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Metabolic Control Analysis Applied to Mitochondrial Networks

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Abstract— **To understand the control and regulation of mitochondrial energy metabolism a generalized matrix method of Metabolic Control Analysis has been applied to a computational model of mitochondrial energetics. The computational model of Cortassa *et al.* (2003) encompasses oxidative phosphorylation, the tricarboxylic acid (TCA) cycle, and ion dynamics across the inner mitochondrial membrane. Control of respiration and ATP synthesis fluxes were found to be distributed among various mitochondrial processes. Control is shared by processes associated with ATP synthesis and ATP/ADP transport, as well as by Ca^{2+} dynamics. The analysis of flux control coefficients and response coefficients has led to the notion of *control by diffuse loops*, that points to the regulatory interactions exerted by processes that are mechanistically only indirectly related with each other. The approach we have utilized demonstrates how properties of integrated systems may be understood through applications of computational modeling and control analysis.**

I. INTRODUCTION

One of the objectives of Systems Biology is to understand the dynamics of networks of processes in living systems. Such understanding encompasses not only the composition of those networks but also their organization in terms of spatio-temporal dynamics and their control and regulation. Metabolic Control Analysis (MCA) addresses the question of what controls, and to what extent, the flux through a metabolic pathway at the steady state [1, 2]. It provides a conceptual framework to quantify the control exerted by a process on metabolic fluxes or metabolite concentrations that could be applied regardless of pathway complexity [3].

Given a network of processes of any complexity, the rates of the individual reactions constituting such a network both influence, and are influenced to a certain extent by, the rates of the other interacting processes. In order to quantify control at the steady state, a series of coefficients have been introduced. The most commonly used is the flux control coefficient, $C^{J_i}_{E_k}$:

$$C^{J_i}_{E_k} = \frac{\partial \ln J_i}{\partial \ln E_k} \tag{1}$$

with J_i representing the flux of interest, and E_k the activity of process k, whose control is quantified by $C^{J_i}_{E_k}$. This analysis requires the system to be continuous (differentiable) in the neighborhood of a steady state.

The elasticity coefficient, $\varepsilon^{v_k}_{Sj}$, quantifies the dependence of the rate of a specific process, k, on the concentration of an intermediate or effector in the network, S_J. The elasticity coefficient as defined in Eq. 2, computes the magnitude by which an enzyme activity (e.g. ATP synthase, v_k) changes upon variation in the level of a substrate or an effector (e.g. ADP, S_j)):

$$\varepsilon^{v_k}_{Sj} = \frac{\partial \ln v_k}{\partial \ln S_J} \tag{2}$$

Control coefficients reflect *global* properties of the network since they dependent on the rates of *all processes* in the system. Unlike control coefficients, elasticities depend upon *local* properties of the enzyme, and the concentrations of its substrates and effectors. In practical terms, elasticities correspond to the slope of the relationship between the initial rate of an enzyme-catalyzed reaction and the concentration of the substrate (or an effector).

On the other hand there are the response coefficients. which measure the fractional change in flux, e.g. respiration, in response to a fractional change in a parameter P (e.g., an effector such as Ca^{2+}) other than enzyme activity [4]. The response of a pathway to an effector depends on two factors [5]: *(i)* the extent of control exerted on the pathway by the enzyme that is the effector's target, and *(ii)* the strength or elasticity of the effect of P on that enzyme. The response coefficient defined in this manner is the product of the control and elasticity coefficients. According to all the definitions stated above, metabolites or ions *regulate*, while changes in enzyme activity or posttranslational modifications *control*.

In the present work, we apply the stoichiometric matrix method of Reder [3] to analyze control exerted by various mitochondrial processes on oxidative phosphorylation (OxPhos). This method generalizes earlier MCA tools [2, 6, 7]. Calculation of the control and response coefficient matrices and changes in steady-state fluxes induced by

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perturbations of the system permit a deeper understanding functional significance in terms of control of different nodes in the network of processes encompassed by the mitochondrial energetics model.

II. Analytical Procedures

In the framework of MCA, Reder [3] developed a generalized linear algebraic method that enables analyzing the sensitivity of metabolic systems to perturbations triggered by either a change in the internal state of the system or by the environment. The departure point of the analysis is the *stoichiometric matrix*, obtained from the set of differential equations of the model. The stoichiometric matrix defines the structural relationships between the processes and the intermediates participating in the metabolic network under consideration. The information in the stoichiometric matrix is independent of both the enzyme kinetics and the parameters that rule the dynamic behavior of the network. The second piece of information required to perform control analysis is the *elasticity matrix* defined by the dependence of each process in the metabolic network on the intermediates (e.g., ions or metabolites) included in the model. The elasticity matrix is quantified through the derivatives of the rates of individual processes with respect to each possible effector

By applying matrix algebra, the corresponding control and response coefficients matrices are obtained. The regulation and control in the network is quantified by both kinds of matrices. The regulation exerted by internal or external effectors to a network can be quantified by the response coefficient [4].

The following matrix relationships were used in the computation of flux and metabolite concentration control coefficients

$$\mathbf{C} = \mathbf{Id_r} - \mathbf{D_x v\, L} \left(\mathbf{N_r\, D_x v\, L}\right)^{-1} \mathbf{N_r} \qquad (3)$$

$$\Gamma = -\mathbf{L} \left(\mathbf{N_r\, D_x v\, L}\right)^{-1} \mathbf{N_r} \qquad (4)$$

with C and Γ referring to the flux- and metabolite concentration control coefficients, respectively; Id_r, the identity matrix of dimension *r*, or the number of processes in the network under study. $D_x v$ the elasticity matrix; N_r the reduced stoichiometric matrix and L, the link matrix that relates the reduced- to the full-stoichiometric matrix of the system (for further details see [8]).

III. Results and Discussion

Figure 1 displays the network of biochemical processes accounted for by the mitochondrial energetics (ME) model. The ME model consists of 11 state variables computed by a system of 11 ordinary differential equation (ODEs). For control analysis the ODEs were integrated until a steady state was achieved and then the elasticities were calculated, i.e. the rate expression of the corresponding process was derived with respect to the effector under study. In the case of the TCA cycle, a single step in the stoichiometric matrix was considered; however, for the quantification of the elasticity coefficients of the TCA cycle with respect to the intermediates, the disaggregated individual rate expressions and their dependence with respect to effectors were taken into account. The individual elasticities were then added together to compute the overall elasticity of the TCA cycle.

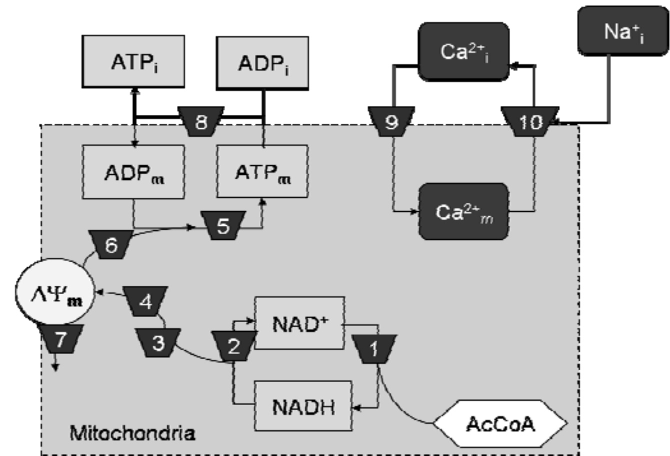

Figure 1. The scheme shows mass transformation interactions between the state variables of the ME model. In the model the TCA cycle (number 1) starts from AcCoA. Number 2 stands for the respiratory electron transport, 3, for proton (H^+) pumping in respiration, 4, succinate-driven H^+ pumping, 5 ATP synthase, 6, H^+ pumping by ATP synthase, 7 H^+ leak, 8 adenine nucleotide translocator, 9, Ca^{2+} uniporter, and 10, mitochondrial Na^+ Ca^{2+} exchanger. State variables are indicated in rectangular (ion or metabolites) while boxes depict a light grey background when the state variables participate in conservation relationships (ATP/ADP, NAD^+/NADH) or a dark grey background for ionic species. $\Delta\Psi_m$ corresponds to the mitochondrial membrane potential. Arrowheads point to the products of the numbered processes, whereas lines without arrowheads indicate inputs to those processes.

Figure 2 shows the control profile of the rate of respiration, V_{O2}, and the F_0,F_1 ATP synthase by different mitochondrial processes,. V_{O2} is mainly controlled by the activity of the respiratory chain carriers (V_{RC}), the H^+ fluxes associated with respiratory electron transport (V_{HNe}) and ATP synthesis (V_{Hu}) (Figure 2A; see also [9]). By discriminating between V_{HNe} and V_{Hu}, we show that mitochondrial respiration is negatively controlled by the build-up of $\Delta\Psi_m$ (via V_{HNe}), and positively controlled by the flux of H^+ associated with ATP synthesis. Additionally, V_{O2} is significantly and positively controlled by the Ca^{2+} uniporter (V_{Cauni}) and the adenine nucleotide translocator (V_{ANT}) (Figure 2A). Minor positive control of respiration is contributed by the TCA cycle (V_{TCA}) and the proton leak (V_{leak}), while negative control is exerted by the proton flux associated with succinate-driven respiration (V_{HFe}) and the ATP synthase (V_{ATPsy}) (Figure 2A).

The ATP synthase control profile mirrors that of respiration, with the exception of V_{ANT} and the TCA cycle (V_{TCA}). For instance, V_{RC} and V_{HNe} exert negative and positive control, respectively, on V_{ATPsy} (Figure 2A and 2B). The positive control displayed by the ANT on V_{ATPsy} is due to the transport of ADP, the substrate for ATP synthesis, to

mitochondria (Figure 2B), whereas in the case of respiration, the ANT exhibits positive control by dissipating $\Delta\Psi_m$, thereby accelerating respiration (Figure 2A).

The results above show that the control of energetics in isolated mitochondria and, more specifically, of respiratory and ATP synthesis fluxes, is distributed. Control is shared by processes associated with adenine nucleotide synthesis and transport, as well as by Ca^{2+} dynamics.

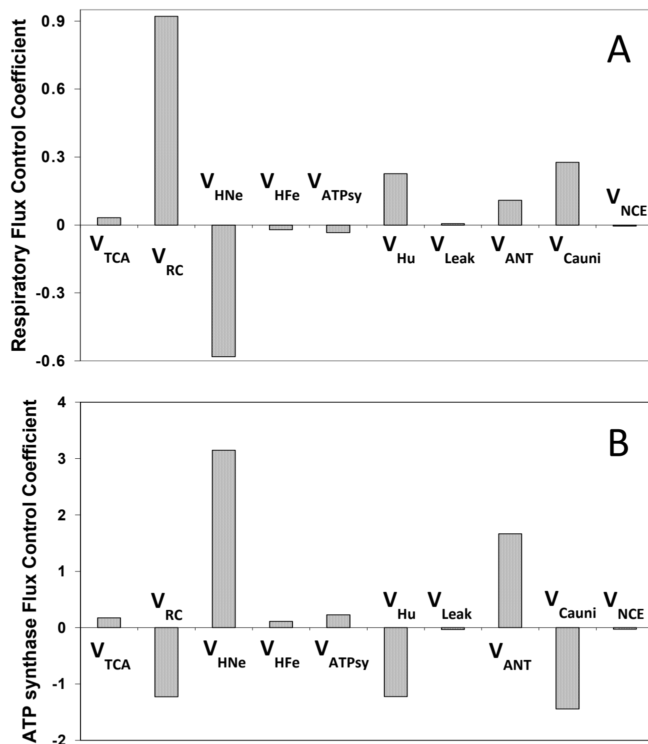

Figure 2. Control of metabolic fluxes in the mitochondrial energetics model. V_{O2} stands for respiratory electron transport (from NADH to O_2) whereas V_{HNe} represents the respiratory proton translocation associated to V_{O2}. V_{HFe} represents the rate of proton translocation associated with succinate-driven respiration. Panel A displays the control profile of electron transport, panel B, the control of the ATP synthase and panel C, the $\Delta\Psi_m$ control coefficients by each of the steps of the ME model [4]

The concept of *control by diffuse loops* emerged from studies attempting to visualize the structure of control of metabolic and transport networks of the myocyte as a whole [8]. We defined *control by diffuse loops* as the control exerted by a process A over another, e.g., C (mechanistically unrelated or indirectly related to process A) through at least one intermediate process B. We pointed out that the existence of diffuse loops provides a rationale for understanding that an action on one part of the network (e.g. by a pharmacological agent) may bring about changes in other parts without obvious direct mechanistic links between them.

Mitochondria also exhibit control by diffuse loops. The control exerted by some mitochondrial processes on the flux of ATP synthesis (Figure 1) can be readily interpreted based on first principles. Discriminating between the proton fluxes associated with respiratory electron transport (V_{HNe} and V_{HFe}), and ATP synthesis (V_{Hu}) we show that ATP synthesis is positively controlled by the buildup of the proton motive force, pmf (V_{HNe} and V_{HFe}), and negatively controlled by the flux of H^+ associated with ATP synthesis. The results indicate that when the pmf is built up by V_{HNe}, it feeds back positively on the ATPase (i.e., higher $\Delta\Psi_m$, higher ATPase activity) whereas when the pmf is dissipated (mainly through $\Delta\Psi_m$), the ATPase activity decreases. In the ME model, V_{O2} and V_{ATPsy} depend upon both $\Delta\Psi_m$ and ΔpH [9]. The overall fluxes of respiration [8] and ATP synthesis (Figure 2A) are strongly dependent on $\Delta\Psi_m$ within a certain range, and follow the general flux-force relationship and dependence upon $\Delta\Psi_m$ and ΔpH described for numerous biological free-energy transduction processes [9-11]. These effects explain the diffuse loop acting as a negative control exerted by the Ca^{2+} uniporter, V_{CaUni}, on ATP synthesis (Figure 2A). The latter control is mediated by $\Delta\Psi_m$ dissipation due to the electrogenic uptake of Ca^{2+} through the uniporter [9]. This $\Delta\Psi_m$–mediated diffuse loop can be further clarified if we take into account the dual effect of Ca^{2+} transport; which on the one hand activates the TCA cycle dehydrogenases thereby stimulating NADH production and respiration, and on the other hand, dissipates $\Delta\Psi_m$ because of the inward transport of positive charges. Quantitatively, the negative control by V_{CaUni} on ATP synthesis happens because $\Delta\Psi_m$ dissipation is larger than the Ca^{2+}-mediated TCA cycle activation.

When the ME model was integrated with the model of excitation-contraction coupling (ECME model) encompassing mechanical activity as well, the pattern of control was found to vary according to working or resting conditions, when the contractile force is close to its maximum, and the energy-consuming pumps are nearly at maximal work during the contraction cycle [8]. The calculations with the ECME model were performed under resting and working conditions, when the contractile force is close to its maximum, and the energy-consuming pumps are nearly at maximal work during the contraction cycle. Although this procedure is rather artificial for a continuous beating heart, this is precisely the usefulness and advantage of a computational model; i.e. it allows you to gain insights into complex processes. Under working conditions, additional control of respiration is exerted by cytoplasmic and sarcolemmal processes, e.g. the myofibrillar and Na/K ATPases. This is especially true under working conditions, when the interaction between cytoplasmic and mitochondrial processes is quantitatively more important. A conspicuous example is the control over respiration exerted by the myofibrillar ATPase, whose degree of share of control appears to be significant only during working conditions. This result underscores the demand-led control of mitochondrial respiration when the energy supply is maximally required.

Uncovering the existence of control by diffuse loops throws new light into the understanding of secondary effects

of pharmacological agents. Profound insights into the rational design of new therapeutic approaches are coming from the utilization of quantitative computational tools to calculate the basic control and regulatory properties of extended networks.

The quantitative tools described herein when applied to computational models or experimental systems will enable a more comprehensive and deeper understanding of the interactions between different processes in a network. Additionally, it helps to gain a quantitative insight into complex processes and their counter-intuitive effects, such as those produced by diffuse loops. Extensive data reported in the literature and our calculations indicate that, in the heart, control by diffuse loops is conspicuously present [12].

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