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How growth affects the fate of cellular metabolites

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Abstract

Cellular metabolites frequently have more than a single function in the cell. For example they may be sources of energy as well as building blocks for several macromolecules. The relative cellular needs for these different functions depend on environmental and intracellular factors. The intermediary products of phosphorylation of pyruvate by mitochondria, for example, are used for growth, while the released ATP is used for both growth and maintenance. Since maintenance has priority over growth, and maintenance is proportional to a cell's mass, a cell's need for ATP vs. building blocks depends on the growth rate, and hence on substrate availability. We show how the concept of Synthesising Units (SUs) in linear and cyclic pathways takes care of the correct variation of the ATP/building block ratio in the context of the Dynamic Energy Budget (DEB) theory. This can only be achieved by an interaction between subsequent SUs in transferring metabolites. Apart from this interaction we also needed an essential feature of the performance of the pathway in the DEB context: the relative amount of enzymes varies with the growth rate in a special way. We solved an important consistency problem between the DEB model at the whole-cell level and a model for pathway dynamics. We observe that alternative whole-cell models, such as the Marr–Pirt model, that keep the relative amount of enzymes constant, and hence independent of the growth rate, will have problems in explaining how pathways can meet cells' growth-dependent needs for building blocks vs. ATP.

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1. Introduction

Here we consider the problem of how to deal quantitatively with the dual function of many cellular compounds: their use as source for energy as well as building blocks. Because of this duality, the fate of metabolites should generally depend on the cellular growth rate. To focus ideas, let us briefly discuss a specific example.

The nine transformations of the linear metabolic pathway of the tricarboxylic acid (TCA) cycle amount to the oxidation of the acetyl group of acetyl-CoA to CO_2:

$$C_2H_3O\text{-SCoA} + 3NAD^+ + FAD + GDP^{3-} + P_i^{2-} + 2H_2O \rightarrow$$
$$2CO_2 + 3NADH + FADH_2 + GTP^{4-} + 2H^+ + \text{H-SCoA}.$$

The H-SCoA re-binds to pyruvate or fatty acid for the next cycle; the reduced coenzymes NADH and $FADH_2$ are re-oxidised by dioxygen in a multi-step transformation. The free energy is used to convert ADP and P_i to ATP via a proton gradient across the mitochondrial inner membrane, as is well known. What is usually less emphasised in text books is that the intermediary metabolites (e.g., citrate, succinate, fumarate, malate) are also used as building blocks. So not all the pyruvate that is passed to mitochondria should be combusted completely. Cells' need for building blocks, relative to that for ATP, depends on the growth rate, and hence on the rate of pyruvate allocation to mitochondria. The six nonmembranebound enzymes of the TCA cycle are released from the gel-like mitochondrial matrix by gentle ultrasonic vibration as a very large multi-protein complex (Lodish et al., 2000). This spatial organisation suggests interactions between the enzymes that might be responsible for the regulation of the proper ATP/building blocks ratio.

We will avoid the use of the concept 'concentration'. This strategy is basic to our reasoning and motivated by the observations that the cell is spatially highly structured, transport is rarely by diffusion (as implied by application of the law of mass action, which is standard in chemical kinetics) and concentrations automatically become state variables of the dynamic system of the cell, which complicates the comparison of models having different state variables. Processes that are directly linked to spatial structure, such as channelling of product to neighbouring enzyme molecules and membrane–cytoplasm interactions, or indirectly linked to spatial structure, such as allocation, are notoriously difficult to address in classical enzyme kinetics.

Rather than enzymes that follow the rules of classical enzyme kinetics, we use Synthesising Units (SUs), which are generalised enzymes with two modifications in their kinetics (Kooijman, 1998): production rates are not formulated in terms of substrate concentrations, but in terms of substrate arrival fluxes, and the dissociation of the enzyme–substrate complex into free enzyme and substrate (without transformation) is assumed to be negligibly small (Kooijman, 1998). An SU thus converts a substrate arrival flux into a flux of products and rejected substrates (Fig. 3); we will take all fluxes to be nonnegative.

We begin the body of the paper by first reformulating the dual function problem more precisely in quantitative terms and place it in the context of the Dynamic Energy Budget theory (Kooijman, 2000). We then show how Synthesising Units can be used to model cells' regulatory functions for linear and cyclic pathways, and finally we set up a Metabolic

Table 1
Table of frequently used symbols for variables

Symbol	Dim	Interpretation
t	t	Time
M_i	(C-)mol	Mass of compound i
m_i,	$\frac{\text{mol } i}{\text{mol } V}$	Structure-specific mass of compound i: M_i/M_V
$y_{i,j}$, $Y_{i,j}$	$\frac{\text{mol } i}{\text{mol } j}$	Mol compound i produced per mol compound j consumed
$J_{i,j}$	mol i t^{-1}	Flux of compound i associated with transformation j
$j_{i,j}$	$\frac{\text{mol } i}{\text{mol } V}\ t^{-1}$	Structure-specific flux of compound i: $J_{i,j}/M_V$
$j_{i,Am}$, $j_{i,K}$	$\frac{\text{mol } i}{\text{mol } V}\ t^{-1}$	Struct-spec. max assim. and satur. flux, of compound i
$n_{i,j}$	–	Chemical coefficient for element i in compound j
r	t^{-1}	Specific growth rate for structural mass: $\frac{\mathrm{d}}{\mathrm{d}t} \ln M_V$
k_E, k_*	t^{-1}	Reserve turnover rate, spec. decay rate for compound $*$
ρ_i	–	Binding probability of compound $i-1$ to ith SU
α_i	–	Handshaking parameter for the ith SU
θ_i	–	Fraction of the ith SU that is unbound

Control Analysis for the situation where metabolites are used to synthesise enzymes that transform these metabolites. See Table 1 for a list of frequently used symbols.

2. Formal problem

Consider an n-step linear metabolic pathway, as illustrated in Fig. 1, which is mediated by enzymes $S_1, \ldots, S_n$ with the following ith step:

$$X_{i-1} \to y_{X_i X_{i-1}} X_i + y_{P_i X_{i-1}} P_i \qquad \text{with } i = 1, \ldots, n. \tag{1}$$

A molecule of intermediary metabolite X_{i-1} is transformed into $y_{X_i X_{i-1}}$ molecules of another intermediary metabolite X_i and $y_{P_i X_{i-1}}$ molecules of product. (Alternatively, the yield coefficients y are probabilities that a metabolite transforms respectively to the next metabolite in the pathway or to the product.) Other substrate molecules might be involved as well, but their availability is assumed to be such that they do not limit the rate of transformation. The product P_i might actually be composed of a set of (possibly different) molecules, rather than a single molecule. Products are, therefore, taken to be *generalised compounds*, which are mixtures of chemical compounds such that the chemical composition of the mixture does not change. Without loss of generality we can identify the last intermediary metabolite X_n with the last product P_n. The substrate flux J_{X_0} to the pathway is given by a model for the whole cell and might vary (slowly) in time. If all intermediary metabolites would follow the full pathway (which they generally do not),

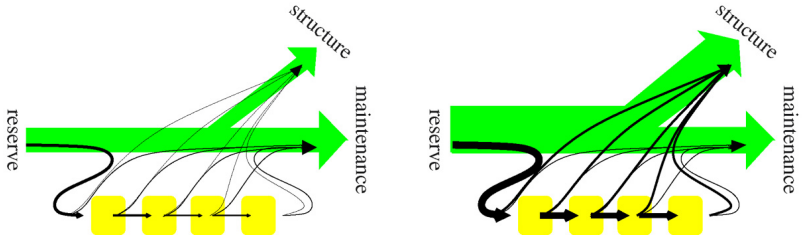

Fig. 1. The resources that are mobilised from the reserve by the catabolic flux are allocated to maintenance and growth, i.e., increase in structure. When the reserve density increases, the catabolic flux and the allocation to growth increase, but not the allocation to maintenance (right panel; widths of arrows indicate the sizes of fluxes). The flux of substrate to the enzymatic pathway is proportional to the catabolic flux. The mixture of products and intermediary metabolites that are released from a linear pathway and allocated to maintenance (or growth) is constant. This paper solves the problem of how the nonlinear dynamics of the pathway should be organised to fulfil this complex task.

we have the overall transformation

$$X_0 \to \sum_{i=1}^{n} y_{P_i X_0} P_i \qquad \text{with } y_{P_i X_0} = y_{P_i X_{i-1}} \prod_{j=1}^{i-1} y_{X_j X_{j-1}} \text{ for } i = 2, \ldots, n. \tag{2}$$

We now consider the situation where some intermediary metabolites follow only part of the pathway and step out of the transformation process at the various nodes of the pathway and become available for two cellular functions: maintenance and growth of structure, see Fig. 1. Cellular maintenance and growth require the intermediary metabolites X_i and products P_i in possibly different relative amounts:

$$\sum_i y_{X_i X_M} X_i + \sum_i y_{P_{i+1} X_M} P_{i+1} \to X_M \tag{3}$$

$$\sum_i y_{X_i X_G} X_i + \sum_i y_{P_{i+1} X_G} P_{i+1} \to X_G \tag{4}$$

where X_M and X_G are taken to be generalised compounds that are involved in the maintenance and growth process, respectively, and the yield coefficients y are taken to be stoichiometric constants (i.e., fixed constants whose values are constrained by mass conservation). *This latter requirement yields the important conclusion that all products and intermediary metabolites that are released from the pathway depend linearly on the growth rate*. To see this, note that the released material at growth rate zero is allocated to maintenance. If more material is released than is needed for maintenance, the extra material is allocated to growth. If, for example, the growth rate is doubled, then twice as much material per unit of time is needed for growth, provided that structure does not change in composition. Maintenance has priority over growth. Accordingly, the flux ratio J_{X_G}/J_{X_M} depends on the flux J_{X_0} in a very special way, as will be discussed below.

The problem now is that the mass balance at the whole-cell level forces us to assume that the chemical composition of the mixture of metabolites and products that is allocated to maintenance is constant. The same applies to the mixture that is allocated to growth,

while the composition of both mixtures will differ. This mass balance does not and cannot account for leaks from a pathway, where leaks are defined to be fluxes that are not associated to maintenance or growth (or any other process that the whole-cell model specifies). What does this imply for the dynamics of the pathway? How is pathway kinetics linked to cellular requirements for particular compounds? The cell has many pathways and if each pathway produced compounds that are not allocated to maintenance or growth, any model at the cellular level would be problematic, unless the cellular model incorporated the details of the then (very large) set of models for all different pathways. Such a complex model would hardly contribute to further insight concerning cellular metabolic functions and would be highly impractical in most applications. Consequently, we here discuss a consistency issue between a whole-cell model and model for the dynamics of a pathway.

The cellular requirements can be expressed in the overall transformation

$$X_0 \to Y_{X_M X_0} X_M + Y_{X_G X_0} X_G \tag{5}$$

where the variable stoichiometric coefficients Y depend on the flux of substrate J_{X_0} to the pathway. Both these coefficients and the flux must be specified by a model for the whole cell, which we will now specify.

3. DEB theory

The one reserve–one structure model for V1-morphs (i.e., organisms that have a surface area that is proportional to the volume of their structure during growth) is the simplest nondegenerate model for a unicellular organism in the context of the Dynamic Energy Budget (DEB) theory. Classical models, such as those of Monod (no reserve, no maintenance), Marr–Pirt [no reserve, Painter and Marr (1968), Pirt (1965)] and Droop [no maintenance, Droop (1973)], are special cases of this simplest DEB model (see Fig. 2). Reserve and structure are assumed to be strongly homeostatic (i.e., they do not change in composition) and are treated as two generalised compounds. Accordingly, a cells' mass at any time is partitioned into M_E C-moles of reserve material and M_V C-moles of structural material. A C-mole is the number of carbon atoms expressed as a multiple of Avogadro's number. The frequency of other chemical elements is expressed relative to carbon and these relative frequencies are constant for generalised compounds; 1 mol glucose = 6 C-mol glucose, since glucose has six carbon atoms. Notice that M_E and M_V are the state variables of the cell; the DEB theory specifies how they change in time as a function of the extracellular substrate concentration X, and the amounts of products that are excreted. Organisms are rarely V1-morphs in detail, but we here focus on unicellular creatures (or cells in tissue cultures) that divide after doubling their structure; such organisms approximate V1-morphs well for most applications. We will now specify all fluxes that are involved. It will be convenient to use structure-specific fluxes $j_* \equiv J_*/M_V$ for any choice of compound $*$.

The DEB model assumes that extra-cellular substrate X is converted to reserve X_E in a process called *assimilation*: for an arrival flux of extra-cellular substrate $J_{X,F}$, the assimilated flux of substrate amounts to $J_{X,A} = f j_{X,Fm} M_V$ with scaled functional

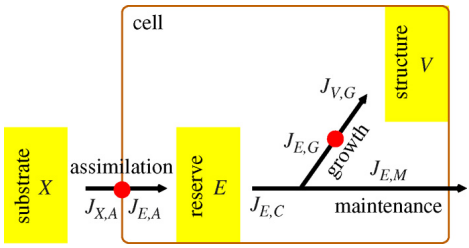

Fig. 2. The DEB theory delineates substrate, X, reserve, E, and structure, V. These pools are connected by a substrate uptake flux, $J_{X,A}$ (disappearance of substrate), an assimilation flux, $J_{E,A}$ (appearance of reserve) and a catabolic flux, $J_{E,C}$ (use of reserve), which is partitioned into a maintenance flux, $J_{E,M}$ (eventually excreted), and a growth flux, $J_{E,G}$ (reserve allocation to growth) and $J_{V,G}$ (appearance of structure). The circles indicate Synthesising Units, which convert substrate into reserve, or reserve into structure.

response $f = (1 + j_{X,K}/j_{X,F})^{-1}$ for some constant specific half-saturation flux $j_{X,K}$. The maximum specific uptake rate $j_{X,Fm}$ is here taken to be constant, although it can be subjected to adaptation processes across generations (Brandt et al., 2003). The assimilation rate of reserve is $J_{E,A} = y_{EX} J_{X,A}$. The energy to drive the transformation from substrate X to reserve X_E is extracted from substrate X and affects the value of the yield coefficient y_{EX}, which implies the existence of waste products in association with assimilation.

The reserve is mobilised for metabolism in a process called *catabolism*. The catabolic flux follows from the dynamics of the reserve density $m_E = M_E/M_V$, which turns out to be $\frac{\mathrm{d}}{\mathrm{d}t} m_E = j_{E,A} - k_E m_E$ for specific assimilation rate $j_{E,A} = J_{E,A}/M_V$ and (constant) specific turnover rate of reserve k_E. This particular kinetics follows from the assumptions that the cell is weakly homeostatic (i.e., m_E approaches a constant value during the cell cycle if the extracellular substrate concentration remains constant), and that the reserve dynamics is partitionable (i.e., reserves can be partitioned into fractions that follow identical kinetics without affecting overall kinetics). See Kooijman (2000, p. 82) for the derivation and motivation and Kooijman et al. (2003) for an evolutionary context.

The reserve $M_E = m_E M_V$ changes because of the difference between the assimilation and the catabolism, $\frac{\mathrm{d}}{\mathrm{d}t} M_E = J_{E,A} - J_{E,C}$. The catabolic flux is

$$J_{E,C} \equiv j_{E,C} M_V = (k_E - r) M_E \tag{6}$$

where cells' (variable) specific growth rate $r \equiv j_{V,G} = \frac{\mathrm{d}}{\mathrm{d}t} \ln M_V$ accounts for the dilution by growth.

The catabolic flux is allocated to maintenance and growth, where maintenance has priority over growth. Maintenance comprises a variety of activities, such as the turnover of compounds in the structure, maintenance of concentration gradients across membranes, movement and transport. The maintenance rate is taken to be proportional to structure,

$$J_{E,M} \equiv j_{E,M} M_V \tag{7}$$

where the specific maintenance cost $j_{E,M}$ is taken to be constant. The fate of this flux is eventually excretion in transformed (mineralised) form. The remaining catabolic flux is

transformed to structure, so that

$$J_{E,G} = J_{E,C} - J_{E,M} = y_{EV} J_{V,G} \qquad \text{and}$$
$$J_{V,G} = r M_V \text{ with } r = \frac{k_E m_E - j_{E,M}}{m_E + y_{EV}} \tag{8}$$

where $y_{VE} = 1/y_{EV}$ is the yield of structure on reserve and $m_E + y_{EV}$ represents the costs for growth, which has a reserve and a structure part, cf. Kooijman (2000, p. 108). The energy to drive the transformation from reserve X_E to structure X_V is extracted from reserve and affects the value of the yield coefficient y_{EV}, which implies the existence of waste products in association with growth.

This completes the specification of the DEB model for our present purpose. We refer to Kooijman (2000) and its citations for tests against experimental data and proceed to work out the link between cells' need for metabolites (as specified by the DEB model) and the release of metabolites from the pathway (that will be specified later).

3.1. Problem in DEB context

To make a clear notational distinction between the two levels of organisation (pathway and cell), we will mark all yield coefficients (i.e., mass–mass couplers) that link the levels with $\circ$.

Substrate X_0 is released from the reserve as part of the catabolic flux, so

$$J_{X_0} = y^\circ_{X_0 E} J_{E,C} \qquad \text{or for } j_{X_0} = J_{X_0}/M_V \qquad j_{X_0} = y^\circ_{X_M E} j_{E,C}. \tag{9}$$

Generalised compound X_M participates in the maintenance flux, so

$$J_{X_M} = y^\circ_{X_M E} J_{E,M} \qquad \text{or for } j_{X_M} = J_{X_M}/M_V \qquad j_{X_M} = y^\circ_{X_M E} j_{E,M} \tag{10}$$

while generalised compound X_G is used for building structure, so

$$J_{X_G} = y^\circ_{X_G V} J_{V,G} \qquad \text{or for } j_{X_G} = J_{X_G}/M_V \qquad j_{X_G} = y^\circ_{X_G V} r. \tag{11}$$

Compound X_G differs from the structure X_V by the inclusion of compounds that are used in the overhead of growth and by that fact that more than one pathway will deliver compounds that are used in growth. For $y_{X_M X_0} = y^\circ_{X_M E}/y^\circ_{X_0 E}$ and $y_{X_G X_0} = y^\circ_{X_G V}/(y^\circ_{X_0 E} y_{EV})$, the variable yield coefficients required in (5) can now be expressed in terms of DEB fluxes as

$$Y_{X_M X_0} = \frac{J_{X_M}}{J_{X_0}} = \frac{y_{X_M X_0}}{1 + y_{EV} r/j_{EM}} \qquad \text{and}$$
$$Y_{X_G X_0} = \frac{J_{X_G}}{J_{X_0}} = \frac{y_{X_G X_0}}{1 + j_{EM}/(y_{EV} r)}. \tag{12}$$

The enzymes that are involved in the metabolic pathway are, by definition, part of the reserve and/or structure since these two components constitute the whole cell. So the amount of the ith enzyme, M_{S_i}, can be written as weighted sums of reserve and structure:

$$M_{S_i} = n_{S_i E} M_E + n_{S_i V} M_V = (n_{S_i E} m_E + n_{S_i V}) M_V \qquad \text{with}$$
$$m_E = \frac{j_{EM} + r y_{EV}}{k_E - r}, \tag{13}$$

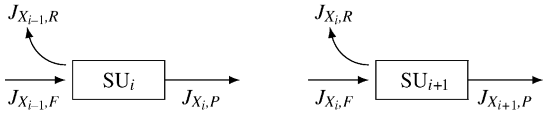

Fig. 3. The arrival (F, for 'feeding'), rejection (R) and production (P) fluxes for two Synthesising Units that are involved in a metabolic pathway. In a linear pathway for $i = 1, \ldots, n$ we have that $J_{X_i,P} = J_{X_i,F}$.

where chemical index n_{ij} specifies how much of i is present in compound j. The first index, i, typically refers to a chemical element present in compound j, but in the situation of a generalised compound j, it can also relate to a compound. The yield coefficient y_{ij} specifies how much of i is formed per (C-)mol of j in a transformation. Both n's and y's are mol mol^{-1}; n's refer to chemical compositions, y's to transformation efficiencies. This notation is standard in microbiology.

The costs for synthesis of the enzymes appear in the yield coefficients for assimilation and growth:

$$y_{XE} = 1/y_{EX} = y^\circ_{XE} + \sum_i n_{S_iE} y_{XS_i} \tag{14}$$

$$y_{EV} = y^\circ_{EV} + \sum_i n_{S_iV} y_{ES_i}. \tag{15}$$

Turnover costs of enzymes that are part of the structure should be included in the specific maintenance costs as

$$j_{E,M} = j^\circ_{E,M} + k_{S_i} n_{S_iV} y^*_{ES_i} \tag{16}$$

where $y^*_{ES_i} = y_{ES_i}$ if no reserve components are saved from the decomposition of enzyme S_i; generally we have $y^*_{ES_i} \leq y_{ES_i}$. The turnover of enzymes that are part of the reserve is implied by the reserve turnover. If both $n_{S_iE} > 0$ and $n_{S_iV} > 0$, we must have that $k_{S_i} = k_E$ to avoid a distinction between enzyme molecules that are part of the reserve and of the structure.

Notice that the intermediary metabolites of the metabolic pathway, X_i, don't appear in the reserve or structure; strict consistency requires that their amounts are negligibly small, and no need exists to evaluate their concentrations in the highly spatially structured internal environment of the cell. The maintenance compound X_M will be excreted in one form or another, just like part of the growth compound X_G, while another part of X_G will be included in the structure X_V. This completes the placement of our dual function problem in the context of the DEB theory for we now have defined and related the various fluxes at the cellular level that specify the fluxes and the variable yield coefficients in (5).

4. Fluxes through linear pathways

We now specify the fluxes through the linear metabolic pathway as a function of the arrival flux of substrate to the pathway, including the branches of rejected fluxes of intermediary metabolites and products. We need interaction between SUs in the pathway, because without interaction, some intermediate metabolites always escape further

transformation, while the cell might not need them for maintenance or growth. For this purpose we introduce $n-1$ *handshaking parameters* α_i, $0 \leq \alpha_i \leq 1$, that affect the release of product and the binding of substrate between SU i and $i+1$. The unbound fraction of the ith SU changes such that if the handshaking parameter $\alpha_i = 0$, the handshaking is *open* and the SUs operate independently. If $\alpha_i = 1$, however, the handshaking is *closed* and no intermediary metabolites X_i are released if the binding probability $\rho_i = 1$; an SU only releases its product if the receiving neighbour SU is in the binding state. This coordinates the activities of all the SUs in the pathway and quantifies the distributed release of products and intermediary metabolites. If the handshaking parameter α_i is set to zero, all control is 'bottom up' because the SUs do not interact and the behaviour of the whole follows (in complex ways) from the behaviour of the units. If the handshaking parameters are increased, the control becomes increasingly 'top down', cf. Segel (2001) since the behaviour of the whole feeds back to the behaviour of the units. If the handshaking is closed for all SUs in the pathway, $\alpha_i = 1$ for $i = 1, \ldots, n-1$, and binding is sure, $\rho_i = 1$ for $i = 1, \ldots, n$, then the full pathway acts as if it is just a single SU (see Appendix A) and all metabolites X_0 that are processed are transformed into products. The behaviour of the units is then fully controlled by the behaviour of the whole.

We use a time scale argument to derive the arrival (F), rejection (R) and production (P) fluxes of intermediary metabolites in terms of the steady state binding fractions of the SUs. (We use F for 'feeding' to indicate arrival rates to avoid confusion with assimilation, which we also need; when a metabolite flux is 'fed' to an SU, it does not mean that all will be 'eaten'.) All intermediary metabolites X_i that are produced by the ith SUs, arrive at the $i+1$th SUs, so $J_{X_i,F} = J_{X_i,P}$. The Appendix A gives the derivation of the pathway kinetics, which amounts for $i = 1, \ldots, n-1$ to the following changes of unbound fractions, θ_i, of SUs:

$$\frac{\mathrm{d}}{\mathrm{d}t}\theta_i = (1-\alpha_i(1-\theta_{i+1})-\theta_i)k_i - (\theta_i + \alpha_{i-1}(1-\theta_i))\rho_i J_{X_{i-1},F}/M_{S_i} \tag{17}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\theta_n = (1-\theta_n)k_n - (\theta_n + \alpha_{n-1}(1-\theta_n))\rho_n J_{X_{n-1},F}/M_{S_n}. \tag{18}$$

Setting the change in the fractions, (17) and (18) equal to zero, we obtain for the unbound fractions of the ith SUs at steady state (denoted by *) as

$$\theta_i^* = \frac{(1-\alpha_i+\alpha_i\theta_{i+1}^*)k_i - \alpha_{i-1}\rho_i J_{X_{i-1},F}/M_{S_i}}{k_i + (1-\alpha_{i-1})\rho_i J_{X_{i-1},F}/M_{S_i}} \qquad \text{for } i = 1, \ldots, n-1 \tag{19}$$

$$\theta_n^* = \frac{k_n - \alpha_{n-1}\rho_n J_{X_{n-1},F}/M_{S_n}}{k_n + (1-\alpha_{n-1})\rho_n J_{X_{n-1},F}/M_{S_n}} \tag{20}$$

where the arrival flux $J_{X_0,F}$ of substrate to the pathway is given. Since SU i exists in M_{S_i} copies, and produces $y_{X_iX_{i-1}}$ intermediary metabolites X_i from each molecule X_{i-1}, the production fluxes are

$$J_{X_i,P} = (1-\alpha_i(1-\theta_{i+1})-\theta_i)k_i y_{X_i,X_{i-1}} M_{S_i} \qquad \text{for } i = 1, \ldots, n-1 \tag{21}$$

$$J_{X_n,P} = (1-\theta_n)k_n y_{X_n,X_{n-1}} M_{S_n}. \tag{22}$$

The set of Eqs. (19)–(22) determine the unbound fractions θ_i and arrival rates $J_{X_i,F}$ for all SUs. Since mass conservation implies that the rejection fluxes equal the difference between

the arrived and the processed fluxes, the rejection fluxes are

$$J_{X_i,R} = J_{X_i,P}(1 - (\theta_{i+1} + \alpha_i(1 - \theta_{i+1}))\rho_{i+1}) \tag{23}$$

$$= J_{X_i,P} - J_{X_{i+1},P}/y_{X_{i+1},X_i} \qquad \text{for } i = 0, \ldots, n-1 \tag{24}$$

$$J_{X_n,R} = J_{X_n,P}. \tag{25}$$

Note that, if $\alpha_i = \rho_i = 1$, no rejection of X_i, $i = 0, \ldots, n-1$, occurs, so $J_{X_i,R} = 0$. This is how closed handshaking is constructed. A nice property of this construction is that more than once a particular enzyme turns out to be a consortium of several smaller ones. As long as the members of the consortium pass metabolites by direct channelling (i.e., the enzyme–product complex does not release the product molecule into the liquid environment, but the molecule is directly bound to a neighbouring enzyme molecule in a enzyme–substrate complex), such a discovery has no consequence for the pathway model. Constraints apply to parameter values; the handshaking parameters restrict the maximum flux that can be processed. With an open handshaking protocol all excess flux is simply rejected, but that possibility becomes increasingly restricted by gradually closing the handshaking. The physical impossibility to allocate more than can be processed leads to unbound fractions outside the interval (0, 1). Any choice of parameter values should be tested for its validity.

This completes the model specification of cells' regulatory functions in terms of the handshaking and the binding parameters. The fluxes and bound fractions can be obtained analytically for $n = 2$, but you don't want to see the result. The result is of little relevance for our purpose, fortunately, because the DEB model already specifies the fluxes. Our interest is in the implied constraints; the next section shows that these can be obtained without explicitly solving for the fluxes.

5. Matching the pathway and the DEB model

In Section 3.1 we specified the flux of substrate to the pathway (9), and the (variable) yield coefficients (12), the maintenance flux (7) and the growth flux (8), which together quantify the transformation at the cellular level (5). In Section 4 we specified how the fluxes of substrates for maintenance and growth (21)–(25) as released by the pathway depend on the flux of substrate to the pathway. Now we are ready for the core of this paper: How does the pathway model for metabolites link with the DEB model for the cell under the various growth conditions? The cell will experience a varying concentration of substrate in the environment, which results in a varying reserve density, and hence a varying allocation to growth. Can we avoid leaks under all conditions? We here use the link between two levels of organisation to extract information about cells' regulatory activities. This exercise will reveal useful constraints on parameter values.

The specific flux of substrate X_0 to the pathway equals by Eqs. (6), (8) and (9)

$$j_{X_0,F} = n_{X_0E}\, j_{E,C} = n_{X_0E}(j_{E,M} + r y_{EV}) = n_{X_0E}(k_E - r)m_E$$

$$= n_{X_0E}\frac{j_{E,M} + k_E y_{EV}}{1 + y_{EV}/m_E} \tag{26}$$

where the specific growth rate r and the reserve density m_E can vary in time. We now equate the release of intermediary metabolites and products from the pathway to their use by the cell. Given (3), (4), (10) and (11), the specific required fluxes of intermediary metabolites and products are,

$$j_{X_i,P} = j_{P_i}/y_{P_iX_i} = y^P_{X_iE}\, j_{EM} + y^P_{X_iV} r \qquad \text{for } i = 1,\ldots,n \tag{27}$$
$$\text{with } y^P_{X_iE} = y_{P_iX_M} y^\circ_{X_ME}/y_{P_iX_i} \text{ and } y^P_{X_iV} = y_{P_iX_G} y^\circ_{X_GV}/y_{P_iX_i}$$
$$j_{X_i,R} = j_{X_i} = y_{X_iE}\, j_{EM} + y_{X_iV} r \qquad \text{for } i = 0,\ldots,n-1 \tag{28}$$
$$\text{with } y_{X_iE} = y_{X_iX_M} y^\circ_{X_ME} = y^P_{X_i,E} - y^P_{X_{i+1},E}/y_{X_{i+1},X_i}$$
$$\text{and } y_{X_iV} = y_{X_iX_G} y^\circ_{X_GV} = y^P_{X_i,V} - y^P_{X_{i+1},V}/y_{X_{i+1},X_i}.$$

The first equality sign in (27), and in (28), is a consequence of the following considerations for the links between the fluxes that are required by the cell and those released by the pathway. The released intermediary metabolites X_i, $i = 0,\ldots,n-1$, are the rejected fluxes $J_{X_i,R}$; the released products P_i, $i = 1,\ldots,n$, are linked to the production fluxes of the metabolites one step earlier. Since both intermediate metabolite X_i and product P_i are stoichiometrically linked to X_{i-1}, product P_i is linked to X_i with yield coefficient $y_{P_iX_i} = y_{P_iX_{i-1}}/y_{X_iX_{i-1}}$.

We must have that $j_{X_i,P} > j_{X_i,R}$ for all growth rates r, which implies from (27) and (28) that $y_{P_iX_M} > y_{P_iX_i} y_{X_iX_M}$ and $y_{P_iX_G} > y_{P_iX_i} y_{X_iX_G}$.

5.1. *Are fluxes of substrates for growth proportional to the growth rate?*

If primes denote differentiation with respect to the specific growth rate r, the linearity of all production and rejection rates in r translates into $j''_{X_i,R} = 0$ and $j''_{X_i,P} = 0$. Double differentiation of (19)–(25) with respect to r results in the following relationships for $j_{X_iP} = J_{X_iP}/M_V$, and $m_{S_i} = M_{S_i}/M_V = n_{S_iE} m_E + n_{S_iV}$ and scaled reserve density $m_E = \frac{j_{E,M}+r y_{EV}}{k_E - r}$.

$$0 = \theta''_i\, j_{X_{i-1}P} + 2\theta'_i\, j'_{X_{i-1}P} \qquad \text{for } i = 1,\ldots,n \tag{29}$$
$$0 = (\alpha_i\theta''_{i+1} - \theta''_i) m_{S_i} + 2(\alpha_i\theta'_{i+1} - \theta'_i) m'_{S_i} + (1 - \alpha_i(1-\theta_{i+1}) - \theta_i) m''_{S_i} \tag{30}$$
$$\text{for } i = 1,\ldots,n-1.$$

Consequently, $m'_E = \frac{y_{EV}+m_E}{k_E - r}$ and $m''_E = 2\frac{m'_E}{k_E - r}$. It follows from condition (29) that θ_i must be a linear fractional function of r, i.e., $\theta_i = (c_{1i} - c_{2i} r)/j_{X_i,P}$ for appropriate values of c_{1i} and c_{2i}. After substitution of (26)–(28), the n relationships (21) and (22) can be rewritten as n equations of the type $0 = \sum_{j=0}^{4} c_{ij} m_E^j$, which only holds for all possible values of m_E if $c_{ij} = 0$ for all $5n$ coefficients c_{ij}. Further analysis shows that this cannot be true if the relative needs for metabolites and products differ between maintenance and growth. Thus our requirement of exact linearity in r cannot be achieved. However, as will now be shown, this requirement can 'almost' be attained.

Numerical studies of (19)–(22) reveal that given values for k_i and n_{S_iE}, and n_{S_iV}, the values for α_i and ρ_i can be chosen such that $j_{X_i,P}$ and $j_{X_i,R}$ are almost perfectly linear in the specific growth rate r for $i = 1,\ldots,n$. The best goodness of fit depends on the abundance parameters; for $n_{S_iE} = 0$, i.e., when the concentrations of enzyme are constant

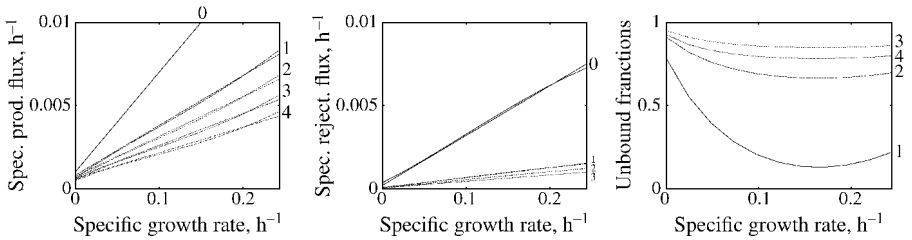

Fig. 4. The production and rejection fluxes, $J_{X_i,P}$ and $J_{X_i,R}$, and the fraction of unbound SUs, θ_i, as functions of the specific growth rate r in a linear pathway of length $n = 4$. The numbers near the curves indicate the position in the pathway, $i = 1, \ldots, 4$. The handshaking and binding parameters are chosen such that the fluxes are 'almost' linear in r (the amount of material required for growth is proportional to the growth rate), which makes the fractions linear fractional functions in r. The linearity is tested by best-fitting linear functions which are plotted as well. The goodness of fit is within the resolution of the figure. The DEB parameters are $y_{EV} = 1.2$, $k_E = 0.4$ h^{-1}, $j_{EM} = 0.02$ h^{-1}, $j_{EAm} = 0.8$ h^{-1} and the substrate concentration for the pathway in the reserve is $n_{X_0E} = 0.05$.

Fixed pathway parameters

i	1	2	3	4
$y_{X_iX_{i-1}}$	1	1	1	1
n_{S_iE}	0.032	0.032	0.032	0.032
n_{S_iV}	0.045	0.045	0.045	0.045
k_i (h^{-1})	0.118	0.186	0.543	0.190

Pathway regulation and yield parameters

i	0	1	2	3	4
α_i	0.7349	0.6652	0.0001	0.2663	
ρ_i		0.6741	0.9134	0.9556	0.9681
$y^P_{X_iE}$	0.0500	0.0400	0.0368	0.0339	0.0311
$y^P_{X_iV}$	0.0600	0.0300	0.0240	0.0192	0.0153
y_{X_iE}	0.0100	0.0032	0.0029	0.0027	0.0311
y_{X_iV}	0.0300	0.0060	0.0048	0.0038	0.0154

and do not depend on the growth rate, the best fit is poor, indeed. The yield coefficients y are in fact parameters at the whole-cell level and have been chosen in Fig. 4, and the abundances of all enzymes have been chosen equal, so $n_{S_iE} = n_{SE}$ and $n_{S_iV} = n_{SV}$ for all i. The reason is that the channelling mechanism of substrate from one enzyme molecule to another is then most simple. Another reason is to remove some of the parameters from the system. The parameter for the maximum specific assimilation rate only served to fix the maximum specific growth rate; the plots show the full range.

Fig. 4 (left panel) shows that the production flux of substrate X_0, which is released from the reserve and fed to the pathway, is analytically linear in the growth rate. This is because the fate of all mobilised reserve, including this substrate, is either maintenance or growth. At growth rate zero, all compounds are used for maintenance. The DEB model fully specifies the flux of substrate X_0 to the pathway and the pathway model does not affect it. Intermediary metabolite 4 is produced by the last type of SU in a linear pathway of four types of SUs, and is passed to the cell (and used for maintenance or growth); it, therefore, does not show up in the second panel for rejected fluxes. The difference between the produced and the rejected fluxes for all compounds is used for further processing in the pathway. The last panel shows that the SUs of the first type are very busy (in processing substrate 0), but the other types are much less busy with this parameter choice. Numerical studies indicate that linearity of product and rejected intermediary metabolite fluxes is more difficult to achieve for increasing degrees of business of the SUs.

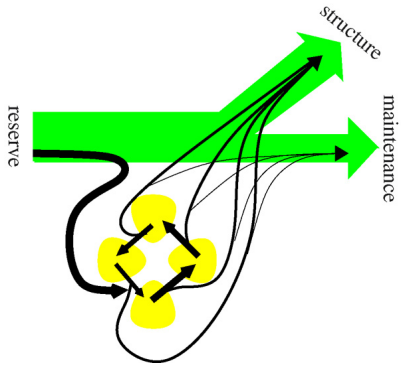

Fig. 5. The problem we try to solve here is the same as that illustrated in Fig. 1, but now for a cyclic pathway. This is to study the robustness of the solution we found for the allocation problem for maintenance and growth.

We checked by simulating (17) and (18) that the pathway is stable for the parameter values that are shown for the full range of growth rates; the speed of convergence to the steady state increases with the specific growth rate. It also holds that, given values for k_i, α_i and ρ_i the values for n_{S_iE}, and n_{S_iV}, can be chosen such that $j_{X_i,P}$ and $j_{X_i,R}$ are close to linear in the specific growth rate r for $i = 1, \ldots, n$. So either the abundance or the control parameters (binding and handshaking parameters) can be fixed, and the other can be chosen such that linearity results approximately. The fit to linearity can be further increased by tuning these four parameters simultaneously for all nodes independently. The significance of this result is that if the reserve is omitted from the cell model, and/or no enzyme is associated with reserve, $n_{S_iE} = 0$, production and rejection fluxes deviate from linearity in the specific growth rate and the pathway model does not match the cell model. The Marr–Pirt model, for instance, which is a limiting case of the DEB model for vanishing reserve, has a consistency problem with this pathway model. We believe that this result is rather general, and applies to a large class of acceptable pathway models.

6. Fluxes through cyclic pathways

We can test the robustness of the matching of the pathway model with the DEB model for the whole cell behaviour by studying a metabolic cycle, rather than a linear pathway, where the compound X_n is identical to X_0 and the last SUs follow a handshaking protocol with the first SUs (see Fig. 5). When α_n denotes the handshaking parameter between SUs number n and 1 we have the following dynamics of the unbound fractions of the first and last SU

$$\frac{\mathrm{d}}{\mathrm{d}t}\theta_1 = (1-\alpha_1(1-\theta_2)-\theta_1)k_1 - (\theta_1+\alpha_0(1-\theta_1))\rho_1 J_{X_0,F}/M_{S_1} + \\ -(\theta_1+\alpha_n(1-\theta_1))\rho_1 J_{X_n,F}/M_{S_1} \tag{31}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\theta_n = (1-\alpha_n(1-\theta_1)-\theta_n)k_n - (\theta_n+\alpha_{n-1}(1-\theta_n))\rho_n J_{X_{n-1},F}/M_{S_n}. \tag{32}$$

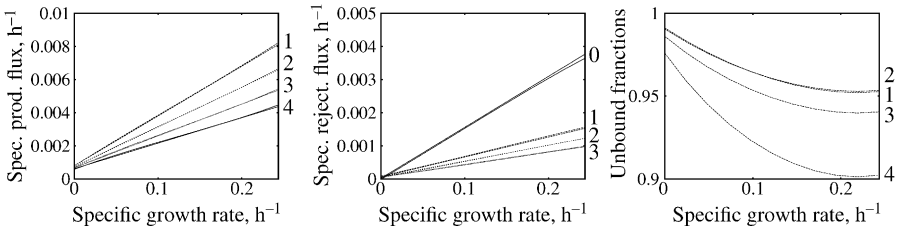

Fig. 6. The production and rejection fluxes, $J_{X_i,P}$ and $J_{X_i,R}$, and the fraction of unbound SUs, θ_i, as functions of the specific growth rate r in a cyclic pathway of length $n = 4$. The numbers near the curves indicate the position in the pathway, $i = 0, 1, \ldots, 4$. The handshaking, α_i, binding, ρ_i, dissociation, k_i and abundance, n_{S_iE} and n_{S_iV}, parameters are chosen such that the fluxes best fitted the preset linear relationships in r, but all enzymes are assumed to be equally abundant. So all yield parameters y were preset, but those for rejection follow from those for production. The linear relationships are also shown, to check the goodness of fit, which is almost within the graphical resolution. The DEB parameters are $y_{EV} = 1.2$, $k_E = 0.4$ h^{-1}, $j_{EM} = 0.02$ h^{-1}, $j_{EAm} = 0.8$ h^{-1} and the substrate concentration for the pathway in the reserve is $n_{X_0E} = 0.05$.

Pathway parameters

i	1	2	3	4
$y_{X_iX_{i-1}}$	0.5	1	1	1
n_{S_iE}	0.065	0.065	0.065	0.065
n_{S_iV}	0.032	0.032	0.032	0.032
k_i (h^{-1})	2.70	1.11	0.71	0.41

Pathway regulation and yield parameters

i	0	1	2	3	4
α_i	0.5600	0.0018	0.0006	0.0015	0.2350
ρ_i		0.8100	0.8400	0.8500	0.9100
$y^P_{X_iE}$	0.0500	0.0400	0.0368	0.0339	0.0311
$y^P_{X_iV}$	0.0600	0.0300	0.0240	0.0192	0.0154
y_{X_iE}	0.0011	0.0032	0.0029	0.0027	
y_{X_iV}	0.0154	0.0060	0.0048	0.0038	

The production flux of metabolite X_n and rejection flux for X_0 are

$$J_{X_n,P} = (1 - \alpha_n(1 - \theta_1) - \theta_n)k_n M_{S_n} y_{X_n,X_{n-1}} \tag{33}$$

$$J_{X_0,R} = J_{X_0,F} + J_{X_n,P} - y_{X_0X_1} J_{X_1,P}. \tag{34}$$

The rejection flux of X_0 must again be linear in the specific growth rate r, so that

$$y_{X_0E} = n_{X_0E} + y^P_{X_nE} - y_{X_0X_1} y^P_{X_1E} \tag{35}$$

$$y_{X_0V} = n_{X_0E} y_{EV} + y^P_{X_nV} - y_{X_0X_1} y^P_{X_1V}. \tag{36}$$

Fig. 6 shows the numerical results for the cyclic pathway; the setup of the figure is similar to that for the linear pathway, Fig. 4. Numerical studies indicate that, compared to linear pathways, the handshaking and binding parameters in cyclic pathways are easier to tune such that the production and rejecting fluxes are (almost) linear in the specific growth rate.

Linearity only seems possible, however, as long as most enzyme is in the unbound state. Although the rejection fluxes are used to minimise the deviations from linearity in the specific growth rate, the computation of both the pathway and the linear fluxes is based on the pathway and linear production fluxes, respectively, using mass conservation. Notice that the yield parameters y_{X_4E} and y_{X_4V} are lacking, because X_4 is assumed to be identical to X_0 and the rejection flux of X_4 is included in that of X_0.

The conclusion must be that the tuning between the pathway model and the whole-cell model is not very sensitive to metabolic 'details' of the pathway. This suggests that, probably, branching pathways could also be tuned to consistency with the DEB model for the whole cell; we did not investigate such pathways in much detail.

7. Cells' design

In what might be called 'the principle of parsimony' one expects that the unbound fractions of SUs are low, otherwise the cell is producing nonfunctional enzymes. These fractions are linear fractional functions of the specific growth rate, which means that for some value of the growth rate, the fractions are minimal, but this value for the growth rate can differ among SUs. Parsimony is a much weaker criterion than optimality, and boils down to the constraint that for each SU a value for the specific growth rate must exist for which the fraction of unbound SU is small. Again we have to rely on numerical analyses to study how the bound fractions of SUs vary as a function of the growth rate. The numerical study is complicated by the fact that it is easy to arrive at combinations of parameter values that result in biologically nonvalid values for certain quantities: α_i and ρ_i must be in the interval (0, 1) for all i, all other parameters must be positive. Even if these conditions are fulfilled, there can be specific growth rates for which some θ_i become negative, which is obviously invalid. As a consequence, the constraints on parameter values must be such that this situation is avoided too. Since the values of the θ's follow implicitly from (19) and (20), these constraints can only be tested retrospectively, after having selected a set of parameter values.

Metabolic control theory is a powerful tool to analyse the relative control of design quantities for fluxes of metabolites in steady state (Heinrich and Schuster, 1996). In Fig. 7 we present the dimensionless (normalised) flux elasticity coefficients $\varepsilon^P_{0i} = \frac{n_{X_0E}}{J_{X_i,P}} \frac{\partial J_{X_i,P}}{\partial n_{X_0E}}$ and $\varepsilon^R_{0i} = \frac{n_{X_0E}}{J_{X_i,R}} \frac{\partial J_{X_i,R}}{\partial n_{X_0E}}$ for all four nodes in a linear pathway as functions of the specific growth rate, using the parameter values given in the legends of Fig. 4. The relative abundance of substrate X_0 in reserve X_E, i.e., n_{X_0E}, is proportional to the flux of substrate X_0 to the pathway for any value of the specific growth rate. The elasticity coefficients for the production fluxes of the linear pathway are in the range of (0.5–1.0), and for the rejection fluxes in the range of (1.0–1.5). The elasticity coefficient for the rejection of substrate 0 is very high for low growth rates.

We can conclude that no simple relationship exists here between the values of the elasticity coefficients and the transformation rates k_i as was observed in linear pathways where all metabolites follow the full pathway (Heinrich and Schuster, 1996). Moreover the specific growth rate modifies the relationships substantially. Major differences between our pathways and the ones studied in the literature (Kacser and Burns, 1973; Heinrich and Rapoport, 1974; Savageau, 1976; Palsson et al., 1985; Hofmeyr, 1989; Heinrich and Schuster, 1996) are that in our case only a fraction of the metabolites follow the full pathway and concentrations of intermediate metabolites do not accumulate. It thus appears that the method is very sensitive for such 'details'.

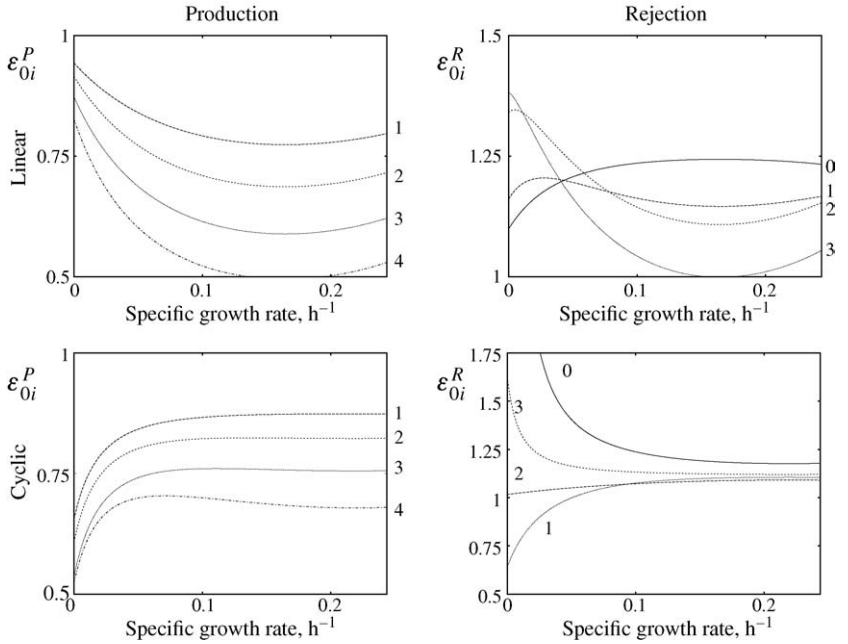

Fig. 7. The elasticity coefficients for production (left) and rejection (right) fluxes, $J_{X_i,P}$, and $J_{X_i,R}$, with respect to the abundance of substrate to the pathway n_{X_0E}. The upper panels show a linear pathway, with parameters as given in Fig. 4; the lower panels show a cyclic pathway, with parameters as given in Fig. 6. We take $\varepsilon^P_{00} = 1$.

A major field of application of metabolic control theory is in the evaluation of the effect of a possible increase or decrease of a particular enzyme on the performance of the whole pathway. Starting from a combination of parameter values for which we have a match between pathway performance and the cell's needs, a change in a concentration of enzyme directly results in a mismatch. This illustrates that the application of control theory in the present context is problematic.

8. Discussion

The problem of dual functions of metabolites and the coupling of the performance of metabolic pathways and cellular needs is much wider then just for those in mitochondria. It not only occurs in all organelles, such as plastids, but also, more abstractly, in all metabolic modules that we might want to delineate in cells' metabolic organisation. The four main modules of the central metabolism in heterotrophic eukaryotes that stepwise decompose carbohydrates (the pentose phosphate cycle, the glycolysis, the TCA cycle and the respiratory chain) probably all ran in reversed mode in their prokaryotic evolutionary history for the purpose of synthesising intermediary metabolites (Kooijman and Hengeveld, 2004). The processing of carbohydrate is a function that evolved later, when carbohydrates took a leading role when the world became oxygenated.

The focus of this paper is on the matching between models at two levels of organization, the whole cell and a single metabolic pathway, with an emphasis on mass balances. The concept 'generalised compound' is hard to avoid for whole-cell models. Strict homeostasis is required for the application of material and energy balances in models that do not specify all compounds dynamically. This means that the stoichiometry for both maintenance and growth of structure must be fixed. The modelled products in the metabolic pathway are not necessarily supposed to have a useful role in metabolism, such as reduced co-enzymes; they can also comprise waste products, such as carbon dioxide. The products in our model primarily serve to close the mass balance. We showed here that in a reasonable model for pathway kinetics, a proper mixture between bottom-up and top-down regulation can be such that all production and rejection fluxes are (almost) linear in the specific growth rate. This is a necessary condition for allocation to maintenance and growth with fixed stoichiometry, while avoiding leaks that are not part of these two fluxes. It also follows that, if the stoichiometries for maintenance and growth differ, reserves are essential to solve the stoichiometric problem of an allocation to maintenance vs. growth that depends on the (variable) growth rate. In other words, the Marr–Pirt model, which has a fixed stoichiometry for biomass and maintenance, implicitly assumes that the stoichiometry for maintenance and growth are identical. This is obviously not realistic; the implication can be seen as the price we have to pay for application of a simple model. Each application of metabolic models has to find the optimal balance between simplicity and realism; the DEB model allows for more metabolic flexibility, but is less simple.

This paper can be regarded as a first step in the study of how a cell allocates resources in the face of changing multiple desiderata. Here we considered only two desiderata, maintenance and growth. We imposed a very simple (but realistic) priority assignment between maintenance and growth, that the first always takes precedence over the second. Given this, we showed how metabolism could be organised to avoid leaks of metabolites. Freedom remained, which we suggested could be employed to diminish the amount of nonfunctional enzyme. It remains for the future to confront more detailed questions in resource allocation.

The chemicals required for the various cell functions are shifting functions of the cells' environment and history. What are the criteria that cells use for resource allocation? We suspect that the scientist can identify several such criteria, 'multiple goals' that overlap and even contradict each other; see Segel (2001) for an illustration of this line of thinking applied to the immune system and Hofmeyr and Cornish-Bowden (2000) for a related approach. Active and regulatory sites on enzymes can be regarded as sensors of information on the internal cellular environment; ligation of these sites modify cellular behaviour. Other sensors modify behaviour as a result of testing conditions exterior to the cell.

An industrial chemical plant can try to arrange production of its numerous products to maximise profits, subject to suitable constraints. The 'boss' of the plant can examine market conditions, to determine policy. A cell can be regarded as acting to maximise its fitness, but progress toward this overarching goal cannot be sensed by the cell and therefore offers no clue to minute by minute behaviour. There is no 'boss' to say what to do, but rather a network organization. Perhaps under stress the cell acts mainly to 'put out fires', that is to skew its activities to raise the production of chemicals that are sensed to be in dangerously short supply. This is just one suggestion that can be examined in a research

program dedicated to the determination of how metabolism is organised to contend with its multiple goals.

We here let maintenance be paid from mobilised reserves, which classifies the DEB model as an assimilation model. A popular class of energy budget models for individual organisms, however, the net production models, first subtract maintenance cost from the assimilatory input, and apply allocation rules on the remaining flux for investment in growth or reproduction; investment into reproduction is via a reserve pool (Lika and Nisbet, 1999). Thus, assimilation and net production models differ in allocation structure in a fundamental way. Apart from problems with paying maintenance costs during starvation, and the impossibility to base body size scaling relationships on net production models (Kooijman, 2000, p. 365), this paper reveals another fundamental problem with net production models: compatibility with a pathway model. The problem is solved in the DEB model that we use here by linking enzyme abundance to both reserve and structure, where the relative amounts depend on the growth rate. This is not an option for net production models. It might be very difficult, if not impossible, to make a net production model compatible with a realistic pathway model.

Acknowledgements

We would like to thank Bob Kooi, Cor Zonneveld, José Ferreira and Hans Westerhoff for their helpful discussion.

Information about the DEB research program and its results can be found at http://www.bio.vu.nl/thb/deb. You can freely download the software package 'DEBtool' from the electronic DEB laboratory, which allows numerical evaluations of the models described in this paper.

Appendix A. Derivation of pathway kinetics

We take $y_{X_i X_{i-1}} = 1$ for simplicity. After an introduction of the behaviour of a single Synthesising Unit (SU), we discuss closed and open handshaking, followed by mixtures of these two extremes. Finally we discuss synthesis from two substrates, to model cyclic pathways.

A.1. Chain of length n = 1

For a given flux $J_{X_0,F}$ of substrate to the M_{S_1} SUs, we have

$$\text{Change in unbound fraction: } \frac{\mathrm{d}}{\mathrm{d}t}\theta_1 = (1-\theta_1)k_1 - \theta_1\rho_1 J_{X_0,F}/M_{S_1} \tag{A.1}$$

$$\text{Steady state unbound fraction: } \theta_1^* = (1+\rho_1 J_{X_0,F}(k_1 M_{S_1})^{-1})^{-1} \tag{A.2}$$

$$\text{Production flux: } J_{X_1,P} = k_1 M_{S_1}(1-\theta_1^*) = \frac{\rho_1 J_{X_0,F}}{1+\rho_1 J_{X_0,F}(k_1 M_{S_1})^{-1}}. \tag{A.3}$$

A.2. Closed handshaking at all nodes

Closed handshaking is defined as an interaction between subsequent SUs in a linear pathway such that, given perfect binding, the product of SU i is directly piped to the SU $i+1$ for further processing. To find appropriate expressions for the dynamics of SUs that have this property, we introduce (yet) unknown functions b_i of the θ_i's that specify the appearance of unbound fractions. The dynamics of the last SU is simple, because the release of product does not depend on the binding state of any other SU. The release rate of product from the last SU-product complex is proportional to the bound fraction, so $b_n = (1-\theta_n)k_n$. We now have

$$\frac{\mathrm{d}}{\mathrm{d}t}\theta_1 = b_1 - \theta_1 \rho_1 J_{X_0,F}/M_{S_1} \tag{A.4}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\theta_i = b_i - b_{i-1} M_{S_{i-1}}/M_{S_i} \qquad \text{for } i = 2,\ldots,n-1 \tag{A.5}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\theta_n = (1-\theta_n)k_n - b_{n-1} M_{S_{n-1}}/M_{S_n} \tag{A.6}$$

$$J_{X_n,P} = k_n M_{S_n}(1-\theta_n^*) = \frac{\rho_1 J_{X_0,F}}{1+\rho_1 J_{X_0,F}\sum_j (k_j M_{S_j})^{-1}}. \tag{A.7}$$

The latter follows from the idea that $(k_i M_{S_i})^{-1}$ acts as a resistance, and that the n-chain should operate as if it is a single SU; compare (A.3) with (A.7). At steady state, we have $b_i^* M_{S_i} = b_{i-1}^* M_{S_{i-1}}$, so $b_1^* M_{S_1} = b_{n-1}^* M_{S_{n-1}}$. From (A.7) follows

$$b_i^* = \frac{\rho_1 J_{X_0,F}/M_{S_i}}{1+\rho_1 J_{X_0,F}\sum_{j=1}^{n}(k_j M_{S_j})^{-1}} \tag{A.8}$$

$$\theta_1^* = \frac{1}{1+\rho_1 J_{X_0,F}\sum_i (k_i M_{S_i})^{-1}} \tag{A.9}$$

$$\theta_n^* = \frac{1+\rho_1 J_{X_0,F}\sum_{j=1}^{n-1}(k_j M_{S_j})^{-1}}{1+\rho_1 J_{X_0,F}\sum_{j=1}^{n}(k_j M_{S_j})^{-1}}. \tag{A.10}$$

We see that

$$\theta_{i+1}^* - \theta_i^* = \frac{\rho_1 J_{X_0,F}(k_i M_{S_i})^{-1}}{1+\rho_1 J_{X_0,F}\sum_{j=1}^{n}(k_j M_{S_j})^{-1}}$$

which suggests

$$b_i = (\theta_{i+1} - \theta_i)k_i. \tag{A.11}$$

Substitution into (A.4)–(A.6) gives

$$\frac{\mathrm{d}}{\mathrm{d}t}\theta_1 = (\theta_2 - \theta_1)k_1 - \theta_1\rho_1 J_{X_0,F}/M_{S_1} \tag{A.12}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\theta_i = (\theta_{i+1} - \theta_i)k_i - (\theta_i - \theta_{i-1})k_{i-1}M_{S_{i-1}}/M_{S_i} \qquad \text{for } i = 2,\ldots,n-1 \tag{A.13}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\theta_n = (1 - \theta_n)k_n - (\theta_n - \theta_{n-1})k_{n-1}M_{S_{n-1}}/M_{S_n}. \tag{A.14}$$

For the purpose of mixing this dynamics with open handshaking, we substitute the feeding fluxes $J_{X_{i-1},F} = J_{X_{i-1},P} = (\theta_i - \theta_{i-1})k_{i-1}M_{S_{i-1}}$ and allow for nonperfect binding ($0 \le \rho_i \le 1$). Moreover, we remove θ_1 in front of the flux of X_0 that arrives to the pathway to avoid leaks of X_0. (This can be done because the open handshaking already has this factor.) The result is

$$\frac{\mathrm{d}}{\mathrm{d}t}\theta_1 = (\theta_2 - \theta_1)k_1 - \rho_1 J_{X_0,F}/M_{S_1} \tag{A.15}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\theta_i = (\theta_{i+1} - \theta_i)k_i - \rho_i J_{X_{i-1},F}/M_{S_i} \qquad \text{for } i = 2,\ldots,n-1 \tag{A.16}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\theta_n = (1 - \theta_n)k_n - \rho_n J_{X_{n-1},F}/M_{S_n}. \tag{A.17}$$

The steady state unbound fractions are

$$\theta_i^* = 1 - J_{X_0,F}\sum_{j=n+1-i}^{n}(k_j M_{S_j})^{-1}\prod_{k=1}^{j}\rho_k = 1 - j_{X_0,F}\sum_{j=n+1-i}^{n}(k_j m_{S_j})^{-1}\prod_{k=1}^{j}\rho_k$$

with $m_{S_j} = M_{S_j}/M_V$ and $j_{X_0,F} = J_{X_0,F}/M_V$. The production fluxes are

$$J_{X_i,P} = k_i M_{S_i}(\theta_{i+1}^* - \theta_i^*) = J_{X_0,F}\prod_{k=1}^{i}\rho_k.$$

A.3. Open handshaking at all nodes

Open handshaking is defined as the lack of any interaction between subsequent SUs in a linear pathway. We here simply have [cf. (A.1)]

$$\frac{\mathrm{d}}{\mathrm{d}t}\theta_i = (1 - \theta_i)k_i - \theta_i\rho_i J_{X_{i-1},F}/M_{S_i} \qquad \text{for } i = 1,\ldots,n \tag{A.18}$$

$$\theta_i^* = (1 + \rho_i J_{X_{i-1},F}(k_i M_{S_i})^{-1})^{-1} \tag{A.19}$$

$$J_{X_i,P} = k_i M_{S_i}(1 - \theta_i^*) = \frac{\rho_i J_{X_{i-1},F}}{1 + \rho_i J_{X_{i-1},F}(k_i M_{S_i})^{-1}}. \tag{A.20}$$

A.4. General handshaking

We now combine the dynamics of Eqs. (A.15)–(A.17) and (A.18) linearly with n handshaking parameters α_i and arrive for $i = 1, \ldots, n-1$ at

$$\frac{\mathrm{d}}{\mathrm{d}t}\theta_i = (1 - \alpha_i(1 - \theta_{i+1}) - \theta_i)k_i - (\theta_i + \alpha_{i-1}(1 - \theta_i))\rho_i J_{X_{i-1},F}/M_{S_i} \tag{A.21}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\theta_n = (1 - \theta_n)k_n - (\theta_n + \alpha_{n-1}(1 - \theta_n))\rho_n J_{X_{n-1},F}/M_{S_n}. \tag{A.22}$$

We can check that the system reduces to closed handshaking for $\alpha_i = 1$ and open handshaking for $\alpha_i = 0$ for $i = 1, \ldots, n-1$. The motivation for the linear combination of the two handshaking protocols is that a fraction α_i of the SUs is following the open handshaking protocol, and a fraction $1 - \alpha_i$ the closed one. Notice that α_0 controls the flux from the cell to the pathway, while the other α_i's only deal with the metabolite traffic between SU i and $i + 1$.

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