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COORDINATED MULTISITE REGULATION OF CELLULAR ENERGY METABOLISM

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PERSPECTIVES AND OVERVIEW

The ability to extract energy from the environment and utilize that energy to organize cell functions and support reproduction is fundamental to all living systems. Animals have evolved mechanisms to ensure adequate energy supply and regulate its delivery to cells according to cellular and organismic demands. In addition, cells have the ability to respond to variations in nutrient supply to optimize utilization of available nutrients. Thus, a dynamic interac-

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tion exists between the supply of foods and the metabolic, transport, and mechanical functions of cells.

This interaction directly or indirectly affects nearly all aspects of biology and medicine. More than 120 scientific reviews have been published on the regulation of mitochondrial function and energy metabolism during the past 5 years. We do not intend to duplicate these, and readers are referred to more comprehensive considerations of the biochemical (8, 9, 29, 33, 50), biophysical (60), genetic (56), pathophysiological (18, 40), and mechanistic (39, 51) aspects of energy supply and utilization. Additional reviews are available on respiratory control and exercise (34), thyroid hormone action (15, 21), nutrient deficiency (57), hyperthermia (53), fatty acid oxidation (23), obesity (49), alcohol metabolism (37), aging (43), and in vivo analysis (24, 46).

As is apparent from this wealth of information, the subject of respiratory control has a rich history. However, a clear understanding of regulation of cellular respiration is not available. Heineman & Balaban (20) concluded in their 1990 review of myocardial respiratory control, "After reviewing the controversies in the literature surrounding the regulation of oxidative phosphorylation, a unifying theory to integrate the disparate results would be welcome." The present review focuses on a new description of the regulation of oxidative phosphorylation, termed coordinated multisite regulation. This regulatory scheme evolved from our studies of mitochondrial regulation during anoxia (26) and expands the concept that control is shared among different control sites, perhaps orchestrated by second messengers (20). It reconciles previously contradictory conclusions and provides a rational basis for interpreting whole body nutritional, physiological, and pathological effects, such as reflected in the diverse list of reviews, cited above, on cellular energy metabolism.

PRINCIPLES OF ENERGY REGULATION

The regulatory scheme is developed in the context of three general principles. Firstly, in terms of general descriptors of energy metabolism, mammalian cell and organ systems function largely as "regulators" rather than "conformers." In higher organisms, cell function does not simply conform to the availability of calories; instead, the utilization of calories is regulated by the physiological demands upon the system. Under most conditions, preparedness is required for any function beyond basal activity. This preparedness has an energy cost and can be affected by the timing and sources of dietary calories.

Secondly, the preparedness for physiological demand is expressed at the cellular level in terms of expression and activities of specific metabolic,

transport, and mechanical machinery. Studies with modern molecular techniques provide new insight into the diversity and expression of this machinery (7). Of considerable importance, data from in vivo and cellular studies suggest that "basal" cellular energy metabolism is variable and can be regulated by mediators of and differential expression of enzyme, transport, and mechanical machinery. This variability allows energy metabolism to be optimized between the degree of preparedness and a more energy-efficient pattern of limited reserve capacity.

Thirdly, a hierarchy of supply of energy to support functions is based upon the immediacy of needs by the cell. This molecular triage provides a flexible yet economical way to optimize energy utilization. However, in addition to specialized functions, such as contraction, absorption, etc, cells are exposed to diverse challenges such as chemical intoxication, oxidative damage, viral infection, and hormonal and development signals to alter cell structure or function. Optimal energy supply for one function may not equate with optimal supply for other functions. Because cells differ in their specialized functions and utilization of oxidizable substrates, adequacy for one cell type may not reflect adequacy for other cell types. Thus, nutritional energy supply to support the hierarchy of nutritional needs must be matched to the specific cellular demands of the physiological and/or pathological state.

ENERGY METABOLISM IN CONFORMERS AND REGULATORS

Contemporary interpretations of the regulation of energy metabolism are derived from early studies of pathways in prokaryotes and yeast. These organisms provided glimpses of elegantly simple feedback inhibition and protein induction mechanisms that allowed self-regulation of pathways and efficient responses to altered nutritional conditions. Although these serve as examples for regulation in higher organisms, there is a fundamental difference in energy metabolism between lower and higher organisms. In lower organisms, cell functions at any given time largely conform to concurrently available nutrient supply (Figure 1). Thus, simple feedback regulation of energy metabolism provides a good description of how the energy metabolism is actually regulated. This regulation is exemplified by the energy charge concept introduced by Atkinson (4) in which activities of energy-producing and energy-consuming systems respond directly to a ratio of high-energy phosphate bond content to total adenine nucleotide content.

In contrast to this elegantly simple means of allowing cell functions to conform to energy status, higher organisms evolved tissue specializations that

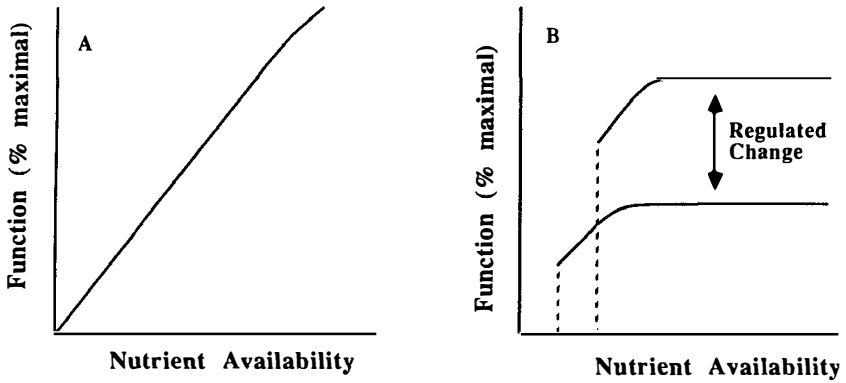

Figure 1 Comparison of functional responses of conformers and regulators to changes in nutrient availability. *A*. In conformers, function is proportional to nutrient availability. For example, growth rate in prokaryotes is proportional to nutrient availability. *B*. In regulators, function is regulated independently of nutrient availability over a wide range. As nutrient availability becomes limiting, only a small change occurs prior to collapse of the function.

allow them to regulate cell functions almost independently of concurrent nutritional energy supply. This concept has been developed in detail for O_2 metabolism and is also applicable to energy metabolism (22, 44). In conformers, energy-related function increases with increasing nutrient availability under essentially all conditions (Figure 1*A*). In contrast, energy-related function in regulators is determined by other factors and is independent of nutrient availability except under conditions of extreme limitation, when the function fails (Figure 1*B*).

Understanding this difference between lower and higher life forms is essential to understanding the regulation of cellular energy metabolism in humans and other higher animals and is illustrated by the energy needs of the mammalian heart. Supply of energy to support cardiac function is of primary importance for organismic survival. This need is so great that during starvation, macromolecules in other tissues are degraded to supply oxidizable substrates to the heart. Clearly, the function of the heart cannot conform to the nutritional energy supply of the organism but must be supplied and regulated independently. Simply put, a hungry animal needs to be able to exert itself to acquire food; it can not slow down and wait until food happens to enter its mouth!

Many strategies have been used by complex multicellular organisms to maintain a reserve capacity to rapidly accommodate altered work demands. A

striking feature of these responses is that in many tissues in higher organisms, changes in work load can be accomplished with little, if any, change in adenylate energy change or in ATP/ADP ratio. Thus, earlier studies demonstrating energy-charge-dependent regulation in higher animals obscured the fact that this is not the primary or most immediate type of response in most cells in these organisms. Moreover, the reliance upon the paradigm of regulation of energy metabolism by high energy phosphates suggested that the regulation was remarkably sensitive to changes in high energy phosphate or was related to other nonmitochondrial O_2-dependent reactions because functional changes occurred under conditions in which changes in high energy phosphates were very small or could not be detected (14).

Heineman & Balaban (20) clearly illustrated this point in their review of the control of mitochondrial respiration in heart with ^{31}P-NMR studies showing that substantial changes in cardiac work load (e.g. from 2 to 10 μmol O_2 consumed per gram per minute) occur with little or no change in ATP, ADP, or inorganic phosphate (P_i) concentrations. They concluded that the two most commonly considered mechanisms of respiratory control, namely, kinetic limitation by ADP or P_i (12) and near-equilibrium (thermodynamic) control by ATP, ADP, and P_i concentrations [kinetically limited at cytochrome oxidase (17)], cannot account for the changes in energy utilization and supply. The unavoidable conclusion from these studies is that ATP, ADP, and P_i are not the most important regulators of energy metabolism in heart and other mammalian tissues.[1] This conclusion is particularly liberating because it allows an unbiased consideration of possible effectors of respiratory control and eliminates constraints upon thermodynamic analyses that have led to conflicts between observation and rational interpretation.

COORDINATED MULTISITE REGULATION

Sites of Regulation

For decades, the prevailing interpretation has been that the redox state, high energy phosphate metabolites, and O_2 are the primary determinants of respiratory control (12, 17, 54). Conditions can be readily established for mitochondrial studies in which each of these could limit (or control) O_2

[1]A wealth of data show that ATP, ADP, AMP, and P_i are effectors of many mammalian enzymes, and thus, this conclusion does not rule out a role for these molecules in regulation. Instead, the conclusion is that the adenylates and phosphate normally are not the primary regulators in controlling energy supply and utilization in response to altered work loads in mammalian cells.

consumption rate and/or ATP production rate. Because no single site could be identified to provide an adequate explanation for respiratory control in cells, the concept of multiple-site regulation was introduced (27, 54). This regulatory scheme provides a mathematical description of respiration in terms of fractional regulation at different sites in the overall process of oxidative phosphorylation (27).

Potential sites for regulation include the adenine nucleotide transporter, NAD^+-linked dehydrogenases, ATP synthase, and cytochrome oxidase (Figure 2). The phosphate transporter is also regulated under anoxic conditions (5). Before considering details of the regulation at each of these sites, it is important to examine their positions in the overall process of oxidative phosphorylation. The coupling mechanism of oxidative phosphorylation involves capture of energy from oxidation of NADH in the form of an electrochemical proton gradient (Δp) across the mitochondrial inner membrane (36, 51). This Δp is composed of two parts, the energy available from the proton gradient (ΔpH) across the membrane and the energy available from moving charges across the membrane in response to the potential difference ($\Delta\Psi$). Mathematically, it is usually expressed in millivolts by the equation $\Delta p = \Delta\Psi - Z\Delta pH$, where Z is a factor to convert ΔpH to millivolts. The expression can be converted to kilocalories per mole or kilojoules per mole of ATP synthesized by the equation

$$\Delta G = -nF\Delta p$$

where n is the stoichiometry of H^+ transported per ATP synthesized and F is Faraday constant. From such calculations, the energy available from the Δp is often not measurably different from that available from the free energy of hydrolysis of ATP to ADP and P_i, which suggests that the oxidative reactions are in equilibrium with the phosphorylation reaction, i.e. a thermodynamic control condition (17).

A usual interpretation is that ATP synthase and phosphate transporter have relatively high rates and contribute little to rate determination. Thus, the amount of energy available for Δp and ATP synthesis is determined by the rate of supply of NADH through the dehydrogenases and cytochrome oxidase to O_2, and the rate of ATP synthesis is limited by either the rate of hydrolysis of ATP in the cytoplasm (equilibrium or thermodynamic control) or the return of ADP by the adenine nucleotide transporter (kinetic control). If one assumes that the oxidative reactions (generating Δp) are in equilibrium with the phosphorylation state, one need not even consider the regulation of dehydrogenases to be important, because these dehydrogenase-catalyzed reactions also could be relatively fast and, hence, in equilibrium with the rest of the electron transport chain and ATP synthase.

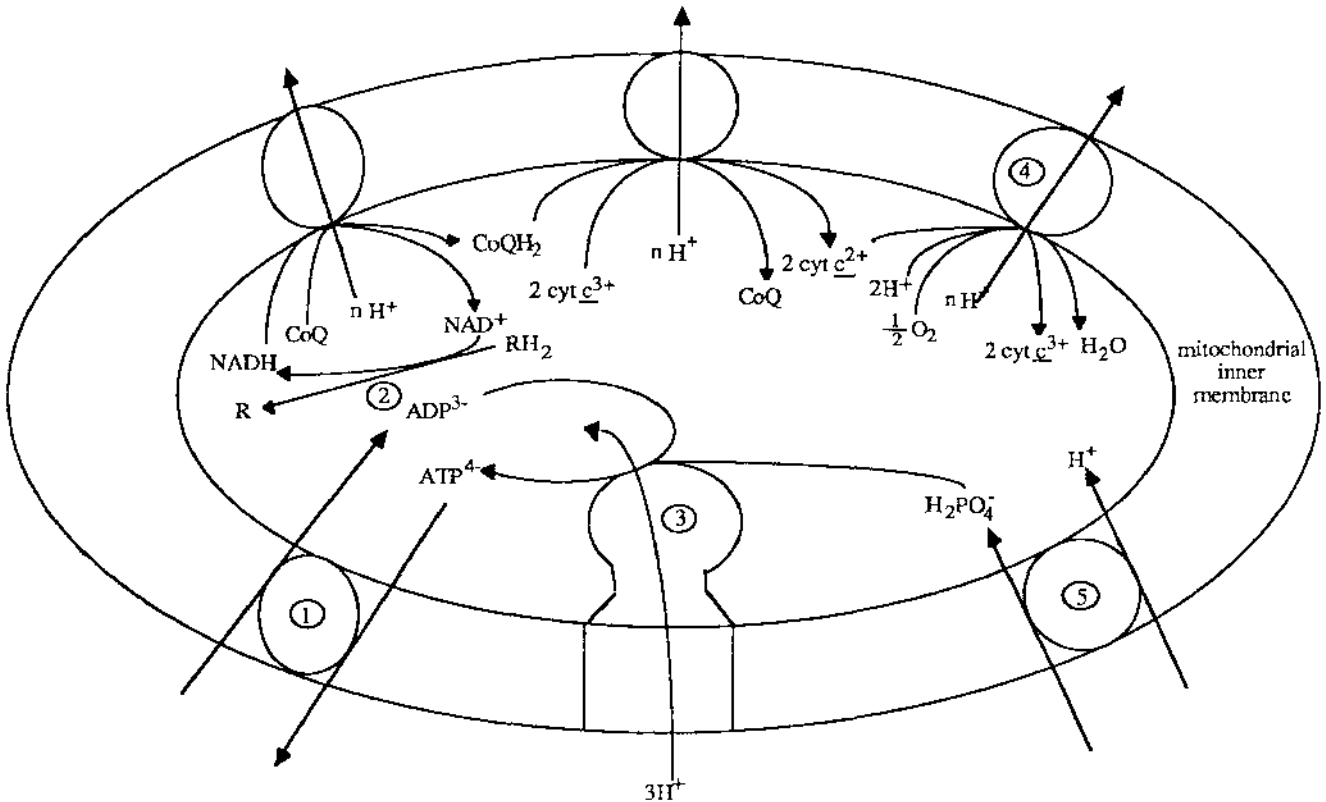

Figure 2 Potential sites for regulation of oxidative phosphorylation. 1. Adenine nucleotide transporter; 2. NAD^+-linked dehydrogenases; 3. ATP synthase; 4. cytochrome oxidase; 5. electroneutral phosphate carrier.

These descriptions are suitable for conformers because when nutrients are ample the system provides energy as rapidly as can be used, whereas when nutrients are limiting, the system limits function to the amount of energy available. However, such an interpretation provides no mechanism to anticipate energy demands or alter work load without changes in ATP/ADP. Mammalian tissues, which regulate metabolism, anticipate work load, and change work load without changes in ATP/ADP, must have additional sites of regulation and regulatory signals.

Inclusion of the five sites listed above allows for regulation of oxidative phosphorylation that can behave like a conformer under some conditions but also can be modulated to anticipate increased work load and to change work load without changes in ATP/ADP (Figure 3). This modulation without changes in ATP/ADP can be accomplished with the following coordinated, multisite regulation mechanism.

The basics of this mechanism are as follows. (*a*) The generation of Δp is regulated by control of the NAD^+-linked dehydrogenases and cytochrome oxidase. Coordinated regulation at these two sites provides the ability to alter the energy available in Δp for chemical, osmotic, and electrical work. (*b*) The utilization of Δp for ATP synthesis is regulated by control of the ATP synthase. (*c*) ATP synthesis in the mitochondria can also be regulated by kinetic control of the adenine nucleotide carrier and phosphate carrier. Coordinated regulation at these sites provides the ability to control the utilization of Δp for ATP synthesis and also to control the extent to which this ATP is maintained within the mitochondria or delivered to the cytoplasm. (*d*) Regulation of the use of Δp for other functions, e.g. Ca^{2+} transport, also provides the ability to control the distribution of utilization of Δp between ATP synthesis and other functions.

Evidence for Multisite Regulation

Two specific issues are important in considering multisite regulation, namely, whether physiological effectors are known for these sites and whether regulation actually occurs at these sites in cells under physiological conditions. During the past decade considerable evidence has accumulated to show that certain NAD^+-linked dehydrogenases are regulated by Ca^{2+} (16, 32). Although earlier studies indicated that mitochondria buffer cytosolic Ca^{2+} (10), we now know that cytosolic Ca^{2+} is an effector of mitochondrial function (16, 38). An increase in cytosolic Ca^{2+} serves as an activating signal for diverse cellular functions (39) and also activates mitochondrial supply of NADH by increasing the activities of pyruvate dehydrogenase, 2-oxoglutarate dehydrogenase, and isocitrate dehydrogenase (16). Variations in O_2 consumption rate in response to changes in intra- and extra-mitochondrial Ca^{2+} have been found in isolated mitochondria (38) and intact cells (60). Measure-

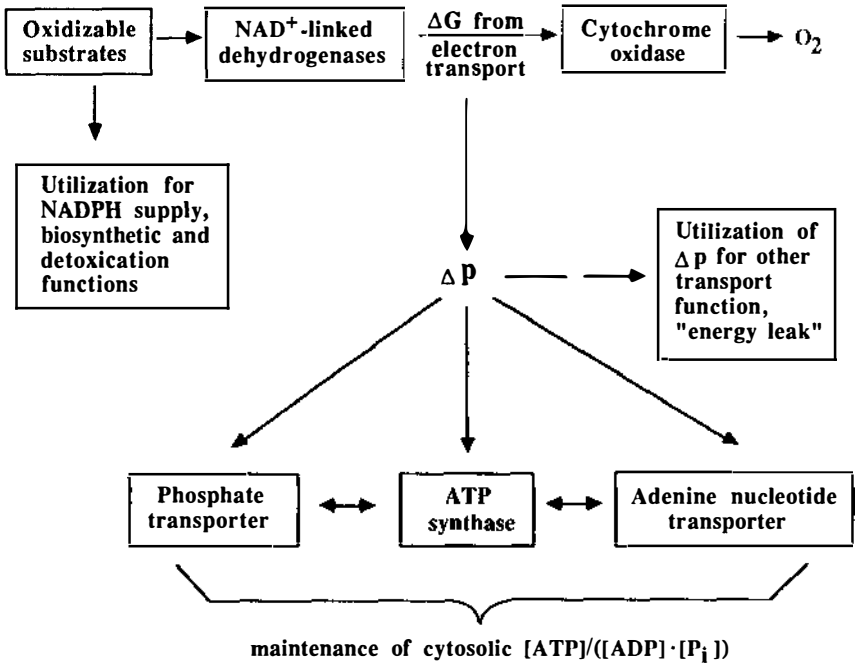

Figure 3 Coordinated multisite regulation of oxidative phosphorylation. Oxidizable substrates can be used for NADPH supply and biosynthetic functions or can be used to support oxidative phosphorylation. Regulation of NAD^+-linked dehydrogenases provides control between these two processes. Coordinated regulation of cytochrome oxidase with NAD^+-linked dehydrogenases provides a means to regulate generation of Δp. Higher Δp is less energy efficient because it increases nonproductive ion leak across the membrane. However, higher Δp also increases the energy available for ATP synthesis and allows for increased ATP/ADP to increase work capacity. Utilization of Δp for ATP synthesis can be controlled by regulating the ATP synthase or by regulating the adenine nucleotide transporter or phosphate carrier. Coordinated regulation of these allows control over cytosolic and mitochondrial $[ATP]/([ADP]\cdot[P_i])$ and the relative proportion of energy from Δp that is used for ATP synthesis as opposed to other mitochondrial functions, e.g. Ca^{2+} cycling or energy-dependent transhydrogenase function.

ments of mitochondrial Ca^{2+} showed that increased Ca^{2+} was associated with positive ionotropic stimulation of heart (13, 59). These results are consistent with regulation of dehydrogenase activity in cells.

Cytochrome oxidase is not usually considered a regulatory enzyme, yet Kadenbach and co-workers have obtained substantial evidence that noncatalytic subunits alter the enzyme kinetics (2, 28). Only two out of the 9 to 13 subunits are involved in the oxidation-reduction reaction and one in proton transport. The functions of the other subunits have not been resolved, but some of these affect the kinetics of the electron transport rate, suggesting that these subunits function in regulation of the oxidase (28). In addition, tissue-

specific isozymes of some subunits have been identified (2); at present, except for regulation, no apparent function for the different isozymes is known. Direct spectral analyses have indicated that a substantial fraction of cytochrome oxidase is reduced in intact tissues (35, 52) even though this extent is not observed in isolated mitochondria (25). This difference may be due to regulatory mechanisms that are lost in vitro.

Electron flow between NADH and O_2 provides the energy available for establishing Δp. In the usual model of respiratory control, the rate of electron flow is thought to be determined by changes in the Δp that are due to alterations in its utilization for ATP synthesis. Coordinated regulation of NADH dehydrogenases and cytochrome oxidase provides a mechanism to increase the rate of O_2 consumption without a decreased Δp.

An endogenous inhibitor of F_1-ATPase (ATP synthase) was described in 1963 (45), and a Ca^{2+}-antagonized inhibitor was subsequently described (62). These inhibitors are usually considered to be safety valves to prevent hydrolysis of ATP by the ATP synthase working in reverse. On the other hand, variations of mitochondrial Ca^{2+} following Ca^{2+} depletion or anoxia in hepatocytes have been associated with altered O_2 consumption rate and altered oligomycin-inhibitable ATPase activity (6). Under anoxic conditions, the energy available from the Δp was substantially more than that necessary for ATP synthesis at prevailing mitochondrial concentrations of ATP, ADP, and P_i (1). Thus, at least under anoxic conditions, the ATP synthase is regulated in intact cells. Similar data for aerobic conditions are consistent with kinetic limitation at this site, but the combined errors from measurement of the various components do not allow certainty in this conclusion. Thus, the data show that regulation of utilization of Δp by the ATP synthase occurs, but they do not clearly establish that this regulation contributes to respiratory control under normal conditions.

The adenine nucleotide transporter is important in limiting oxidative phosphorylation in many in vitro experiments (19, 20, 54). Different isozymic forms are present in different tissues, further suggesting that this is an important control site (48). The activity appears to be inhibited by anoxia (5) and is inhibitable by acyl CoA's (41). The adenine nucleotide transporter is associated with an inner membrane-outer membrane complex that includes porin and other associated proteins (11). Doubtlessly, it is a central component in the regulation of oxidative phosphorylation. The primary effectors of the transporter remain unknown, however, and the ways in which regulation at this site are integrated with other sites of regulation also remain undefined.

Regulation of the phosphate transporter has not been considered extensively in respiratory control, perhaps because its activity is so great that it does not appear to be a likely site of regulation. However, under anoxic

conditions, cytosolic phosphate accumulates dramatically because of the hydrolysis of ATP (5). Continued activity of the phosphate transporter would result in accumulations of up to 100 mM matrix phosphate and would be expected to cause extensive mitochondrial swelling. This phosphate loading and mitochondrial swelling does not occur, thus indicating that the transporter is dramatically inhibited under anoxic conditions (1). In principle, regulation of the phosphate transporter could regulate the production of ATP by limiting mitochondrial phosphate concentration. It could also function to regulate matrix Ca^{2+} or availability of matrix anions, such as dicarboxylates. Thus, evidence suggests that the phosphate carrier is regulated, but the conditions (other than anoxia) and mechanisms involved remain unknown.

Coordination of Multisite Regulation

While there is little doubt that multisite regulation of oxidative phosphorylation occurs, little is known about how this multisite regulation is coordinated, especially since it does not involved high energy phosphates. One of the most important challenges ahead is to identify the signalling agents and their molecular mechanisms of action. Two specific examples provide a framework for more detailed consideration, effects of Ca^{2+} and of di-calciphor, a synthetic prostaglandin B_1 analogue.

As discussed above, increased cytosolic Ca^{2+} is an intracellular second messenger that signals activation of diverse, specialized cell responses. Most of these responses either directly or indirectly require increased energy production, e.g. for cytoskeletal changes, altered gene expression, altered ion transport. Thus, it is reasonable to assume that mitochondria respond to the same activating signal. Indeed, mechanisms are known whereby increased Ca^{2+} increases NAD^+-linked dehydrogenase (16), ATP synthase (62), and adenine nucleotide carrier (6) activities. Increased Ca^{2+} also increases mitochondrial P_i uptake (47) and stimulates mitochondrial O_2 consumption (6, 38). Thus, Ca^{2+} appears to be a likely agonist for coordinated activation of mitochondrial respiration.

Studies of anoxia provide evidence for a second type of coordinated regulation, one involving simultaneous inhibition of ATP synthase, P_i uptake, and adenine nucleotide exchange (1, 5, 6). Recent studies suggest that di-calciphor, the dimer of 16,16-dimethyl-15-dehydroprostaglandin B_1, inhibits each of these activities in cyanide-treated liver cells (42). The mechanism of this inhibition is not known but may occur through a fatty acid binding site that appears to be common to several of the mitochondrial inner membrane transport systems (3, 31). Such a pattern of coordinated regulation provides a means to adjust oxidative phosphorylation to a reduced functional state in opposition to activation provided by Ca^{2+} or other positive effectors.

NUTRITIONAL RELEVANCE OF THE COORDINATED MULTISITE REGULATION OF MITOCHONDRIAL RESPIRATION

Coordinated regulation of mitochondrial respiration is of fundamental importance to nutritional support of higher organisms. Cells in higher organisms are usually regulators, not conformers. Thus, provision of oxidizable substrates without consideration of the regulatory factors may be of little or no benefit.

The most general implication of the coordinated multisite regulation is that cells and organisms can be adjusted between relatively energy-inefficient, high work capacity machines and highly efficient, low capacity machines. This concept is easiest to apply to muscle, but it is also applicable to all cells and tissues that can vary work load, e.g. kidney, lungs, etc. A high work capacity can be achieved through two distinct mechanisms---expression of more metabolic, transport, and mechanical machinery (61) and operation of that machinery at higher rates of oxidation, higher Δp, and higher [ATP]/([ADP]·[P_i]). Increases such as these reduce energy efficiency at a low work load because of the energy required to maintain the machinery and metabolic and ionic homeostasis. For example, at higher Δp, more ion leak occurs. At higher ATP/ADP, more spontaneous hydrolysis occurs. Coordinated multisite activation of cellular respiration allows changes in Δp and ATP/ADP to anticipate work load and/or respond rapidly and efficiently to an imposed work load.

In contrast, chronic lack of work load allows acclimatization to a more highly efficient, reduced capacity state. This physiological state not only has decreased work machinery but also has oxidative phosphorylation machinery in a lower state of readiness for function. Substrate oxidation is less, Δp is lower, and [ATP]/([ADP]·[P_i]) is lower; overall utilization of energy is more energy efficient, but work capacity is considerably reduced.

This view of respiratory control has far-reaching implications for human health and disease. Firstly, it provides a rational basis for different "set points" of metabolism, where "set point" refers to the prevailing basal condition ranging from a low-capacity, high efficiency state to a high-capacity, low efficiency state determined by coordinated multisite regulation. The need for different set points of oxidative phosphorylation can be readily seen from consideration of the balance between NADPH supply and NADH supply. NADPH is required for most biosynthetic and detoxication pathways; thus, conditions of growth and repair have much higher requirements for NADPH than do other conditions. The pentose phosphate pathway, which competes with glycolysis for utilization of glucose (55), produces much of the NADPH in cells. NADPH is also produced from oxidative decarboxylation of iso-

citrate and 2-oxoglutarate by enzymes that are $NADP^+$-specific (30) and by an energy-linked transhydrogenase that utilizes NADH as the reductant in the mitochondria (58). Because all of these major reactions that produce NADPH compete with provision of NADH for oxidative phosphorylation, mechanisms must be present to control the flux through these competing pathways. By controlling the amount of substrate oxidation directed toward NADH generation, the balance between substrate utilization for ATP production and NADPH supply is effectively regulated. The set point for oxidative phosphorylation must be variable to accomplish such shifts in metabolic needs of cells.

Changes in molecular machinery in response to altered work load, such as hypertrophy of muscle with exercise or tissue recovery following surgery, are well known to adjust the cells to a different work capacity and efficiency. Of considerable importance is the fact that coordinated multisite regulation can alter work capacity and energy efficiency without changes in constitutive molecular machinery. Under pathological conditions where detoxication and repair is critical, it may not be possible or even appropriate to therapeutically modulate this regulation of oxidative phosphorylation. On the other hand, supply of precursors for NADPH, such as glucose and citrate, may be beneficial to oxidative phosphorylation indirectly because of their use for NADPH production. Direct support of oxidative phosphorylation, bypassing the NAD^+-linked dehydrogenases, may also be affected by supply of succinate. Succinate is oxidized by succinate dehydrogenase and can, in principle, support oxidative phosphorylation and ATP production even though NAD^+-linked dehydrogenases are inhibited.

Until more is known about the mechanisms of regulation, therapy aimed at altering this response may not be very effective. On the other hand, variations in this regulation may underlie the contradictory results obtained with efforts to regulate oxidative metabolism. An example is the use of chloroacetate in an effort to stimulate pyruvate dehydrogenase activity and thereby stimulate energy production in septicemia and other conditions of apparent bioenergetic failure. Activation at a single site cannot activate the overall process.

A second implication of coordinated multisite regulation is that it provides a simple explanation for genetic differences in basal metabolic rate. Variations in expression of the machinery would be expected to alter capacity and efficiency of cell function because of the energy cost of maintaining more machinery. In addition, genetic differences in the regulatory components that result in altered steady-state concentrations of intermediates, increased membrane ion leak, or increased futile cycling would also affect the basal metabolic rate even without changes in the expression of molecular machinery.

Genetic differences can affect these responses and determine both the range of set point changes that are possible and the conditions under which these

occur. Similarly, differences between sexes and age-related differences in expression and regulation may result in an altered set point and, hence, may account for differences in basal metabolic rate. A range of set points within a population provides adaptability of the population because it assures that some individuals have a high degree of metabolic preparedness for physiological challenges (high energy demands) and other individuals have a high energy efficiency to survive periods of starvation but have a relatively poor preparedness for physiological challenges.

A third area of importance for the concept of coordinated multisite regulation is that it emphasizes the need for exercise, in conjunction with proper nutrition, for optimal health. The metabolic set point of oxidative phosphorylation is variable and at least partially determined by the need to be prepared for altered work loads. In the absence of imposed work, the systems shift to a more highly efficient, low work-capacity level because of the lack of need to maintain the machinery to accommodate work. Thus, sedentary individuals are expected to be more energy efficient, i.e. have a lower basal metabolic rate and require fewer calories for weight maintenance. This is an extremely important issue because changes in calorie expenditure of only 1–2% per day can result in a 1 to 2 kg change in body weight per year. Changes in energy efficiency of 1 to 2% in forming Δp or using it to generate $[ATP]/([ADP]\cdot[P_i])$ would not be measurable with current techniques. Thus, improved methodologies will be needed to rigorously test whether coordinated, multisite regulation of oxidative phosphorylation controls the energy efficiency of nutrient oxidation and thereby contributes to differing tendencies of individuals toward obesity.

CONCLUSIONS

Contemporary studies of cellular energy metabolism in higher organisms indicate that primary regulation occurs independently of ATP, ADP, and inorganic phosphate concentration changes. Results indicate that regulation occurs at multiple sites and that coordinated regulation at these sites allows optimization of cell function through a range of metabolic set points. At one extreme, metabolic systems are at a high state of readiness to accommodate increased work loads, but this is relatively inefficient with regard to energy expenditure under basal conditions. At the other extreme, a low work capacity is associated with highly efficient energy utilization.

The sites of regulation include NAD^+-linked dehydrogenases, cytochrome oxidase, ATP synthase, adenine nucleotide transporter, and the mitochondrial phosphate carrier. Coordinated regulation at these sites has the ability to balance utilization of nutrients between NADPH supply for biosynthetic and detoxication functions and the need of ATP for these and other aspects of cell

function. Coordinated regulation also provides the means to anticipate an increased work load and to adjust to increased work without decreases in $[ATP]/([ADP]\cdot[P_i])$. In addition, control at these sites allows mitochondrial function to be suppressed during anoxia to preserve osmotic stability and prolong the duration of anoxic tolerance.

The existence of this type of control has diverse implications for nutrition because it affects the strategies used for nutrient supply to individuals under various pathological and physiological states. It brings us to the conclusion that all calories are not equivalent with regard to cell and organ function and, hence, to human health. Numerous epidemiologic studies have arrived at this same conclusion: isocaloric supply of different types of fats, of different mixtures of fats, sugars, and protein, and of different types of proteins and carbohydrates are associated with different human health benefits and risks. Thus, the challenge before us is to identify the molecular factors involved in coordinated multisite regulation so that these factors can be nutritionally or therapeutically manipulated to improve human health. Recognition of this type of regulation directs our attention to certain precursors, such as citrate and succinate, which may be useful because their pathways of metabolism allow them to be used for NADPH supply or improved ATP production independently of the respiratory control state. Finally, recognition of coordinated multisite regulation reemphasizes the interplay between diet and exercise as determinants of health because it defines metabolic readiness in terms of metabolic conditioning.

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