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Review

How understanding the control of energy metabolism can help investigation of mitochondrial dysfunction, regulation and pharmacology

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Abstract

Understanding the control of mitochondrial energy metabolism is central to knowing how mitochondria function within cells. Metabolic control analysis is the best approach available for studying the control of mitochondrial energy metabolism. Here I outline how metabolic control analysis has been used to help understand mitochondrial regulation, damage and pharmacology.

Keywords: Metabolic control analysis; Mitochondrial energy metabolism; Mitochondrial dysfunction

1. Introduction

Historically there has been considerable interest in understanding how mitochondrial energy metabolism is controlled (e.g. [1] and reviewed in [2–4]). This has led to insights into the mechanism and regulation of oxidative phosphorylation and energy metabolism at the cell, tissue and whole organism level. In other studies, metabolic control analysis (MCA) was developed as an effective theoretical and experimental framework for investigating the control and regulation of metabolic systems and was then applied to oxidative phosphorylation. In addition to oxidative phosphorylation mitochondria are involved in thermogenesis, cell death, radical production, modulation of calcium homeostasis and mitochondrial damage contributes to a number of pathologies [5]. Here I discuss how understanding the control of mitochondrial energy metabolism can be used to investigate physiological, pathological and pharmacological changes in mammalian mitochondria.

2. What controls mitochondrial energy metabolism?

There have been many extensive reviews of mitochondrial energy metabolism and the following is a summary of their findings; I encourage you to read these reviews [2–4,6–8].

2.1. A brief historical perspective

Early in the study of mitochondrial function it was recognised that respiration and ATP synthesis were coupled [9] (Fig. 1). Chance and colleagues showed that respiration rate increased when mitochondria synthesised more ATP and this led to the concept of respiratory control, in which ADP supply to mitochondria stimulated respiration and ATP synthesis

Abbreviations: MCA, metabolic control analysis; ΔpH, pH gradient; $\Delta\mu_H$, proton electrochemical potential gradient

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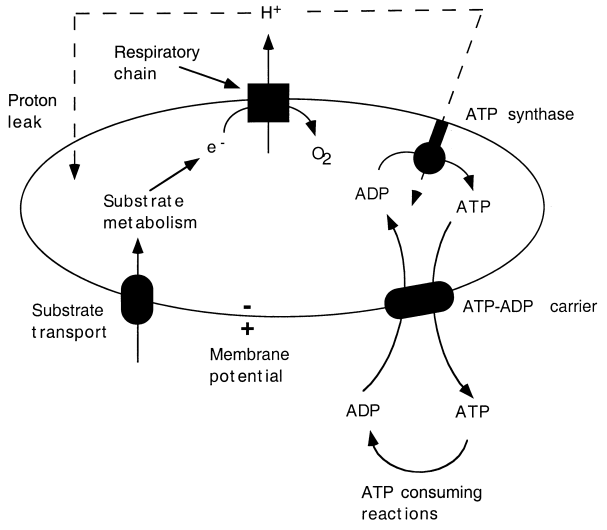
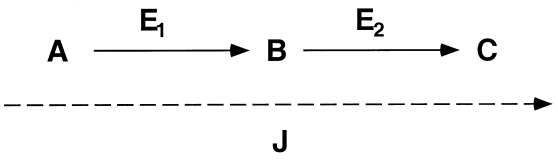


Fig. 1. Mitochondrial oxidative phosphorylation.

[1]. This useful idea linked the rate of mitochondrial ATP synthesis to cellular ATP demand by a feedback mechanism. It was extended to consider whether transport of ATP across the mitochondrial inner membrane by the adenine nucleotide carrier was an important control [10], and whether ATP demand was sensed by mitochondria through ADP concentration, cellular or mitochondrial ATP/ADP ratio, the phosphorylation potential or the energy charge (reviewed in [3,4,6]). The mechanism by which ATP demand affected respiration became clear with the development of the chemiosmotic coupling hypothesis: ATP synthesis decreased $\Delta\mu_H$ thus stimulating the respiratory chain to pump more protons across the mitochondrial inner membrane and maintain $\Delta\mu_H$ [11]. However, control of mitochondrial energy metabolism by ATP demand alone was incomplete because electron supply to the respiratory chain also affected respiration and ATP synthesis. For example, Denton and coworkers showed that calcium stimulated mitochondrial matrix dehydrogenases, increasing electron supply to the respiratory chain and thus the rates of respiration and ATP synthesis [12,13]. Therefore, respiration and ATP synthesis respond to both the 'pull' of ATP consumption and the 'push' of increased electron supply to the respiratory chain. One approach to integrating these two forces was the near-equilibrium hypothesis, which considered the reactions of oxidative phosphorylation prior to cytochrome oxidase to be close to equilibrium with all control residing in the non-equilibrium step catalysed by cytochrome oxidase [2]. Another approach was to use non-equilibrium thermodynamics to represent fluxes such as respiration as functions of the thermodynamic driving forces for ATP synthesis (ΔG_p) and of the redox reactions between reduced substrates and oxygen (ΔE_h) [14]. While these, along with many other studies, laid the foundations of our understanding of the control and regulation of mitochondrial energy metabolism, they did not lead directly to a comprehensive and widely applicable description of the control of mitochondrial oxidative phosphorylation. Instead, MCA emerged as the most useful theory for understanding the control of oxidative phosphorylation.

2.2. MCA

MCA arose in response to the ambiguities inherent in discussing the control of metabolic processes [15–21]. A key first step in applying MCA to a metabolic system is to define what is meant by control, which is generally used vaguely and often interchangeably with regulation. As shown in Fig. 2, an activity such as an enzyme (E_1) controls the overall flux (J), another enzyme activity (E_2) or the size of a metabolite pool (B) only if changing the activity of E_1 alters the magnitude of J, the activity of E_2 or the size of B. Control is quite distinct from regulation, which implies a telelogical endpoint that is physiologically relevant, and system components with substantial control may not regulate the system in vivo. The system being studied by MCA must be explicitly defined, because the question 'what controls a flux?' depends critically on the system limits. For example, the answer to 'what controls fatty acid oxidation?' depends on whether the system is the whole body, the liver or the mitochondria: for the body adipose tissue lipases have significant control while for the liver the plasma fatty acid concentration is critical and for mitochondria membrane transport becomes important. In practice, system limits are usually set by boundary substrate and product metabolites which are at constant concentrations (e.g. A and C in Fig. 2). The system is in a steady state and within it are a series of enzymes and transport processes connected by metabolite pools. The final consideration is that control of the fluxes and metabolite con-



$$\text{Control Coefficient} = C_p^v = \lim_{\delta P \to 0} \frac{\delta V/V}{\delta P/P} = \frac{\partial V}{\partial P} \cdot \frac{P}{V} = \frac{\partial \ln |V|}{\partial \ln P}$$

$$\text{Elasticity Coefficient} = \varepsilon_S^E = \frac{\partial v_E}{\partial S} \cdot \frac{S}{v} = \frac{\partial \ln v_E}{\partial \ln S}$$

$$\text{Summation Theorem} = \sum_{i=1}^{n} C_i^J = 1$$

$$\text{Connectivity Theorem} = \sum_{i=1}^{n} C_i^J \varepsilon_S^i = 0$$

Fig. 2. Application of MCA to a simple metabolic pathway. The upper section shows a simple metabolic pathway comprising the metabolite pools A, B and C interconnected by the enzymes E_1 and E_2 while J is the overall flux through the pathway. In the lower section some of the equations used in MCA are shown. The control coefficient (C_P^v) describes the fractional change in a system property ($\delta V/V$) in response to a fractional change in a system parameter ($\delta P/P$) as the change in the system parameter tends to zero and all else is unchanged. For example, the flux control coefficient of E_1 over the flux J for the metabolic pathway shown above is $C^J{}_{E1} = \partial \ln J/\partial \ln E_1$. The elasticity coefficient ε_S^E describes the change in the activity of the enzyme E in response to changes in the concentration of its substrate S. For the simple metabolic pathway above the elasticity of E_2 to its substrate B is $\varepsilon_B^{E2} = \partial \ln E_2/\partial \ln B$. The summation theorem states that the sum of the flux control coefficients for enzymes in the pathway is one. For the pathway above, $C^J{}_{E1} + C^J{}_{E2} = 1$. The connectivity theorem states that the products of the flux control coefficient and the elasticity to a substrate S for all enzymes connected by that substrate S sum to zero. For example, for the pathway above, $C^J{}_{E1} \cdot \varepsilon_B^{E1} + C^J{}_{E1} \cdot \varepsilon_B^{E2} = 0$.

centrations within this system is a property of the whole system, not an isolated property of a particular enzyme. When the activity of an enzyme changes in a metabolic system, all connected metabolite pools and enzyme activities alter as the system adjusts to a new steady state. This idea is central to MCA and is a major shift from the text book idea of one rate limiting step within a metabolic pathway.

The mathematical formulation of MCA was developed in parallel with these concepts [15,17] and the nomenclature is now agreed upon [19,21] (see Fig. 2). The flux control coefficient relates changes in a pathway flux to alterations in the activity of a particular enzyme or transporter. This is defined as the fractional change in flux that occurs following a fractional change in the amount of the enzyme, hence a step with a substantial flux control coefficient has significant control over flux. The elasticity coefficient indicates how the activity of an individual enzyme or transporter responds to changes in its substrates, products or other effectors. This is defined as the fractional change in enzyme activity in response to a fractional change in the effector. The response coefficient indicates how a system flux changes in response to an external effector. It is defined as the fractional change in flux in response to a fractional change in the effector, or alternatively as the product of the flux control coefficient and the elasticity of that enzyme to the effector. The flux control and elasticity coefficients of a system are all interrelated. From the summation theorem, all the control coefficients over a flux in a pathway sum to one, making explicit that control is distributed throughout the system. The connectivity theorem relates the flux control coefficients of steps connected by a common intermediate to their elasticities to that intermediate. Hence a step with substantial elasticity to its substrates is unlikely to have a high flux control coefficient because lowering its activity is opposed by the build up of substrate. There are many further definitions and theorems that have been developed to account for branched pathways, feedback loops and displacement from the steady state that are beyond the scope of this outline [19,21].

The application of MCA to metabolism has helped to refine and clarify thinking about metabolic control, but most importantly it has enabled the design and interpretation of experiments. Even so there are a number of theoretical and practical limitations to MCA. The pattern of control described by MCA is explicitly defined for one set of conditions, therefore even a small change could radically alter the distribution of control within the system. While this can make it difficult to use the information obtained under different conditions, the distribution of control obtained by MCA is often sufficiently similar over a range of conditions to make it useful in practice. A further limitation is that control and regulation are quite distinct concepts and it can be difficult to use MCA to study how metabolic systems change in response to physiological signals. The final limitation is experimental in that measurement of the flux control

coefficients and elasticities in complicated systems can be difficult. Many of these difficulties can now be overcome by theoretical and experimental developments, such as application of the top-down approach. MCA and our understanding of the control of mitochondrial oxidative phosphorylation have developed in parallel, as is discussed in the Section 2.3.

2.3. Application of MCA to mitochondrial oxidative phosphorylation

The first application of MCA to mitochondrial oxidative phosphorylation was to determine the flux control coefficients of the various components of mitochondrial oxidative phosphorylation over respiration in isolated mitochondria [22,23]. This was done by titration with irreversible inhibitors of mitochondrial enzymes and transporters, relating the decrease in their activity to changes in respiration. This approach is now termed the bottom up approach, because the description of control develops from analysing individual system components [7,8]. These classic experiments gave two significant insights: the control of respiration and ATP synthesis was distributed throughout the mitochondrial oxidative phosphorylation system and this distribution of control shifted as the metabolic state of the mitochondria changed [22,23]. The redistribution of control with change in metabolic state was important because mitochondria change from state 4, where respiration is low, $\Delta\mu_H$ is high and there is no ATP synthesis, to state 3 where respiration is high and $\Delta\mu_H$ is lowered by maximal ATP synthesis. Within cells mitochondria are most often intermediate between states 3 and 4. In isolated mitochondria the major control over state 4 respiration is the proton leak through the mitochondrial inner membrane. As mitochondria shift to a state intermediate between state 4 and state 3 control by the proton leak decreases, and that by the adenine nucleotide carrier, the dicarboxylate carrier, cytochrome oxidase and the ATP consuming reactions increase. In state 3, where ATP synthesis is maximal, most of the control is by the respiratory chain and substrate transport [6,22–24]. The bottom up approach has been applied to a range of mitochondria from different species and tissues and the same general pattern of distribution of control between proton leak, the respiratory chain and the phosphorylating system is retained, although the distribution of flux control between the steps within these systems varies [6,23–26].

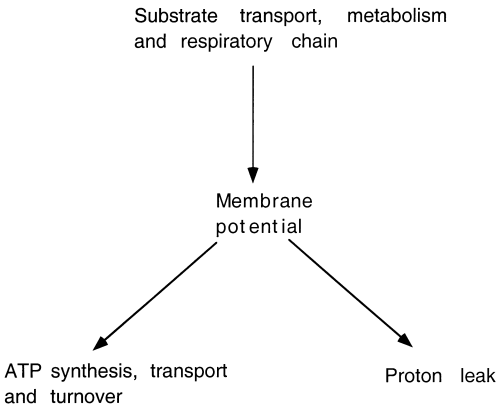


Fig. 3. Top-down analysis of oxidative phosphorylation.

While the bottom up approach is useful for studying the distribution of control in isolated mitochondria, limitations of this approach are that the system components must be known and that measuring the flux control coefficients by titration with irreversible inhibitors is difficult in cells and organs. This led to the development of the top-down approach, also known as top-down elasticity analysis or modular analysis [7,27,28]. In top-down elasticity analysis the metabolic system is grouped into a few blocks of reactions that either feed into or consume a common intermediate [27]. By determining the elasticities of these blocks to the common intermediate the overall flux control coefficients of the blocks can be determined [27]. In applying the top-down approach to mitochondrial oxidative phosphorylation the system is usually divided into three blocks connected by the membrane potential (Fig. 3; the ΔpH component of $\Delta\mu_H$ is usually considered negligible relative to the far larger and more easily measured membrane potential [28]). The membrane potential is generated by one block of reactions (substrate transport, metabolism and the respiratory chain) and consumed by two other blocks, the proton leak through the mitochondrial inner membrane and the reactions of ATP synthesis, transport and turnover [27,28]. The elasticities of the three subsystems to their common intermediate are then determined from appropriate uncoupler or inhibitor titrations and the connectivity theorem used to determine the flux control coefficients of the

three blocks over respiration, ATP synthesis or the proton leak [28].

This approach simplifies complex systems both conceptually and experimentally, making it useful for analysing metabolic control in cells and organs as well as isolated mitochondria [29]. Furthermore, it does not make assumptions about the composition of the blocks of reactions in the system and the experiments are relatively straightforward, even in complicated systems. However, some potential weaknesses of this approach are that all the pathways generating and consuming the common intermediate must be known, the various reaction blocks can only interact through the single intermediate and any changes in ΔpH must be negligible [85]. When top-down analysis is applied to isolated mitochondria, the major control over respiration in state 4 is the proton leak with some control by the substrate oxidation system [30], while in state 3 the major controls are the phosphorylating system and the respiratory chain. In states intermediate between states 3 and 4 the control shifts from leak to the phosphorylating system with some control by the substrate oxidation system [28]. This distribution of control is similar for mitochondria isolated from a range of tissues and organisms and is consistent with the distribution of control within isolated mitochondria determined by bottom up analysis [6,22]. Because only a few variables are measured, the top-down approach can be applied to the control of respiration and ATP synthesis in more complicated systems such as intact cells, perfused organs and whole organisms [29,31,32]. For example, in intact rat hepatocytes from fed or starved animals the control of respiration was largely by ATP synthesis and consumption with the rest of the control distributed between the proton leak and the reactions that generate the membrane potential [29]. The top-down approach can also extend outwards from mitochondria to integrate the control of mitochondrial function with that of related metabolic blocks such as glycolysis, pyruvate oxidation and cellular ATP consumption [31,33].

3. Regulation of oxidative phosphorylation

The application of MCA to oxidative phosphorylation gives a good description of how control is distributed throughout the system, and how this distribution changes when the system shifts to a new steady state. A challenge now is to use this understanding of control to study how mitochondria are regulated under physiological conditions. We know that mitochondrial function is regulated by a number of factors over a range of time scales [4]. These include rapid, reversible signals such as calcium stimulating NADH supply to the respiratory chain [12] or by directly or indirectly affecting oxidative phosphorylation complex activities [34,35], nitric oxide inhibiting cytochrome oxidase [36,37], or thyroid hormones binding to cytochrome oxidase [38]. There are also long-term alterations to mitochondrial function following changes in the expression of nuclear or mitochondrial genes in response to hormonal or developmental signals: some examples are the long-term effects of thyroid hormones on mitochondrial function [39,40], the changes in mitochondrial content and activity on exercising [41] and the alterations to mitochondria that occur during development [42]. The physiological endpoint for this regulation of mitochondria is often to match ATP supply to changes in workload, but it may also be for other reasons such as to modulate thermogenesis, biogenesis or cell death [5,43].

In all these situations we want to understand how the changes in mitochondrial function are brought about. At first sight it may seem easy to use the distribution of the control of oxidative phosphorylation provided by MCA to study regulation. However, regulation implies a physiological outcome brought about by manipulating mitochondrial function, and this is quite distinct from control. Even so, it might be expected that physiological effectors would manipulate mitochondrial function by altering system components with substantial flux control coefficients. However, many physiological signals to mitochondria cause large alterations in activity associated with a major redistribution of control, such as in the shift from state 4 to state 3. Consequently, MCA has little power to predict where within a system external effectors will act to bring about a physiological outcome. Instead MCA describes the new pattern of control that arises when the system reaches a steady state after stimulation. The widespread assumption that effectors only act through steps with high flux control coefficients indicates

how pervasive the concept of a single rate limiting step remains.

This lack of predictive power of MCA is a frustration that is being addressed in a number of ways. One approach is to integrate MCA into mathematical models of metabolic systems (e.g. [44–46]). A developing approach that has been applied experimentally is that of regulation analysis which can be used to determine the interactions that are important in mediating flux changes [8,47]. This approach describes how the system responds to external effectors and indicates which changes in intermediates bring about the new steady state. Determination of partial response coefficients quantitates how a factor interacts with the system and thereby indicates the relative importance of changes in key intermediates. This approach yields a better understanding of how systems respond to effectors, and extends MCA to help understand how the physiological response to an effector occurs. However, it is too early to say whether this approach will have sufficient predictive power to indicate how and where to manipulate a metabolic system to bring about a desired therapeutic or biotechnological outcome.

4. Determining where changes in mitochondria occur

An important question in investigating mitochondrial physiology, pathology and pharmacology is to determine where a physiological effector, drug or damaging agent interacts with mitochondria. Often the first step is to measure respiration rate, membrane potential or the activity of respiratory complexes. These experiments frequently find changes in a range of activities, but the significance of these changes is often unclear. MCA has been used to help determine how damage to individual mitochondrial components affects overall function. In using MCA

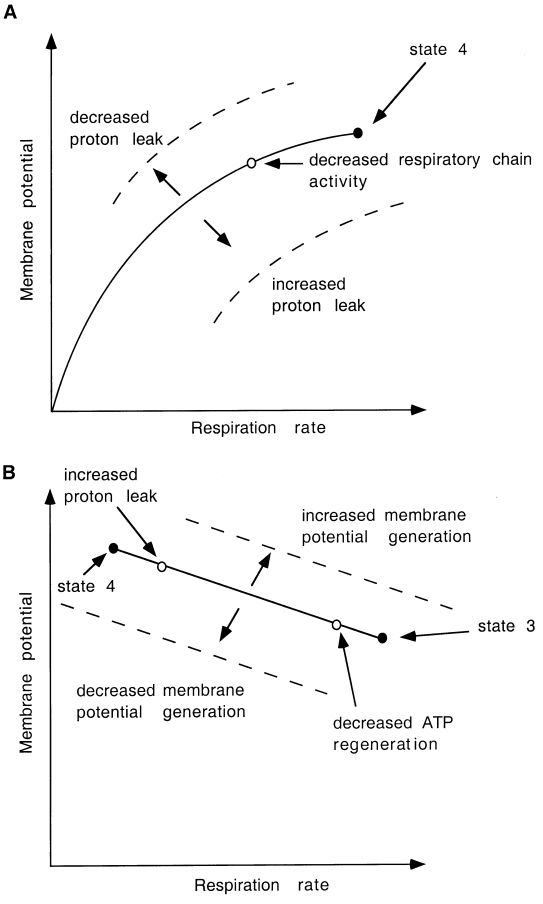


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Fig. 4. Elasticity analysis of mitochondrial respiration. In A, the solid line shows how the membrane potential and respiration rate vary as mitochondria respiring in state 4 (filled circle) are gradually inhibited by titration with a respiratory inhibitor. This curve describes the kinetics of the proton leak across the mitochondrial inner membrane as a function of membrane potential. Factors that increase the proton leak will cause such curves to shift down and to the right while factors decreasing the proton leak will shift such curves up and to the left (dashed lines). In contrast, a decrease in the activity of the respiratory chain would decrease both the respiration rate and membrane potential, but the new steady state would lie on the original curve (open circle). In B, the solid line shows how the membrane potential and respiration rate vary as mitochondria respiring in state 4 (closed circle) are exposed to a gradual increase in ATP turnover until increasing ATP turnover no longer increases respiration rate (state 3; closed circle). This line describes the kinetics of the mitochondrial membrane potential generating system as a function of membrane potential. Factors that decrease the activity of the membrane potential generating system will cause the curve to shift down and to the left while factors increasing the activity of the membrane potential generating system will shift such curves up and to the right (dashed lines). In contrast, an increase in the proton leak would increase state 4 respiration rate and decrease the state 4 membrane potential, but the new steady state would lie on the original curve (open circle). Similarly, a decrease in the activity of the ATP regenerating system would increase the membrane potential and decrease respiration rate, but the new steady state would lie on the same curve (open circle). The ATP regenerating system can be analysed in similar ways to the proton leak and the membrane potential generating systems [50].

to address these questions it is sometimes assumed that damage to steps with large flux control coefficients in the undamaged system will be most important. However, damage often leads to a redistribution of control as the system settles down to a new steady state [48]. This makes it difficult to use measurements of the flux control coefficients in the undamaged state, or the new pattern of control in the damaged state, to infer where damage occurred. This is similar to the difficulty of using flux control coefficients to predict where physiological effectors interact with mitochondria.

However, elasticity analysis can be used to show where an effector has acted on mitochondria [49]. For this, the elasticities of the three mitochondrial subsystems (respiratory chain/substrate transport, the phosphorylation system and the proton leak) to the membrane potential are determined in control and affected mitochondria, just as described in top-down analysis [7,50]. These experiments lead to plots of the membrane potential as a function of the respiration rate due to the activity of the subsystem (Fig. 4). Alterations to the activity of any of the subsystems show up as a shift to a new curve of membrane potential against respiration rate (Fig. 4). This analysis is particularly useful for effectors that alter more than one subsystem, and enables their disparate effects to be distinguished. Once the site(s) of action are localised within a particular subsystem, this analysis can be extended to find where the effector acts within the system. For example, if elasticity analysis indicates that the respiratory chain/substrate transport subsystem is affected, then the elasticities of the individual respiratory complexes to their substrates and products can be determined by appropriate inhibitor, substrate or uncoupler titrations [42,51]. As was the case for the analysis of the subsystems, a displacement from the control curve indicates that the step has been directly altered.

Elasticity analysis has been used to determine where a number of effectors interact with mitochondria. These include long-term alterations that occur in response to changes in circulating thyroid hormones [39,40], chronic exposure to ethanol [52,53] and during the shift from weaning to adulthood in rats [42]. Elasticity analysis has also helped to pinpoint where damage occurs to heart mitochondria during ischaemia [48]. Another use of elasticity analysis is to determine how toxins and drugs interact with mitochondria. For example, this approach was used to show that chloroform affects both the proton leak and the respiratory chain [54], that some antioxidants can act as both uncouplers and respiratory inhibitors [55] and that cadmium both increased proton leak and inhibited respiration in potato mitochondria [50,56]. The potential of elasticity analysis to infer where an effector acts on mitochondria and its simplicity of use make it by far the best method available to find out where effectors and damaging agents interact with mitochondria.

5. Threshold effects and the tissue specific expression of mitochondrial dysfunction

Disruption to mitochondrial function due to the accumulation of oxidative damage and mutations to mitochondrial DNA (mtDNA) contributes to ageing and degenerative diseases [5,57,58]. There is particular interest in understanding the pathology of diseases caused by accumulation of mtDNA mutations [5,59]. Because there are several hundred mtDNA molecules per cell, a feature of mtDNA diseases is heteroplasmy whereby the proportions of mutant and undamaged mtDNA molecules vary [5,59]. A consequence of heteroplasmy is that a patient must accumulate a mutant load above a threshold value before the phenotype is expressed [5,60,61]. This threshold can be as high as 90–95% mutation load for tRNA point mutations in cybrids [62,63] or 40–60% for tRNA point mutations in fibroblasts or for mtDNA deletions in cybrids [64–66]. The proportion of mtDNA molecules mutated usually correlates with decreased specific activity of mitochondrial complexes, therefore threshold values for mtDNA mutations most likely reflect thresholds for disruption to mitochondrial function [5,60,67].

A further feature of mtDNA diseases is their tissue specificity for expression of phenotype, with post-mitotic tissues of high energy demand such as neurones and muscle cells being most affected [5,59,61]. Tissue to tissue and cell to cell mosaicism for a particular mtDNA mutation may contribute, however, homoplasmic mtDNA mutations and mutations in nuclear genes for mitochondrial proteins also show tissue specific expression of phenotype [5,68]. Furthermore,

similar tissue sensitivity to mitochondrial dysfunction also seems to occur in other diseases associated with respiratory complex dysfunction, such as neurodegenerative diseases and in ageing [5,69–71]. The substantial ATP demands of the affected tissues no doubt contribute to their sensitivity to defective mitochondria [25,72]. As metabolic processes are differentially affected by ATP depletion, variations in these may also render particular tissues vulnerable to defects in mitochondrial ATP production [31,33,73]. Finally, mitochondrial damage affects other processes, such as calcium buffering, radical production and susceptibility to cell death [66,74]. Therefore, mitochondrial dysfunction may also kill post-mitotic cells because of their vulnerability to changes in calcium homeostasis, radical production, or turnover of damaged mitochondria.

Respiratory complexes have thresholds for inhibition whereby mitochondrial function is only disrupted when a certain proportion of the complex has been inactivated [24,25,75–78]. As these thresholds are often lower in those tissues most affected by mitochondrial damage, they may contribute to the tissue specific expression of mitochondrial damage [24,25,75–78]. The threshold for depletion of respiratory complexes has been studied in vitro by titrating complex activity with irreversible inhibitors and determining how this affects respiration and ATP synthesis rates [24,25,75–78]. For example, the activities of some respiratory complexes can be inhibited by 25–80% before affecting respiration or ATP synthesis in brain mitochondria [24,25] and the activity of rat liver complex III can be decreased by 45% before respiration is affected [75]. Most interestingly, these thresholds vary with tissue, for example the threshold for complex III is about 45% in rat liver but only about 5% in rat muscle [75] and similar differences have also been found for other tissues and complexes [76,77]. These tissue variations in threshold did not correlate with the specific activities of the complexes, and low inhibition thresholds did not always correlate with high flux control coefficients [24,25,75]. The threshold effects for respiratory complexes may be related to their elasticities to their substrates and products [75]. For example, partial inhibition of complex III will increase the ubiquinol/ubiquinone ratio marginally, increasing the activity of uninhibited complexes because of their high elasticity to their substrate [75]. This buffers respiration in response to complex inhibition until a certain proportion of the complexes has been inhibited. The threshold for inhibition occurs when the elasticity of the complex to its substrate decreases, consequently the complex now has substantial flux control and further inhibition decreases respiration. Complexes with moiety conserved substrates such as ubiquinol/ubiquinone, NADH/NAD or ferro-/ferricytochrome *c* may be susceptible to an abrupt decrease in elasticity after a certain proportion has been inhibited. This decrease in elasticity may arise because the rate of electron supply to the uninhibited complexes no longer responds to increasing reduction of the substrate pool after a certain proportion of the pool has been reduced. Thus tissue specific threshold effects may be due to variations in the size or redox states of metabolite pools such as ubiquinol/ubiquinone [75] and in the elasticities of complexes to these pools as a function of pool redox state [79].

More work is necessary to clarify the nature of thresholds as those found for isolated mitochondria may not reflect the situation in cells and whole animals, because of the saturating substrate concentrations used in vitro and other changes between the cell and the isolated mitochondria [67,80]. A further question is why mitochondrial complexes should have an apparent excess of capacity. It seems unlikely that excess capacity is built into the system to allow for damage and the apparent excess activity may reflect other constraints, such as maintaining metabolite pool size to allow other pathways to work or to prevent unwanted side reactions, such as excessive radical production if the ubiquinol pool becomes too reduced.

6. Summary

The application of MCA to mitochondria has led to a system to describe how control is distributed throughout mitochondria. This analysis does not predict how systems are regulated in vivo, however, this lack of predictive power is being addressed by the development of regulation analysis. An extension of MCA that could predict drug targets within mitochondria [81,82] or where to modulate metabolic pathways in industrially or agriculturally useful or-

ganisms would be useful. The final component of MCA is elasticity analysis and this elegant methodology shows where effectors interact with mitochondria. There is an increasing need to apply MCA when studying the role of mitochondria in pathological and physiological situations. This is happening to some extent but the use of MCA is still not widespread, particularly outside Europe.

There are interesting challenges facing the application of MCA to mitochondria. Up to now MCA has been used to study the control of respiration and ATP synthesis but it will be utilised to study the control of other aspects of mitochondrial function such as radical production and the modulation of thermogenesis and calcium homeostasis. Another challenge will be to integrate understanding of the control of mitochondria with the information emerging from genomic and proteomic studies. For example, the expression patterns of large numbers of genes involved in mitochondrial function change in response to p53 activation [83], and during ageing and caloric restriction [84]. How can these large scale changes in gene expression be interpreted and their consequences for mitochondrial function determined? Elasticity and regulation analysis may be able to cope when the number of genes involved is small, but this may become impractical for a large number of changes uncovered by oligonucleotide arrays and related technologies.

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