Part I
Molecular System Bioenergetics: Basic Principles, Organization, and Dynamics of Cellular Energetics
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Cellular Energy Metabolism and Integrated Oxidative Phosphorylation

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Abstract

Energy metabolism in living organisms is supported by the oxidation of carbohydrates and lipids, which are metabolized with several similarities as well as major differences. Conversely to prokaryotes and inferior eukaryotes, a complete oxidation leading to CO₂ and H₂O formation is mandatory. Our knowledge about oxidative phosphorylation is mostly based on simplified in vitro models, i.e., isolated mitochondria where only those parameters included in the experimental systems can be appreciated. However, relationships between mitochondria and the host cell are of major importance in the regulation of the pathway of ATP synthesis and oxygen consumption by the respiratory chain. By determining the respective rate of glucose or fatty acid oxidation, cellular intermediary metabolism affects the ratio between NADH and FADH₂, upstream of the Krebs cycle, thus affecting the yield ATP synthesis. The mechanism for translocating reducing equivalents across the mitochondrial inner membrane (the malate–aspartate and glycerol-3-phosphate–dihydroxyacetone phosphate shuttle) also plays an important role. Indeed, because of such characteristics of electron supply to the respiratory chain, oxidative phosphorylation activity also participates in the determination of the ratio of NADH to FADH₂ oxidation, by modulation of the protonmotive force. Besides the role of thermodynamic and kinetic constraints applied to the oxidative phosphorylation pathway, it now appears that the supramolecular organization of oxidative phosphorylation and of cellular energy circuits introduces new regulatory factors of oxidative phosphorylation.

1.1

Introduction

Energy metabolism in living organisms is supported by the oxidation of two substrates: carbohydrates and lipids. Amino acids are also good oxidative substrates;
however, after deamination, they enter the pathway of carbohydrate oxidation. Interestingly, the specific metabolism of these two families of substrates exhibits several similarities, while some major differences explain the advantage of maintaining these two different pathways throughout evolution. In particular physiological or pathological situations, lipid and/or glucose oxidation has alternatively both advantages and drawbacks, and choosing the right substrate may confer a substantial advantage. In prokaryotes, as in inferior eukaryotes, a complete oxidation, i.e., involving a respiratory chain, is not mandatory because these living organisms can release an excess of reducing equivalents in the medium. By contrast, in mammals, as in all superior eukaryotes, full oxidation of energetic substrates is required, leading to CO₂ and H₂O formation. Actually, while some cells lacking mitochondria (such as red blood cells and a few other cells) can survive with glucose fermentation to lactate as a unique energetic pathway, the reducing compound lactate is further oxidized in other aerobic cells of the same organism in such a way that, as a whole, energy metabolism in these organisms is completely aerobic [1].

From a strictly bioenergetic point of view, carbohydrates and lipids exhibit major differences regarding rate and efficiency of oxidative phosphorylation, while they both contribute to the reduction of NAD⁺ and FAD, although not in the same proportion. Because of the characteristics of the pathways of electron supply to the respiratory chain, oxidative phosphorylation activity participates in the determination of the ratio of NADH to FADH₂ oxidation, by the level of the generated steady-state protonmotive force. Reciprocally, the nature of the electron donors (NADH or FADH₂) regulates the rate and efficiency of oxidative phosphorylation as well as their relationship with the protonmotive force.

Besides such thermodynamic and kinetic constraints applied to the oxidative phosphorylation pathway, other important parameters are emerging, such as the supramolecular organization of oxidative phosphorylation and of cellular energy circuits. The highly dynamic characteristics of supramolecular organization introduce new regulatory factors of oxidative phosphorylation in addition to classical kinetic and thermodynamic parameters. Most of our knowledge about energy metabolism is based on simplified in vitro models, where the number of significant regulatory parameters is artificially limited. Hence, only those parameters included in the considered systems can be appreciated, and thus the others are ignored. Furthermore, minor parameters are often overemphasized because of the characteristics of the considered experimental system. In fact, the difference between in vitro and in vivo situations regarding glucose or lipid as a preferred substrate for oxidation and ATP synthesis is a good example. When carbohydrates (glucose) and lipids (octanoate) are provided simultaneously to isolated cells (hepatocytes), lipid oxidation will be preferred and pyruvate oxidation powerfully inhibited. This is due to the negative feedback effect of β-oxidation provided by acetyl-CoA on pyruvate dehydrogenase [2, 3]. However, the same competition between lipids and glucose in vivo (in humans) results in preferred glucose oxidation, while lipids are stored as a consequence of a rise in insulin [4]. Hence, the competition between the two major metabolites as substrate for ATP synthesis
results in opposite pictures \textit{in vivo} and \textit{in vitro}. Of course, this chapter will also suffer from these limitations; however, we will attempt to present the most integrative perspective.

1.2 Membrane Transport and Initial Activation

Plasma membrane transports followed by activation are the initial steps of glucose and fatty acid metabolism through glycolysis and \(\beta\)-oxidation, respectively. Glucose transport is allowed through a family of 13 carriers (GLUT), which differ in their kinetic characteristics [5]. Among these different carriers, GLUT4 is recognized as being regulated by insulin and other effectors and is connected to cellular energy status via AMP-activated protein kinase (AMPK) phosphorylation. GLUT4 carriers are stored in cytoplasmic vesicles and translocated to the plasma membrane in response to appropriate signaling [6]. Interestingly, similar events occur during fat transport across plasma membrane. FAT/CD36, the fatty acid carrier, is stored in the cytoplasm and translocated to the membrane upon appropriate signaling events. Furthermore, AMPK activation is also involved in the initiation of such translocation, reinforcing the similarity between both pathways [7]. Fatty acid activation by acyl-CoA synthetase is a cytosolic step requiring ATP and free CoA, while AMP and PP\(_i\) are released. Hence, fatty acid activation affects AMP levels and thus may participate in AMP kinase signaling processes (see Chapter 7). Glucose phosphorylation is permitted by a family of four enzymes (hexokinase) with different kinetic characteristics [8]. Interestingly, it has been shown that these enzymes are present as free compounds in the cytoplasm or bound to the outer mitochondrial membrane voltage-dependent anion channel (VDAC) (see Chapter 6). Interestingly, these enzymes, except glucokinase, are powerfully inhibited by the product glucose 6-phosphate (high elasticity) when present in the free form, while they are insensitive to this product when bound to the mitochondrial membrane [9]. This feature of hexokinase when bound to mitochondria is of major importance in explaining high glycolytic rates in some conditions, such as in pancreatic \(\beta\)-cells, in cancer cells, or the occurrence of cellular energy deficits.

1.3 Cytosolic Pathway

Numerous effectors regulate glycolysis, and it is not the purpose of this chapter to describe this as it has already been summarized in several good reviews [10–12]. However, we would like to focus on two important thermodynamic parameters: redox and phosphate potentials. Two successive steps work at near-equilibrium downstream of triose phosphate: glyceraldehyde-3-phosphate dehydrogenase (GAPdh) and phosphoglycerate kinase. Glycolysis is activated when the cytosolic
compartment is oxidized and when phosphate potential is lowered. The last step of glycolysis, pyruvate kinase, is far from equilibrium and is allosterically activated by ADP (see Fig. 1.1). A defect in ATP supply by oxidative phosphorylation induces a decrease in phosphate potential, while the cytosolic and mitochondrial compartments are even more reduced, a situation that would induce opposite effects on glycolysis: a low phosphate potential favors high phosphoenolpyruvate...
concentration, while a high NADH:NAD$^+$ ratio favors high glyceraldehyde-3-phosphate concentration. However, the allosteric activation of pyruvate kinase by ADP [10] results in increasing the glycolytic flux, allowing a cellular release of reducing equivalents via pyruvate fermentation to lactate. Hence, it appears that the glycolytic rate is finely tuned by both the cytosolic redox state and phosphate potential and that oxidative phosphorylation is tightly connected to glycolytic rate. Three main pathways represent the cytosolic fate of fatty acyl-CoA metabolism: (1) mitochondrial transport and subsequent $\beta$-oxidation, (2) phospholipid synthesis, and (3) cholesteryl ester synthesis, plus two others in some specific tissues: triglyceride synthesis and peroxisomal metabolism (Fig. 1.1). Recent data indicate that cytosolic metabolism of acyl-CoA is, at least partly, dependent on channeling processes at the level of acyl-CoA synthetase [13].

1.4 Mitochondrial Transport and Metabolism

The next step is represented by the transport across the mitochondrial membrane of both pyruvate and acyl-CoA, which are ultimately oxidized in the matrix. Fatty acid translocation across the mitochondrial inner membrane has been extensively studied and represents a major controlling step of long-chain fatty acid oxidation [14, 15]. Non-activated medium-chain fatty acids (non-esterified medium-chain fatty acids) can cross the inner mitochondrial membrane; therefore, they can be oxidized in a carnitine-independent manner. However, such a process requires prior matricial activation by mitochondrial medium-chain acyl-CoA synthetase, which is present mostly in liver [16, 17]. Therefore, the carnitine-independent oxidation of medium-chain fatty acids occurs mainly in liver. Besides being the major controlling step of acyl-CoA translocation into the matrix, the mitochondrial NADH:NAD$^+$ ratio also plays a key role in the control of $\beta$-oxidation, mainly through the redox state of enzymes directly involved in this pathway [14]. As in the respiratory chain, the pathway of $\beta$-oxidation involves several electron carriers, and the requirement of a simultaneous oxidation of both NADH and FADH$_2$ in order to complete the entire pathway is a very important feature (see Fig. 1.2). Because NADH oxidation must occur at the complex I level (except for 3-hydroxybutyrate dehydrogenase, see below), this respiratory chain complex represents a major controlling step for $\beta$-oxidation, and NADH oxidation by complex I must parallel the rate of $\beta$-oxidation in all tissues, except liver. In liver mitochondria, NADH can also be substantially oxidized by reducing acetoacetate to $\beta$-hydroxybutyrate in the ketogenic pathway. It is therefore possible to compare glycolysis with lactate fermentation (anaerobic glycolysis) versus liver $\beta$-oxidation with ketogenesis: both pathways generate reducing equivalents (NADH production), which in turn negatively control the rate, while in both cases NADH can be oxidized in the last step (lactate dehydrogenase or $\beta$-hydroxybutyrate dehydrogenase), thus allowing maintenance of the flux (Fig. 1.1). However, a striking dif-
ference remains between the two pathways: the net redox balance of glycolysis and fermentation is null, while there is a net production of reducing equivalents with \( \beta \)-oxidation, even when followed by ketogenesis. In conclusion, fatty acid \( \beta \)-oxidation requires mitochondrial respiratory chain activity.

Pyruvate entry into the matrix via the pyruvate carrier has long been studied [18]. This electroneutral transport involves one proton; therefore, pyruvate transport is affected by the difference in pH through the inner membrane. The next step is represented by the oxidative decarboxylation of pyruvate by pyruvate dehydrogenase, a step highly regulated by many effectors including two major forces related to oxidative phosphorylation: redox and phosphate potentials [3, 19]. It is important to note that the product of this step, acetyl-CoA, represents the ultimate and single common compound of both pathways. Indeed, the negative feedback by acetyl-CoA, provided by the \( \beta \)-oxidation, towards pyruvate oxidation represents the reciprocal metabolic control of \( \beta \)-oxidation on glucose oxidation (Fig. 1.1). However, as stated above, numerous effectors are involved in the regulation of the TCA cycle, ketogenesis, and steroidogenesis.
of both pathways, such as insulin, resulting in a much more complicated physiological response.

1.5 Respiratory Chain and Oxidative Phosphorylation

Respiratory chain activity has three main functions: (1) to oxidize reduced coenzymes, (2) to lower cellular oxygen concentration, and (3) to maintain a high protonmotive force. In addition, a high protonmotive force allows several mitochondrial enzymatic activities, including, of course, ATP synthesis, the net result being a chemiosmotic coupling between oxidation and phosphorylation. Because the cellular ATP requirement is not stoichiometrically linked to the need of reoxidation of reduced equivalent production, it is therefore of importance to finely adjust phosphorylation and oxidation separately, i.e., to modulate the ratio of ATP to O. There are three physiological ways to disjointedly tune oxidation and phosphorylation: (1) the site of electron supply to the respiratory chain; (2) the intrinsic stoichiometry of respiratory chain proton pumps, and (3) the degree of the proton conductance of the inner mitochondrial membrane [20].

1.6 Electron Supply

Electron supply to the respiratory chain is provided either upstream (NADH) or downstream (FADH₂) of complex I. This difference has important consequences because in the former case there are three coupling sites (complexes I, III, and IV), while in the latter only two coupling sites are involved (complexes III and IV). Hence, the yield of ATP synthesis is lowered by approximately 40% when FADH₂ is oxidized as compared with NADH. The nature of the cellular substrates (i.e., fatty acids versus carbohydrate) affects the stoichiometry of oxidative phosphorylation by affecting the ratio between NADH and FADH₂. Conversely to carbohydrate metabolism, fatty-acid β-oxidation results in the formation of equimolar amounts of NADH and FADH₂. Regarding the β-oxidation pathway, electrons are provided both to complex I from 3-hydroxyacyl-CoA dehydrogenase, via the bulk phase or by a channeling process (Fig. 1.2), and downstream of complex I to the quinone pool via the electron transfer flavin (ETF). Hence, the stoichiometry of ATP synthesis to oxygen consumption is lower when lipids rather than carbohydrates are oxidized. In the case of acetyl-CoA oxidation by the Krebs cycle, which is common to both carbohydrate and lipid oxidation, reducing equivalents are provided simultaneously to complex I (3 NADH), either via the bulk phase or by channeling, and to the quinone pool via complex 2 (1 FADH₂). The net result is then 10 NADH:2 FADH₂ for the complete glucose oxidation and 12 NADH:6 FADH₂ for the complete oxidation of hexanoate, a six-carbon fatty acid, by β-oxidation.
Fig. 1.3 Mitochondrial reducing power translocation and oxidative phosphorylation. The oxidative phosphorylation pathway consists of successive transductions of potentials from the chemical energy contained in the nutrient-to-phosphate potential (ATP:ADP-Pi), which is the energy source for the different biological functions. The chemical energy, supplied as reducing equivalent (NADH at complex I and FADH2 at complex 2), is first converted in membrane potential (∆p) by the respiratory chain, which links redox reaction to proton extrusion from the matrix to the intermembrane space. The high electrochemical gradient (−180 mV) generated permits ATP synthesis from ADP and Pi, as well as other functions such as Ca2+ uptake and substrate transport. The inner membrane is impermeable to NADH/NAD+ and to ATP/ADP; therefore, these compounds must be translocated by carrier systems: the malate–aspartate shuttle and adenine nucleotide translocase. These metabolite exchanges across the mitochondrial membrane are electrogenic and therefore depend on an electrochemical gradient. The gradient allows the entry of reducing equivalents in the matrix and the transport of ATP into cytosol. Hence, the net result is that the higher the electrochemical gradient is, the higher the matricial NADH:NAD+ ratio will be, allowing a high concentration of respiratory substrate NADH. Similarly, the higher the gradient, the higher the export of ATP, which helps to maintain a low ATP:ADP-Pi ratio in the matrix (facilitating ATP synthesis) and a high ATP:ADP-Pi ratio in the cytosol (facilitating ATP hydrolysis and energy utilization). (1) malate–aspartate shuttle; (2) complex I; (3) mitochondrial glycerol-3-phosphate dehydrogenase; (4) respiratory chain complex 2 – succinoo-dehydrogenase; (5) complex III; (6) complex IV; (7) ATP synthetase; (8) adenine nucleotide translocator; (9) cytosolic glycerol-3-phosphate dehydrogenase.
Reducing Power Shuttling Across the Mitochondrial Membrane

Because the mitochondrial inner membrane is impermeable to NADH, shuttle systems are required to carry the reducing power into the mitochondrial matrix. Two shuttles are involved in this exchange: the malate–aspartate shuttle, which depends on protonmotive force (Δp), and the glycerol-3-phosphate–dihydroxyacetone phosphate shuttle, which does not (see Fig. 1.3). While the former system provides electrons to complex I (i.e., as NADH), the latter supplies electrons directly to the quinone pool from the mitochondrial glycerol-3-phosphate dehydrogenase (FADH$_2$). Thus, by adjusting the flux through these two shuttles, the yield of oxidative phosphorylation (i.e., the cellular metabolism of oxygen and ATP) can be regulated. One of the major effects of thyroid hormones on mitochondrial energy metabolism is achieved through this mechanism because these hormones affect transcription of the mitochondrial glycerol-3-phosphate dehydrogenase, which regulates the flux through the glycerol-3-phosphate–dihydroxyacetone phosphate shuttle [21–23].

Electron Transfer in the Respiratory Chain: Prominent Role of Complex I in the Regulation of the Nature of Substrate

Because complex I is the final common obligatory step for NADH oxidation, all pathways leading to NADH production are in competition at this level, which must be a location of tight control. When considering carbohydrate and lipid mitochondrial oxidation, the dehydrogenases of the specific pathways (pyruvate dehydrogenase and 3-hydroxyacyl-CoA dehydrogenase) as well as the Krebs cycle dehydrogenases (isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase) compete. Furthermore, because malate dehydrogenase also represents the matricial NADH supplier of the malate–aspartate shuttle, this step represents a crossroad between (1) cytosolic and (2) mitochondrial redox state, (3) mitochondrial protonmotive force, (4) pyruvate, and (5) fatty acyl oxidation.

Considering this highly composite situation of multiple reciprocal regulations of different and interconnected pathways, two opposite and extreme pictures can be envisaged. First, the redox state of the bulk phase of the matricial compartment represents the common intermediate. In this situation the flux control of the different pathways depends on the capacity of complex I to oxidize NADH and on the specific elasticity of each dehydrogenase of the whole system towards the matricial NADH:NAD ratio. The second possibility is based on channeling of electron transfers between each dehydrogenase (or some of them) of the whole system and complex I. In this situation the supramolecular organization represents the main controlling factor (see below). Most likely, the actual situation is the result of a combination of these two extreme possibilities in a dynamic compromise, which is continuously adjusted and resettled. Nevertheless, the oxidation-reduction status of complex I probably represents the key regulator of these complex and interconnected pathways.
Fig. 1.4 (legend see p. 21)
Complex I is well known to work at near-equilibrium: it equilibrates the matricial redox state (NADH:NAD) and the protonmotive force [24]. Complex I generates a high protonmotive force while oxidizing NADH to NAD, i.e., when electron flux is transported in the forward direction. When electrons are transported in the reverse direction, it reduces NAD to NADH at the expense of the high protonmotive force generated at other coupling sites of the respiratory chain (complexes III and IV) and of electron supply downstream of complex I (i.e., FADH$_2$). Hence, thanks to this near-equilibrium between redox state and protonmotive force by complex I, mitochondrial protonmotive force appears to be the key regulatory factor in determining both the rate and the nature of the substrate used for fuelling the cell.

1.9 Modulation of Oxidative Phosphorylation by Respiratory Chain Slipping and Proton Leak

The occurrence of slipping processes between electron flux and proton transfer at the level of the respiratory chain is now well established [20, 25–28]. Recent data on the structure of cytochrome oxidase support the occurrence of slipping processes at this level [29–32]. In contrast to a proton leak, a slipping mechanism permits modulation of the rate of oxidation while the protonmotive force and the rate of ATP synthesis are not modified. Despite the low permeability of the inner membrane, proton leaks across the membrane do occur and result in the uncoupling of oxygen consumption from ATP synthesis, the energy being dissipated as heat (see Fig. 1.4). Uncoupling through proton leak permits dissociation of respiratory chain activities. In the presence of fatty acids, the metabolic effects of uncoupling are different. In this case the production of FADH$_2$ in the matrix by $\beta$-oxidation allows the supplying of substrates directly to complex II, even when the electrochemical gradient is collapsed. High levels of substrate supply to the respiratory chain lead to a strong activity, as evidenced by the very large increase in oxygen consumption. This high-level respiration activity permits maintenance, to some extent, of the electrochemical gradient, and therefore some ATP synthesis is maintained. In this case the main effect of uncoupling is an increase in oxygen consumption and heat production.

**Fig. 1.4** Schematic view of oxidative phosphorylation and its uncoupling. (A) Coupled oxidative phosphorylation (see legend to Fig. 1.3). (B) Uncoupling with carbohydrates. By permitting the protons to freely reenter into the matrix, the uncoupling process (via uncoupling protein, for instance) creates a “futile cycling,” dissipating energy into heat at the expense of oxygen consumption and water production. In the presence of carbohydrate as exogenous source of energy, reduced substrates are supplied to the respiratory chain as NADH, and energy-dependent import of NADH is required. Because of the uncoupling, the electrochemical gradient collapses, impairing the active transport of NADH. Therefore, a sufficiently high reducing state in the matrix may not be sustained. In these conditions the net result is a collapse of $\Delta\psi$ and the ATP/ADP-P$_i$ ratio, while oxygen consumption is low, despite the uncoupling state. (C) Uncoupling with fatty acids. In the presence of fatty acids, the metabolic effects of uncoupling are different. In this case the production of FADH$_2$ in the matrix by $\beta$-oxidation allows the supplying of substrates directly to complex II, even when the electrochemical gradient is collapsed. High levels of substrate supply to the respiratory chain lead to a strong activity, as evidenced by the very large increase in oxygen consumption. This high-level respiration activity permits maintenance, to some extent, of the electrochemical gradient, and therefore some ATP synthesis is maintained. In this case the main effect of uncoupling is an increase in oxygen consumption and heat production.
of the rate of oxidation from that of phosphorylation and thus a decrease in the yield of oxidative phosphorylation. This mechanism is similar to uncoupling through uncoupling proteins. The discovery of the physiological function of brown fat in mammals, related to the presence of uncoupling protein-1 (UCP1), has opened a new era in our understanding of the regulation of oxidative phosphorylation by describing a role for energy waste. Several other UCPs have recently been described [33, 34], and some of these (UCP2 and UCP3) have been found in most tissues, including white adipose tissue, muscle, macrophages, spleen, thymus, Kupffer cells, etc. [35, 36]. Whether proton leak occurs through these UCPs appears to be a legitimate question to ask.

In summary, the primary effects of slippage of proton pumps appear to modulate the rate of oxidation at a given level of protonmotive force, while proton leak primarily affects the level of protonmotive force. The secondary effects of slipping are related to its effect on redox state, and the physiological result is an increased reoxidation rate without a major effect on the nature of substrate involved (NADH or FADH₂). By contrast, the secondary effect of proton leak is related to the change in Δp with all the consequences related to it, thus including the effect mediated by complex I (i.e., a modulation of the nature of substrate supply [NADH versus FADH₂]).

1.10 The Nature of Cellular Substrates Interferes with the Metabolic Consequences of Uncoupling

Irrespective of the molecular mechanism(s), the metabolic consequences of a protonophoric leak (uncoupling) can be classified into three categories: (1) those related to the change in oxidation rate and redox state, (2) those related to the change in protonmotive force, and (3) those related to the change in ATP synthesis and phosphate potentials. In isolated mitochondria incubated in the presence of saturating concentrations of respiratory substrates, uncouplers invariably decrease Δp and redox and phosphate potentials and consequently increase respiratory rates. By contrast, in intact cells these forces are involved in a complex metabolic network that may significantly affect the outcome of uncoupling on the same parameters. On the one hand, when uncoupling is achieved without fatty acid, it results in a profound decrease in both Δp and cytosolic and mitochondrial ATP:ADP ratios, while the rate of respiration is not increased. This is due to a decline in the matricial reducing state linked to the collapsed protonmotive force. On the other hand, in the presence of octanoate, a large increase in respiration is associated with limited effects on Δp and ATP:ADP ratios because of the matricial supply of reducing equivalents downstream of complex I (FADH₂) [37–39]. Hence, the metabolic consequences of uncoupling in intact liver cells are variable and critically depend on the metabolic state of the cells. In the presence of a large supply of fatty acids and oxygen, the main effect of uncoupling is a dramatic in-
crease in oxygen consumption as well as energy waste. The active mitochondrial 
β-oxidation permits the sustaining of a very high rate of mitochondrial respiration 
and a high membrane potential, while ATP synthesis can be at least partially 
maintained because of this high respiratory chain activity. When glycolysis is the 
unique pathway for substrate supply to the respiratory chain, the decreased mito-
chondrial membrane potential resulting from uncoupling strongly affects the mi-
tochondrial redox potential, because the malate–aspartate shuttle, which depends 
on maintenance of Δp, is not able to sustain a highly reduced redox potential in 
the matrix. Hence, under these conditions, uncouplers do not significantly affect 
the respiratory rate, because the supply of reducing equivalents to complex I be-
comes controlling [40]. The main effect of uncoupling would be a striking de-
crease in Δp and ATP:ADP ratio, with an overall decrease in cell metabolic activ-
ity. It is not surprising that uncoupling by UCPI results in a huge increase in the 
rate of fatty acid oxidation, oxygen consumption, and heat production in brown 
fat, where the storage of triglycerides is associated with a large number of mito-
chondria with high oxidative capacity. Thus, depending on substrate oxidation 
and heat production, uncoupling in intact cells may have very different effects 
on mitochondrial depolarization and on its consequences on cell energy status. 
Hence, on the one hand, uncoupling may be a very efficient way of decreasing 
oxgen concentration by reducing it to water; on the other hand, by decreasing 
mitochondrial membrane potential and the ATP:ADP ratio, uncoupling may 
ffect all cellular pathways related to these potentials.

1.11 Dynamic Supramolecular Arrangement of Respiratory Chain 
and Regulation of 
Oxidative Phosphorylation

Considering the complex situation resulting from the numerous interactions of 
various parameters involved in many steps, either common or specific to these 
different pathways, the large number of common controlling steps may lead to 
excessive reciprocal dependence of these interconnected pathways. Hence, it 
seems important to maintain some degree of independence between these con-
sidered pathways, even if a high degree of coordination is mandatory. A biological 
response to this crucial question is given by a supramolecular organization of the 
pathway. Indeed, channeling in the glycolysis pathway has long been recognized. 
The cellular plasma membrane and cytoskeleton binding of the glycolytic enzyme 
lead to channeling of NADH to the respiratory chain in yeast, thanks to the pres-
ence of an external NADH dehydrogenase at the outer surface of the inner mem-
brane [41, 42]. In mammals, including humans, such an organization has been 
shown to play a role in the compartmentation of the glycolytic supply of ATP for 
fuelling the sodium–potassium ATPase [43]. More recently the impact of chan-
neling on fatty acid metabolism has been emphasized for both the cytosolic and 
mitochondrial parts of the pathway [13, 44]. Indeed, the fate of fatty acids appears
to be determined by supramolecular organization immediately after cellular entry, i.e., activation and orientation towards the main pathways (mitochondrial oxidation, phospholipids synthesis, cholesterol esterification, etc.). In addition, similar processes of channeling are also involved in mitochondrion translocation and matrrial \(\beta\)-oxidation. At the end of the pathway, electrons are probably channeled to the respiratory chain, and supramolecular organization of Krebs cycle enzymes has long been reported [45–48]. Interestingly, one of the Krebs cycle dehydrogenases, malate dehydrogenase, which is also involved in the reducing equivalent’s translocation across the inner mitochondrial membrane, has been reported to preferentially provide NADH towards complex I [49]. Finally, the organization of the respiratory chain is another example of metabolic channeling between reduced coenzymes and oxygen. In the classical model of the respiratory chain arrangement, several multiproteic blocks are defined (complexes I, III, and IV), which are interconnected by small and mobile electron carriers (i.e., quinone and cytochrome \(c\)). In such a view, a “common” quinone pool interconnects complexes I and III, as complexes III and IV are connected by a “common” cytochrome \(c\) pool (“liquid-state” model). Such an organization has been challenged by two kinds of experimental data. First, experiments with mild detergents have permitted the obtaining of several types of supramolecular organizations of the respiratory chain with different fixed stoichiometry, including, for instance, complexes I, III, and IV associated with quinones and cytochrome \(c\) or complexes III and IV associated with quinone and cytochrome \(c\) (see [50] for review). Of course, such a “unit of electron transfer” is not fixed but represents a dynamic supramolecular organization that can be modulated depending on environmental conditions. This view of a “solid-state” model in which orderly sequences of redox compounds catalyze electron flux is also supported by kinetics analysis [51, 52].

Secondly, the origin of electron supply, i.e., from the different dehydrogenases, may or may not lead to competition. Hence, in yeast mitochondria, NADH supply by external NADH dehydrogenase inhibits all matrical dehydrogenases except succinate dehydrogenase, indicating a preferred channeling pathway [53, 54].

From these considerations, it appears that besides the long-recognized role of the various regulatory effectors involved in the tight reciprocal control of the two main substrates involved in cellular energy metabolism (carbohydrates and lipids), the global organization of the system is also a major parameter that, like metabolic effectors, is subject to continuous adaptation.

In view of several data sets, already published or not, it seems unlikely, at least for liver metabolism, that succinate is oxidized in the absence of complex I, because of the importance of the reverse electron flux from succinate to NADH [24, 55]. By contrast, the lack of evidence of such a reverse electron flux on complex I when fatty acid oxidation represents the electron source does not favor the presence of complex I in such a respiratory chain organization. Of course, NADH formation by 3-hydroxyacyl-CoA dehydrogenase in the \(\beta\)-oxidation must oxidized; however, in such a view, this could be achieved in a different respiratory chain organization.
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References


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