiv \mathrm{d}v_i/\mathrm{d}S_k$ describe how the reaction velocities depend on the metabolite concentrations, in a linear approximation. Thus, the columns of ϵL contain the immediate change in reaction rates, as the concentration of an independent metabolite is changed. The response coefficients R_E^S and R_E^J describe the linear influence of enzyme concentrations (regarded as parameters) on steady state quantities S and J and can be decomposed into a product $R_E^J = C^J \pi_E$ (similar for R_E^S). The elasticities π_E describe the linear influence of the enzyme concentrations on the reaction rates. Systemic effects of the local perturbation are expressed by the control coefficients C^J and C^S describing the change of steady-state concentrations or fluxes due to a small parameter change affecting only the kth reaction.

$$(C^J)_k^i \equiv \frac{\partial J_i/\partial p}{\partial v_k/\partial p} \tag{27}$$

$$(C^S)_k^i \equiv \frac{\partial S_i/\partial p}{\partial v_k/\partial p} \tag{28}$$

The control coefficients can be calculated by (see Heinrich et al., 1996)

$$C^S = -L(M^0)^{-1} N^0 \quad \text{where} \quad M^0 = N^0 \epsilon L \tag{29}$$

$$C^J = 1 + \epsilon C^S$$

They fulfil the summation and connectivity theorems (see Heinrich et al., 1996) of metabolic control theory

$$\begin{pmatrix} C^J \\ C^S \end{pmatrix} (K \quad \epsilon L) = \begin{pmatrix} K & 0 \\ 0 & -L \end{pmatrix} \tag{30}$$

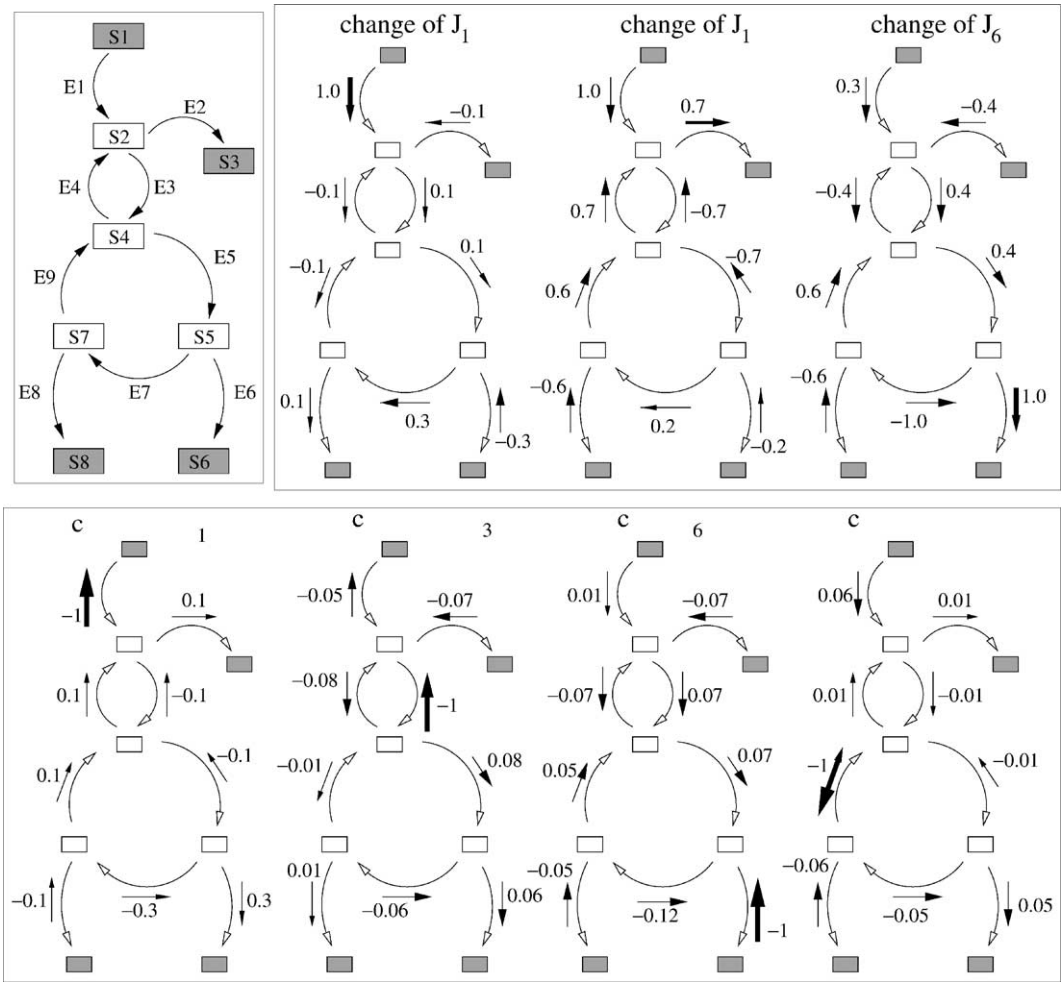


Fig. 5. Optimal regulation of a metabolic model system. We consider a simple network of irreversible reactions (top left box) containing eight metabolites (shown as rectangles). The metabolites S_1, S_3, S_6, and S_8 (shaded) are considered external. Each reaction J_i is catalyzed by an enzyme (regulator) E_i. The fitness function depends on the fluxes J_1, J_2, and J_6, and on the enzyme concentrations. Top right: Each diagram shows the optimal response to a specific perturbation of J_1, J_2, or J_6, respectively. The effect of the adaptation is shown by the arrows: arrowheads indicate the direction of the immediate flux change $R_E^t \mathrm{d}\bar{E}$ caused by regulation. The numbers denote the adaptations $\mathrm{d}\bar{E}_i$, normalised to $\max(|\mathrm{d}\bar{E}_i|) = 1$ for each diagram. All enzymes are involved in the systemic response, which counteracts the initial perturbation. Bottom: In each diagram, one of the enzymes E_1, E_3, E_6, E_9 (indicated by a thick arrow) is inhibited, that is, constrained to a lower value. The remaining enzymes adapt themselves and damp the perturbation.

Optimal regulation of metabolic fluxes is illustrated in Fig. 5: as an example, we consider a simple network of irreversible reactions, containing 8 metabolites, four of which are external. Each reaction J_i is catalyzed by an enzyme E_i. A value of one was chosen for the elasticity between a reaction and its substrate, while all other elasticities vanish. The fitness function depends on the fluxes J_1, J_2, and J_6, and on the enzyme concentrations. The "relevant" fluxes J_1, J_2, and J_6 are evaluated by a fitness function with the local curvature matrix $V_{JJ} = -I$. A function U with equal curvatures $U_{EE} = -I$ describes the fitness contribution of the enzyme levels E_i. The slopes of the fitness do not appear in the formulae and thus need not be specified. For illustration, we assume specific external perturbations that decrease one of the relevant fluxes

while leaving the others unchanged. We consider the two scenarios from Sections 2.2 and 2.4: the diagrams in the upper box show the optimal response (according to Eq. (14)) to a specific perturbation of J_1, J_2, or J_6, respectively. In each diagram in the lower box, one of the enzymes E_1, E_3, E_6, E_9 is inhibited, that is, constrained to a lower value. The remaining enzymes adapt themselves optimally, according to Eq. (19). For both scenarios, all enzymes respond in a coordinated way: fluxes in the whole system are redirected to increase the perturbed flux, and thus to damp the perturbation.

3.3. Consequences of the metabolic theorems

If the output variables describe metabolic fluxes or concentrations, then the theorems of metabolic control theory (Eq. (30)) lead to sum rules for the differential regulation profiles.

We shall now consider the optimal profiles (enzyme activities or the respective expression values) to achieve a change $\mathrm{d}Y$ of metabolic variables, for instance $\mathrm{d}Y = \mathrm{d}\bar{Y} - \mathrm{d}\hat{Y}$ in the presence of a perturbation $\mathrm{d}\hat{Y}$. If the output variables y describe metabolic fluxes or concentrations, then the theorems (30) of metabolic control theory lead to sum rules for the differential regulation profiles. In this section, the regulatory variables are supposed to describe enzyme concentrations E_i. The elasticity matrix π_E is considered invertible, which holds, for instance, if each enzyme catalyzes exactly one reaction. According to Eq. (18), the optimal regulation profile $\mathrm{d}\bar{E}$ fulfils

$$(\pi_E^T)^{-1} F_{EE}\, \mathrm{d}\bar{E} = C^{Y^T} (R_E^Y F_{EE}^{-1} R_E^{Y^T})^{-1}\, \mathrm{d}Y \tag{31}$$

If the costs of different enzymes are independent of each other and each enzyme catalyzes exactly one reaction, then both F_{EE} and π_E^T are diagonal. In this case, $\mathrm{d}\bar{E}^* \equiv (\pi_E^T)^{-1} F_{EE}\, \mathrm{d}\bar{E}$ equals $\mathrm{d}\bar{E}$ up to a rescaling of the individual elements. The first term on the right-hand side of Eq. (31) is the transposed control coefficients matrix: so, like any metabolic flux distribution is a linear combination of the columns of C^J, $\mathrm{d}\bar{E}^*$ is a linear combination of control profiles (the transposed rows of C^J).

If the output variables represent either only fluxes or only concentrations, then Eq. (31) leads to sum rules for $\mathrm{d}\bar{E}^*$:

(1) If the fitness term V depends only on concentrations, the summation theorem $C^S K = 0$ yields

$$\mathrm{d}\bar{E}^{*^T} K = 0 \tag{32}$$

For the proof, we transpose Eq. (31), postmultiply with K, and apply the summation theorem:

$$\mathrm{d}\bar{E}^{*^T} K = \mathrm{d}S^T (R_E^S F_{EE}^{-1} R_E^{S^T})^{-1} C^S K = 0 \tag{33}$$

A similar argument yields $\mathrm{d}\bar{E}^{*^T} C^J = 0$.

(2) If the fitness term V depends only on fluxes, the connectivity theorem yields the sum rule

$$\mathrm{d}\bar{E}^{*^T} \epsilon L = 0 \tag{34}$$

because

$$\mathrm{d}\bar{E}^{*^T} \epsilon L = \mathrm{d}J^T (R_E^J F_{EE}^{-1} R_E^{J^T})^{-1} C^J \epsilon L = 0 \tag{35}$$

Similarly, we obtain $\mathrm{d}\bar{E}^{*^T} C^J = \mathrm{d}\bar{E}^{*^T}$ and $\mathrm{d}\bar{E}^{*^T} \epsilon C^S = 0$. These results resemble the statements for optimal enzyme concentrations derived in (Klipp and Heinrich, 1999), where the sum of enzyme concentrations was kept fixed.

What is the meaning of the above sum rules? The first one, for the control of metabolites, implies that the elements of $\mathrm{d}\bar{E}^{*^T}$, summed over any stationary flux distribution, vanish. This holds, in particular, for the sum over any elementary mode (Schuster et al., 2000). As an example, let us consider the regulation of a metabolite in an unbranched chain: the stationary flux is described by $K = (1, 1, \ldots, 1, 1)^T$. According to the sum rule, the scaled differential expression values in the chain sum to zero:

$$\sum_i (\pi_E)_{ii}^{-1} (F_{EE})_{ii}\, \mathrm{d}\bar{E}_i^T = 0 \tag{36}$$

Indeed, the most efficient way to accumulate the metabolite is to activate the upstream enzymes and to inhibit the downstream enzymes.

The second rule, for the regulation of fluxes, predicts dependencies among the regulation patterns of neighbouring enzymes. If no conservation relations hold among the metabolites ($L = I$), then the ith column of ϵL describes the reaction elasticities with respect to the ith metabolite. If the reaction velocities depend only on the concentrations of their own substrates and products, then all elements of the column vanish, except for the reactions of this metabolite. The

sum rule Eq. (34) yields one linear equation for each metabolite: if the metabolite participates in n reactions (subscripted by i), then the scaled expression values $\mathrm{d}\bar{E}^*$ for the respective enzymes fulfil

$$\sum_i \mathrm{d}\bar{E}_i^{*T} \epsilon_i = 0 \tag{37}$$

In a series of experiments, the expression data of the n enzymes, will be confined to an $(n-1)$-dimensional subspace. If a metabolite is involved in two reactions only, the ratio of the expression values $\mathrm{d}\bar{E}_i^*$ is fixed, that is, they are strictly correlated. In an unbranched reaction chain, each metabolite will usually exert a negative and a positive elasticity on the producing and on the consuming reaction, so the changes of enzyme expression will all have the same sign and will be strictly correlated.

It is sometimes convenient to represent regulators, fluxes, and concentrations by logarithmic values. Then, the control coefficients have to be replaced by normalised control coefficients $\mathrm{dg}(J)^{-1} C^J \mathrm{dg}(J)$ and $\mathrm{dg}(S)^{-1} C^S \mathrm{dg}(J)$ (see Heinrich et al., 1996) in all formulae of this section. In addition, K and L have to be normalised by the stationary fluxes and concentrations, yielding $\mathrm{dg}(J)^{-1} K$ and $\mathrm{dg}(S)^{-1} L$.

3.4. Functional modules

The statistical properties of the response coefficients reflect the system's large-scale structure. Let us assume that the cell contains specialised subsystems (Kahn and Westerhoff, 1991), such as protein complexes or reaction networks maintaining particular metabolic fluxes. In this case, the response coefficients will assume almost sparse values, concentrated within functional subsystems (see Schuster and Schuster, 1992). For enzymes acting in modules or complexes, Eq. (31) has an interesting consequence: a module of n regulators which affects only $m < n$ of the output variables will show differential expression that is confined to an m-dimensional subspace. If the proteins form complexes and if each protein belongs to one complex only, the response coefficients matrix can be decomposed into a product $R_x^y = R_c^y R_x^c$ where R_x^c has a block structure. In this case, Eq. (24) implies that genes acting in the same complex show proportional differential expression, that is, their linear correlation is ± 1.

3.5. Predictions for expression profiles

The proposed model yields a quantitative relation between response coefficients, a fitness function, and the optimal response of regulators to small perturbations. Unfortunately, at present, we cannot test the theory by predicting real gene expression patterns, because only few response coefficients are known for appropriate systems, and the fitness function can only be guessed. Experimental expression values may carry considerable measurement errors, and moreover, enzyme activities would be better candidate regulators because they determine the cell's performance more directly. Expression usually has an effect on activity, but empirically, the correlation between them may be weak. For all these reasons, we shall restrict ourselves to summarising some qualitative predictions from the model.

Some properties of expression patterns follow from the model structure without involving optimality, and could also be derived from a linear causal model the linear response implies linear dose response curves and a linear superposition of different perturbations. Asymptotic responses after the onset and after the end of a perturbation, or to perturbations of opposite sign, are symmetric. Asymptotically, a perturbation is buffered and distributed by a cascade of responses. Thus, perturbation may affect subsystems which do not seem directly concerned. For instance, a heat shock response may be supported by an increase of energy production (Mensonides et al., 2002).

Other predictions reflect the relation between function and expression induced by the optimality assumption: the response is an appropriate answer to the perturbation, and is likely to contribute to homoeostasis. Expression patterns reflect the response coefficients on the relevant variables: if F_{xx} and π_x are diagonal, differential expression patterns after a perturbation of cell variables are linear combinations of regulatory profiles. Even if the response coefficients are not known, this can be used for qualitative predictions: genes that do not affect the concerned output variables remain unchanged. Gene products that always act together are coregulated. Superfluous gene products are downregulated so that resources can be allocated to other, more important processes. These intuitive assertions are qualitatively backed by expression data from several experiments (for

instance, DeRisi et al., 1997; Gasch et al., 2000; Causton et al., 2001). If the genes' response coefficients on important variables are sparse or almost sparse, they might be reconstructed from expression profiles using analysis methods like the plaid model (Lazzeroni and Owen, 2002) or independent component analysis (Liebermeister, 2002). However, such a decomposition would also be possible for a causal model if the genes' regulatory functions were linear with sparse input weights w_y^x. For metabolic systems, the model predicts coregulation of genes with a high control on important fluxes. The chemical reactions exerting large control on a particular flux or concentration are often localised in a small region of the metabolic network, so the same should hold for some of the coregulated genes. Quantitative relations to the structure and kinetics of the metabolic network were described in Section 3.2.

4. Implementation and value of regulators

4.1. Optimal control realised by feedback

Until this point, we studied the optimal behaviour of regulators without considering how it is realised. Biological regulators often receive signals from the processes to be regulated: this phenomenon is known as feedback. Gene expression, for instance, is controlled by transcription factors that provide information about the cell status. It is a basic assumption of the present analysis that during evolution, adaptation mechanisms for coping with variable environmental conditions have developed and can be described by optimality principles. This assumption is now used for describing feedback systems: the objective is to derive a feedback system that realises the optimal behaviour of regulators defined above.

Let us consider a system of interacting regulators x and cell variables y in a stationary environment α: if α is replaced by $\alpha + \mathrm{d}a$, then the stationary state values of x and y exhibit changes (see Fig. 2, left bottom)

$$\begin{aligned} \mathrm{d}x &= w_y^x \,\mathrm{d}y \\ \mathrm{d}y &= R_x^y \,\mathrm{d}x + R_\alpha^y \,\mathrm{d}\alpha \end{aligned} \tag{38}$$

The linear coefficients w_y^y represent the partial derivatives of a (possibly nonlinear) feedback function. For example, the activity of an enzyme can be affected by a metabolite concentration via allosteric control. At steady state, this concentration, in turn, is a function of all enzyme concentrations in the reaction network.

A small perturbation $\mathrm{d}\alpha$ results in a response

$$\mathrm{d}x = (1 - w_y^x R_x^y)^{-1} (w_y^x R_\alpha^y)\,\mathrm{d}\alpha \tag{39}$$

With an appropriate choice

$$w_x^y = -F_{xx}^{-1} R_x^{y^T} F_{yy} \tag{40}$$

this feedback model realises optimal regulation, that is, it maximises the fitness for all possible perturbations $\mathrm{d}a$ (Proof: compare Eqs. (24) and (38)). However, this only holds if the second term in 14, describing a perturbation of the response coefficients, is neglected. The feedback to a regulator depends on the regulator's influence w_y^x weighted by the fitness curvatures. An output variable with large negative fitness curvature will send strong feedback signals, a regulator with large negative fitness curvature will receive weak signals. So, feedback signals represent the most important variables and affects the most efficient regulators. Let us consider again allosteric control in metabolism: if homoeostasis in metabolism is to be ensured, Eq. (40) predicts feedback from metabolites to those reactions exerting a considerable control on the metabolite. If the curvatures F_{xx} and F_{yy} are negative and the reaction exerts a positive control on the metabolite, a negative feedback is predicted.

4.2. The value of regulators

What quantitative advantage does a regulatory system mean the organism? To answer this question, we have to refer to an specific ensemble of external conditions: if the perturbations $\mathrm{d}\alpha$ are small and normally distributed with mean $\langle \mathrm{d}\alpha \rangle = 0$ and covariance matrix $\mathrm{cov}(\mathrm{d}\alpha) = \langle \mathrm{d}\alpha\, \mathrm{d}\alpha^T \rangle$, the presence of the regulating system raises the fitness, on average, by

$$\langle \bar{G} - \hat{G} \rangle = -\frac{1}{2} \mathrm{Tr}(G_{ax} G_{xx}^{-1} G_{xa}\, \mathrm{cov}(\mathrm{d}\alpha)) \tag{41}$$

As G_{xx} has no positive eigenvalues, the value $\langle \bar{G} - \hat{G} \rangle$ of the regulatory system is nonnegative. The name "value" has been chosen in analogy to the value of information defined in Bayesian decision theory (Pearl, 1988): the value of an information source is defined as the average advantage (increase in expected payoff) if signals from the information source can be used

for the decisions. Evolution is likely to develop regulators of high value: if the very presence of a regulatory system involves additional costs, it should only be maintained if its value exceeds the costs. Like the value of information, which depends on the presence of other information sources, the value of regulators may be influenced by the presence of other regulators. For instance, adding copies of existing regulators to the system will not yield much additional fitness.

4.3. Growth of deletion mutants

After a change of the environmental conditions, some gene products may become especially important for surviving. They should be activated, and their loss by a deletion should have a strong impact on the growth rate, while the loss of a dispensable gene should play a minor role. Thus a relation between expression data and the growth rates in deletion experiments may be hypothesised. Giaever et al. (2002) studied the growth rate of yeast deletion mutants under different experimental conditions and compared the results to expression data for the same conditions: except for the growth on galactose, their experiments gave only weak evidence for such a relation, but this was seen as a surprise. The model of optimal regulation, though, supports the initial hypothesis, predicting a quantitative relation between the data from expression and deletion experiments.

How should a deletion influence the growth rate under different conditions? A small environmental perturbation $\Delta\alpha$ and a small regulatory change Δx lead to a fitness change

$$\begin{aligned}\Delta G &\approx G_x^T \Delta x + G_\alpha^T \Delta\alpha + \frac{1}{2}(\Delta x^T G_{xx} \Delta x \\ &\quad + \Delta\alpha^T G_\alpha \Delta\alpha + 2\Delta x^T G_{x\alpha} \Delta\alpha) \\ &= \left[G_x^T \Delta x + \frac{1}{2} \Delta x^T G_{xx} \Delta x \right] \\ &\quad + \left[G_\alpha^T \Delta\alpha + \frac{1}{2} \Delta\alpha^T G_\alpha \Delta\alpha \right] + \Delta x^T G_{x\alpha} \Delta\alpha \end{aligned} \tag{42}$$

The fitness change consists of three terms, one caused by the deletion, one due to the changed conditions, and one representing the interaction between both effects, which should manifest themselves in the data matrix. If the rows and columns of the data matrix are centred, the matrix will basically represent the interaction term. According to Eq. (19), the optimal response to a deletion $\Delta\hat{x}_i$ is $\Delta\bar{x} = 1/(G_{xx}^{-1})_{ii} G_{xx}^{-1} \Delta\hat{x}$. Inserting this into the interaction term from (42) yields the fitness loss

$$\frac{1}{(G_{xx}^{-1})_{ii}} \Delta\hat{x}_i^T G_{xx}^{-1} G_{x\alpha} \Delta\alpha \tag{43}$$

For each gene i, this term is proportional to the differential expression under the different conditions described by $\Delta\alpha$ (see Appendix, Eq. (A.4)).

5. Discussion

We have presented a theoretical apparatus for describing the adaptation of living cells to perturbations of environmental or internal parameters. We have made a distinction between regulatory variables and output variables. Specific examples could be the concentrations of gene products (e.g., enzymes) and metabolic fluxes. Accordingly, we have used gene expression as a running example. However, the presented theoretical tool is far more general. Moreover, it is applicable to systems of any size. A promising application of our method is the analysis of DNA microarray experiments where healthy states are compared with perturbed (e.g., diseased) states. However, the proposed model is not limited to gene expression: it may be applied to the design of various regulatory systems on different timescales, such as enzyme kinetics, allosteric control, adaptation of receptors, and even evolution of enzyme properties.

The success of the method largely depends on the choice of the fitness function. This is a general problem in the modelling of optimal properties of living organisms (Allen, 1999; Heinrich, 1985; Heinrich et al., 1987). In any case, the biological costs for the regulatory variables should be taken into account. This can be done (and has been done here) by including, in the fitness function, a negative term expressing these costs. In unbranched enzymatic chains, equating the fitness function with the metabolic flux minus a linear combination of enzyme concentrations is a reasonable choice (Reich, 1983). In either case, biological behaviour is regarded as the solution to an economical problem (Reich, 1983), namely to choose an optimal compromise between possible actions

which maximises a utility function (Henderson and Quandt, 1980). A related optimisation problem also appears in biotechnology, namely to increase the yield of a metabolite by the modification of single genes: the costs depend on the number of genes to be engineered, so only th genes which exert the highest control on the respective metabolite will be modified. On the contrary, the present model, in which the number of responding genes does not play a role, claims that all genes should be adapted, but those with the highest control should be adapted most strongly.

Considering small perturbations has allowed us to use differential calculus. As has been shown earlier in Metabolic Control Analysis, the large changes occurring in biological systems can, in many cases, be described by linearly extrapolating small changes; in other cases, they cannot. Here, we included first-order and second-order terms. However, for deriving simple predictions, we neglected second-order terms in the fitness coupling $\mathrm{d}x$ and $\mathrm{d}y$, and also second-order terms in $y(x, \alpha)$ which couple $\mathrm{d}x$ and $\mathrm{d}\alpha$.

5.1. Structure–function relation of regulators

An interesting result is that optimal regulatory profiles tend to portray aspects of the system to be regulated. The regulators' response reflects the functions of the regulators, that is, their influence on relevant variables, as well as the local shape of the fitness landscape. If the fitness function is isotropic with respect to the regulators, the differential expression pattern is a linear combination of regulatory profiles, i.e., rows of the response coefficients matrix. As an important example, we have analysed metabolic systems: among other things, we have derived a sum rule for the enzymes within metabolic flux modes (metabolic pathways), and a relation between enzymes that catalyze the reactions of a metabolite. In both cases, we have used the summation and connectivity theorems of Metabolic Control Analysis.

A convenient way of self-regulation of biological systems is by feedback. We have applied our method to feedback systems and have obtained the result that also optimal feedback signals reflect the function of regulators. Thus the proposed model predicts a general relation between a gene's function, its optimal expression behaviour, and its regulatory program. In this framework, the task of a gene is to maximise the organism's fitness under typical evolutionary conditions by exerting its function. The behaviour of x depends on a dual variable, namely the marginal fitness $G_x = \nabla_x G = F_x + F_y R^y_x$, which has to remain zero despite any perturbation. The marginal fitness reflects the response coefficients, so during evolutionary learning, information about the functional structure of the cell becomes implicitly stored in the regulatory system. This information can be read by probing the regulatory system with perturbations, or by measurements of biological fluctuations.

An example of this structure–function relation are operons, where sets of cooperating genes are controlled by the same transcriptional machinery, thus functional relations are portrayed qualitatively by the regulatory structure. Another example can be found in the regulation of amino acid synthesis: the aspartate kinase is the first enzyme in the pathway for the synthesis of threonine, isoleucine, lysine, and methionine. The three isoenzymes AspKI, AspKII, and AspKIII receive negative feedback signals from the amino acids, thus "portraying" the strong control of aspartate kinase on amino acid levels. This pattern of regulation even appears on two levels of regulation, as the feedback signals are realised by both allosteric inhibition and repression of gene expression (see Lengeler et al., 1999).

5.2. Validity of the optimality assumption

When expression data are published, authors often relate expression patterns to biological purpose, that is, the function of the genes being up- or downregulated. Our model is meant to formalise such assertions by deriving them from explicit assumptions, in order to find out what can be predicted from an optimality principle alone. However, it is not clear to which extent biological regulators realise an optimal behaviour. Segrè et al. (2002) found evidence for non-optimal adaptation of metabolic fluxes after gene deletions in *E. coli*, but their ansatz for the fitness function does not account for costs of the expression machinery, so it cannot be compared directly to the approach of this work. Experiments (Hughes et al., 2000; Giaever et al., 2002) have shown that gene deletions can increase the growth rate of yeast. According to our theory, the cell would anticipate any possible advantageous deletion by downregulating the respective genes, so no further increase would be possible. The present theory may

fail here for various reasons: either no steady-state function is optimised by the cells, or the growth rate is not the (only) optimisation target. Moreover, the experimental conditions possibly did not reflect the typical environment during evolution, or the deletion had side effects that could not be achieved by a change in expression alone.

One cannot hope to deduce all biological behaviour from optimality principles, and it is an open question in which cases optimality assumptions are valid. At least, two conditions should be met: the experiment must probe the cell with physiological conditions to which the system has accustomed during evolution, and for our analysis, the perturbations must be small. In fact, if a regulator is only indirectly concerned, it will experience an effective perturbation that has already been sufficiently buffered by the other regulators, and then even a large or unphysiological perturbation like a gene deletion may be described by a linear theory.

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Appendix A. Optimal response

We consider an effective fitness $G(x, \alpha) \equiv F(x, y(x, \alpha))$ and expand $G_x \equiv \nabla_x G(x, \alpha)$ to first order

$$G_x(x + \mathrm{d}x, \alpha + \mathrm{d}\alpha) \approx G_x(x, \alpha) + G_{xx}(x, \alpha)\,\mathrm{d}x + G_{x\alpha}(x, \alpha)\,\mathrm{d}\alpha \quad (A.1)$$

The total differential reads

$$\mathrm{d}G_x = G_x(x + \mathrm{d}x, \alpha + \mathrm{d}\alpha) - G_x(x, \alpha) = G_{xx}\,\mathrm{d}x + G_{x\alpha}\,\mathrm{d}\alpha \quad (A.2)$$

An optimal initial state with $G_x(x, \alpha) = 0$ becomes perturbed by $\mathrm{d}\alpha$. Without response d, this implies three changes

$$\mathrm{d}\hat{R}^y_x \equiv R^y_{x\alpha}\,\mathrm{d}\alpha \quad (A.3)$$

$$\mathrm{d}\hat{F}_x \equiv F_{xy} R^y_\alpha\,\mathrm{d}\alpha$$

$$\mathrm{d}\hat{F}_y \equiv F_{yy} R^y_\alpha \mathrm{d}\alpha$$

The optimal response $\mathrm{d}\bar{x}$ must ensure that $\mathrm{d}G_x$ vanishes, so

$$\mathrm{d}\bar{x} = -G_{xx}^{-1}(G_{x\alpha}\,\mathrm{d}\alpha) \quad (A.4)$$

with

$$G_{xx} \equiv F_{xx} + T_{xx} + R_x^{y^T} F_{yy} R^y_x + F_{xy} R^y_x + (F_{xy} R^y_x)^T$$
$$G_{x\alpha} \equiv R_x^{y^T} F_{yy} R^y_\alpha + F_{xy} R^y_\alpha + T_{x\alpha} \quad (A.5)$$

The matrices $(T_{xx})_{ik} = (F_y)_l (R^y_{xx})^l_{ik}$ and $(T_{x\alpha})_{ik} = (F_y)_l (R^y_{x\alpha})^l_{ik}$ are calculated from the tensors R^y_{xx} and $R^y_{x\alpha}$ containing the second derivatives of $y(x, \alpha)$. We assume that G_{xx} has negative eigenvalues, so it is invertible. Rewriting the term in brackets from Eq. (A.4)

$$\mathrm{d}\hat{G}_x = R_x^{y^T}(F_{yy} R^y_\alpha\,\mathrm{d}\alpha) + (F_{xy} R^y_\alpha\,\mathrm{d}\alpha) + (R^y_{x\alpha}\,\mathrm{d}\alpha)^T F_y = R_x^{y^T}\,\mathrm{d}\hat{F}_y + \mathrm{d}\hat{F}_x + \mathrm{d}\hat{R}_x^{y^T} F_y \quad (A.6)$$

yields

$$\mathrm{d}\bar{x} = -G_{xx}^{-1}[R_x^{y^T}\,\mathrm{d}\hat{F}_y + \mathrm{d}\hat{F}_x + \mathrm{d}\hat{R}_x^{y^T} F_y] \quad (A.7)$$

Thus, the regulators react to the three effects (see Eq. (A.3)) of the perturbation. For simplicity, we assume that $F(x, y) = U(x) + V(y)$ implying $F_{xy} = 0$, so $\mathrm{d}\hat{F}_x = 0$. This yields

$$\mathrm{d}\bar{x} = -(F_{xx} + T_{xx} + R_x^{y^T} F_{yy} R^y_x)^{-1} \times (R_x^{y^T}\,\mathrm{d}\hat{F}_y + \mathrm{d}\hat{R}_x^{y^T} F_y) \quad (A.8)$$

Appendix B. Symbols used

x	Regulatory variables	Vector
α	Environmental variables	Vector
$y(x, \alpha)$	Regulated variables	Vector
$(R^y_x)_{ik} \equiv \partial y_i \partial x_k$	Response coefficients	Matrix
$(R^y_{ab})^k_{il}$	Second-order response coefficients w.r.t. a and b	Tensor

Appendix B (*Continued*)

$F(x, y)$	Fitness function	Scalar
$F_x \equiv \nabla_x F$	Marginal fitness of x	Vector
$F_y \equiv \nabla_y F$	Marginal fitness of y	Vector
$G(x, \alpha)$ $\equiv F(x, y(x, \alpha))$	Effective fitness	scalar
$G_x \equiv \nabla_x G$	Effective marginal fitness of x	Vector
$(G_{xx})_{ik}$ $\equiv \partial^2 G/(\partial x_i \partial x_k)$	Effective fitness curvature	Matrix
$T_{ab} \equiv F_y^T R_{ab}^y$	Effective fitness curvature due to second-order response	Matrix
$\mathrm{d}\alpha$	Perturbation of α	Vector
$\mathrm{d}\bar{x}$	Optimal response of x	Vector
R_y	Feedback coefficients	Matrix

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