Integrated and Organized Cellular Energetic Systems

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Advanced Article

Article Contents

Bioenergetics as a part of biophysical chemistry and biophysics is a quantitative science that is based on several fundamental theories. The definition of energy itself is given by the first law of thermodynamics, and application of the second law of thermodynamics shows that the living cells can only function as open systems in which internal order (low entropy state) is maintained by increasing the entropy in surrounding medium (Schrödinger's principle of negentropy). For this mechanism, exchange of mass is needed that yields metabolism as the sum of catabolism and anabolism. The free-energy changes taking place during metabolic reactions obey rules of chemical thermodynamics, which deals with Gibbs free energy of chemical reactions and with electrochemical potentials. For application of these theories to the integrated systems *in vivo*, complex cellular organization should be accounted for: macromolecular crowding, metabolic channeling and functional coupling mechanisms caused by close and tight protein–protein interactions, compartmentation of enzymes, as well as both macrocompartmentation and microcompartmentation of substrates and metabolites in cells should be considered. For integration of these system components, effective methods of communication, including compartmentalized energy transfer systems, are required. These systems are represented in muscle cells by phosphotransfer networks, mostly by creatine kinase and adenylate kinase systems. System analysis of these networks shows their central role both in energy transfer and metabolic feedback regulation of mitochondrial function, in regulation of ion fluxes and cellular energetics during increased workload by synchronizing the cellular electrical and mechanical activities with energy supply and substrate oxidation.

Introduction

Living cells belong to the group of open thermodynamic systems, because they exchange both energy and mass with their environment (1–6). This exchange is necessary to maintain cell structure and function and to avoid their decay into equilibrium, which would mean death. This thermodynamic principle as a physical basis of life was first discovered and described some 60 years ago by Erwin Schrödinger in his famous book "What is Life?" (7). Mass and energy exchange is exactly what metabolism does (7), which includes both catabolism and anabolism (see below), and it is a lawful way for cells to live in a thermodynamic sense. At the same time, metabolism is the basis of the biological energy transformations in the cells, because any chemical or biochemical reaction is inevitably linked to free-energy changes, according to the laws of chemical thermodynamics. In the first part of this article, we generally describe these theoretical bases of free-energy conversion and metabolism. In the second part, we analyze the energy-transfer networks in muscle and brain cells. Because of structural organization and macromolecular crowding, almost

all processes important for the cell life are compartmentalized, and for their integration, effective ways of communication are required, which include compartmentalized energy transfer systems. These systems are represented in muscle cells by the phosphotransfer networks mostly by creatine kinase and adenylate kinase systems. Normal muscle function depends on the fine interplay of energy metabolism and calcium metabolism inside the cell. System analysis of integrated energy metabolism shows that highly organized phosphotransfer pathways play a central role in intracellular communication and signaling. The last part of this article describes the mechanisms of metabolic feedback regulation of respiration and substrate supply, as well as the mechanisms of energy sensing by which the energy state of the cell controls ion fluxes, including those of calcium.

Theoretical Basis of Cellular Metabolism and Bioenergetics

Thermodynamic laws, energy metabolism, and cellular organization

The first law of thermodynamics formulated by Mayer in 1842 is the law of energy conservation for isolated systems. It is also useful for defining of energy itself as the capacity to perform any kind of work and/or produce heat. This law says that the change of the internal energy of a system dE

$$dE = E_f - E_0 = \delta q + \delta w \qquad (1)$$

is the result of heat exchanged and taken up by system δq, and work done by the surrounding medium on the system δw (E_f and E_0 are the final and initial energy levels, correspondingly). By convention, if the system performs work, then the sign of δw is negative.

The second law of thermodynamics describes a direction in which all processes can occur spontaneously. It tells us that irreversible processes move in the direction of the increase of entropy. The entropy function S was introduced by Clausius as $dS = \delta q/T$ (for reversible processes) to describe the Carnot cycle, and Boltzmann gave its statistical explanation in terms of molecular kinetic theory:

$$S = k_B \log W \qquad (2)$$

Here k_B is Boltzmann constant and W is the thermodynamic probability function associated with the number of possible arrangements of molecules (each arrangement is called "microstate") in the given macrostate of a system (1, 4). Thus, the less organized a system is, the larger the number of possible arrangement of molecules in it (or number of "microstates") and the higher the entropy of system will be. The second law of thermodynamics tells us that under irreversible conditions, all processes in all systems move toward a state of a greater probability or greater disorder.

The two thermodynamic laws taken together describe the relationship between changes in the functions of the thermodynamic state of a system—Gibbs free energy G (which is the capacity to do useful work), enthalpy H (which is the maximal amount of exchangeable heat a system can produce), and entropy S (related to the amount of heat that cannot be used to perform useful work, but related to the order exhibited by the system):

$$dG = dH - TdS = dE + pdV - TdS \leq 0 \qquad (3)$$

According to these laws, all irreversible processes can proceed spontaneously only when the free energy decreases in the direction of equilibrium at which it reaches a minimum and does not change anymore ($dG = 0$). The decrease of free energy in any spontaneous process is mainly caused by an increase in entropy. If the system is under nonequilibrium steady-state conditions, then the free energy is continuously dissipated (see below) (1, 2, 4–6).

Thus, the universal laws of thermodynamics described by the Equation (3), allow all processes and reactions to proceed only in the direction in which the free energy decreases and entropy increases and thus in which disorder increases. This law seems to be in contradiction with what is known about cell life. In cells, biochemical catalysis needs protein catalysts with precise conformational structure; transmission of genetic information needs the permanence of the structure of DNA and RNA; metabolic pathways are organized into supramolecular complexes, metabolons, and so on. Schrödinger (7) showed that the apparent contradiction is overcome if we consider that living cells are open systems, which by means of metabolism decrease or maintain their low entropy state by increasing the entropy of the medium. This mechanism is shown by the general scheme in Fig. 1 that describes the energetics of the cell as an open system that exchanges both energy and masses with surrounding medium. Catabolic reactions through coupling to anabolic reactions (biosynthesis) maintain cell structure and organization as an expression of the decrease in entropy. They are also the source of metabolic energy for the performance of any kind of cellular work.

Chemical and electrochemical potentials: energy of transmembrane transport and metabolic reactions

In general terms, the capacity to do useful work, which is quantified by the Gibbs free energy, is a function of the pressure p, temperature T, and chemical composition (1), and its full differential can be written as follows:

$$dG = \left(\frac{\partial G}{\partial p}\right)_{T,n_i} \cdot dp + \left(\frac{\partial G}{\partial T}\right)_{p,n_i} \cdot dT + \sum_i \left(\frac{\partial G}{\partial n_i}\right)_{p,T} \cdot dn_i \qquad (4)$$

where $\frac{\partial G}{\partial n_i} = \mu_i$ is a chemical potential of a component i.

Usually, biochemical reactions occur at constant temperature and pressure, and thus $dP = 0$, and $dT = 0$. Under these conditions, the free-energy changes are caused by changes in the chemical composition, that is, the free-energy changes in

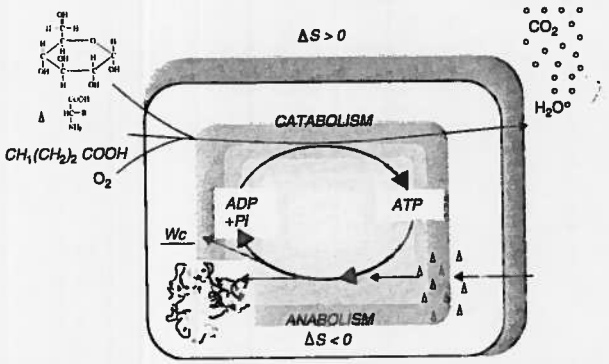


Figure 1 General scheme of cellular metabolism. A cell is a thermodynamically open system. In accordance with the Schrödinger's principle of negentropy, extraction (increasing the entropy in extracellular medium, and decreasing it in the cell) via metabolism is necessary for maintenance of the structural organization of both biopolymers as proteins, DNA and RNA, as well as maintenance of the fine structural organization of the cell for effectively running compartmentalized metabolic processes. In this way, the cell can live in agreement with the thermodynamic laws (see text).

the system are directly related to the chemical or biochemical reactions (1, 2):

$$dG = \sum_i \left(\frac{\partial G}{\partial n_i}\right)_{T,p} \cdot dn_i = \sum_i \mu_i \cdot dn_i \qquad (5)$$

Or in the integral form:

$$G = \sum_i \mu_i \cdot n_i \qquad (6)$$

The first derivative of the Gibbs free energy with respect to the molar concentration of component n_i, its chemical potential $\mu_i = (\partial G/\partial n_i)_{T,p}$ [see Equation (4)], is function of the molar concentration C_i of component n_i. For species bearing electric charges z (cations or anions) in the presence of electrical potential Ψ, it becomes an electrochemical potential (1–3):

$$\tilde{\mu}_i = \mu_i^0 + RT \ln C_i + zF\Psi \qquad (7)$$

$F = 0.0965\ \text{kJ mol}^{-1}\text{mV}^{-1}$ is the Faraday's constant (3), and $\mu^0{}_i{}_n$ a standard chemical potential, which corresponds to the standard reference state of system, in which the concentration is $C^0 = 1$ M. To remember that all concentrations should be given in reference to this state, the equation can be rewritten as follows:

$$\tilde{\mu}_i = \mu_i^0 + RT \ln \frac{C_i}{C^0} + zF\Psi \qquad (8)$$

Equations (8)–(9) are the basic equations of bioenergetics and describe the energy conversion in biophysical or biochemical processes (1–6). Thus, according to Mitchell's chemiosmotic theory, the protons translocated by the respiratory chain result in a transmembrane gradient of H^+ or ΔpH and other ions (e.g., K^+, Na^+, Ca^{2+}) indirectly coupled to the H^+ gradient or $\Delta\Psi$, which together represent an electrochemical potential of ions or protonmotive force:

$$\Delta p = \frac{\Delta\mu_{H^+}}{F} = \Delta\Psi - \frac{2.3RT}{F} \cdot \Delta pH \qquad (9)$$

The free-energy changes in the metabolic reactions are in similar way explained by changes of chemical potentials of reaction components, substrates, and products. Thus, free-energy change in the reaction of the conversion of substrate A into product P:

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} P$$

Is given by a difference of chemical potentials of P and A,

$$\begin{aligned}\Delta G &= \mu_P - \mu_A \\ &= \left(\mu_P^0 + RT \ln \frac{[P]}{C^0}\right) - \left(\mu_A^0 + RT \ln \frac{[A]}{C^0}\right) \\ &= (\mu_P^0 - \mu_A^0) + RT \ln \frac{[P]}{[A]}\end{aligned} \qquad (10)$$

$\Delta G^0 = (\mu_P^0 - \mu_A^0)$ is the standard free-energy change and $\Gamma = \frac{[P]}{[A]}$ is the mass-action ratio of the reaction (for multireactant reactions this ratio is defined as $\Gamma = \frac{\Pi_i[P]_i}{\Pi_i[A]_i}$), and therefore:

$$\Delta G = \Delta G^0 + RT \ln \Gamma \qquad (11)$$

At equilibrium,

$$\Delta G = \Delta G^0 + RT \ln \Gamma = 0 \qquad (12)$$

and Γ corresponds to the equilibrium constant, Keq:

$$\Gamma_{eq} = K_{eq} = \frac{[P]_{eq}}{[A]_{eq}} = \frac{k_1}{k_{-1}} = e^{\frac{-(\mu_P^0 - \mu_A^0)}{RT}} \qquad (13)$$

That value is typically written as:

$$\Delta G^0 = -RT \ln K_{eq} \qquad (14)$$

This equation is the famous equation that relates the equilibrium constant to standard free-energy change, which was first described by van't Hoff. Thus, the final equation for the free-energy change in any reaction is as follows:

$$\Delta G = -RT \ln K_{eq} + RT \ln \Gamma \qquad (15)$$

This equation can be rearranged in the following way:

$$\Delta G = RT \ln \frac{\Gamma}{K_{eq}} \qquad (16)$$

Figure 2a shows the plot of the free energy G of a system calculated on the basis of Equation (6) as functions of log Γ/K_{eq} (the ratio Γ/K_{eq} is shown in logarithmic scale), and **Fig. 2b** shows the corresponding ΔG. In **Fig. 2**, one can observe a minimum of free energy G in the equilibrium position, where $\Delta G = 0$ (8). If the reaction is shifted to the right in direction of an increase of Γ/K_{eq}, then free energy and ΔG increase (**Fig. 2**). Free energy of a system is higher also on the left from equilibrium position of the reaction (**Fig. 2a**), but in this case ΔG is negative (**Fig. 2b**). That means that on the left from equilibrium the reaction in direction A → P is exergonic (spontaneous), and on the right it is endergonic (nonspontaneous). The opposite is true for the reaction P → A, which is endergonic on the left and exergonic on the right

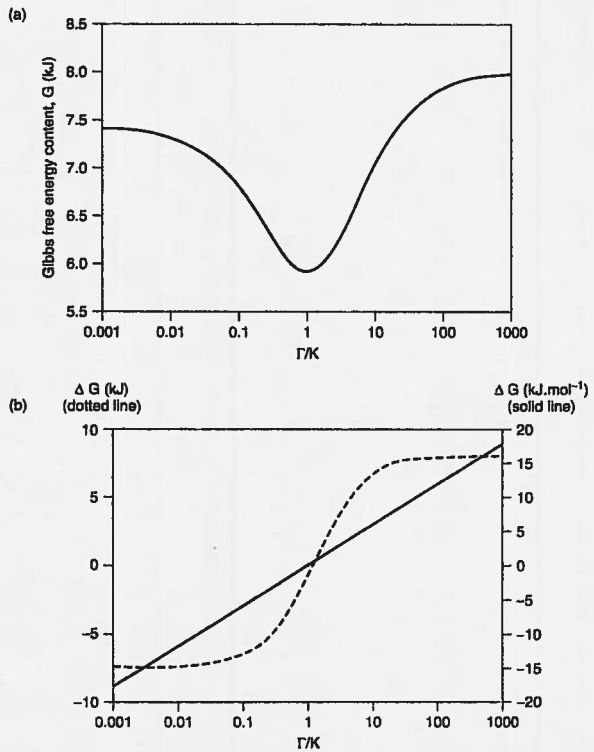


Figure 2 (a) The free energy of a system as a function of the log Γ/K_{eq} (Γ/K_{eq} on the abscissa axis is given in the logarithmic scale). Letters A and P indicate the free energies of pure compounds A and P, respectively. Γ is shown for the direction of conversion of A into P. Free energy of the system was calculated according to Equation 6: $G = n_A\mu_A + n_P\mu_P$. (b) ΔG as a function of log Γ/K for two cases. The solid line shows ΔG calculated by using Equation 16 and expressed in kJ per mole. This line shows the amount of the free energy, which could be obtained (in exergonic reactions) or used (in endergonic reactions) for conversion of one mole of substrate under given conditions. The dotted line shows the ΔG available in the "real" system that corresponds to our calculations, taking into account an amount of substrate present, $\Delta G = n_P\mu_P - n_A\mu_A$, in kJ. The calculations were made by Marc Jamin (Joseph Fourier University, France) using the following arbitrary conditions: $\mu_A^o = 7.4\ \text{kJ.mol}^{-1}$, $\mu_P^o = 8\ \text{kJ.mol}^{-1}$, $K = 0.8$, $[A]_0 = 1$ M, $[P]_0 = 0$ M (adapted from Reference 8 and is a courtesy of Marc Jamin).

from equllibrium. Figure 2b shows two ways of to express ΔG: A solid line shows this parameter in kJ per mole of a substrate, which is a linear function of log Γ/K in accordance with Equation (16). This value is the free-energy change during conversion of one mole of substrate into product under given conditions (given value of Γ). The dotted line shows ΔG available in the actual system by taking into account the number of moles present in the system, $\Delta G = n_P\,\mu_P - n_A\,\mu_A$, where nA and nP are the number of mole of A and P, respectively. This latter value of ΔG is directly related to "real" changes in G shown in Fig. 2a.

These relationships mean that any biochemical reaction in the cell that is away from equilibrium is a potential source of free energy.

Nonequilibrium steady-state conditions

As it has recently been shown by Qian and Beard (6), In the case of biochemical networks in the living systems, when the metabolic processes are in the steady state far from equillbrium, Equation (16) can be modified to include fluxes, or reaction rates (5, 6). For processes that are in the nonequilibrium steady state, for example the reaction

$$\rightarrow \quad A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} P \quad \rightarrow$$

the forward and backward fluxes are $J_+ = k_1(A)$, $J_- = k_{-1}(P)$, correspondingly and by definition $J = J_+ - J_- \neq 0$ (only in equilibrium $J = 0$). The equilibrium constant is $K_{eq} = k_1/k_{-1}$, and replacing K_{eq} in Equation (16) by this expression, we may write for chemical potential differences in steady state of this reaction in the system:

$$\Delta\mu = RT \ln \frac{J_-}{J_+} \tag{17}$$

This equation represents the chemical driving force under steady-state conditions. In an open system, Equation (17) also shows the amount of energy to be applied to the system to maintain the reactions in the steady state (constant concentrations of A and P), and in the absence of work performed this is the energy dissipated as heat and transformed into entropy of surrounding (5, 6). This result is explained by nonequilibrium thermodynamics (2, 9–11), according to which for open systems the entropy may be split into two contributing parts (9, 10)

$$dS = d_iS + d_eS \tag{18}$$

where d_iS is the entropy production from irreversible catabolic processes inside the system that always increases; d_eS is the entropy flow from the surroundings whose contribution is null in an isolated system because entropy always increases irreversibly in such a system. However, in systems open to the exchange of matter and energy like biological systems, d_eS may be negative, so that the system may decrease its entropy because statistical fluctuations may favor the organized state. The stabilization of an organized state after statistical fluctuations or instabilities (order through fluctuations) may occur because of the existence of nonlinear kinetic laws. These states are maintained by energy and matter dissipation, therefore they are also known as *dissipative structures* (9). This example clarifies the answer given by Schrödinger to the question: How does the living organism avoid decay? Or, otherwise stated, how do they avoid contradicting the second law of thermodynamics? The basic answer given by Schrödinger is that organisms do not disobey the second law because as first formulated, it applies to closed rather than open systems. More explicitly, although a living organism continually increases entropy (positive entropy or d_iS) and tends to approach the dangerous state of maximum entropy (or death), it keeps away from danger by continually drawing from its environment negative entropy (d_eS) (7) or more rigorously, from free energy to keep its organization. See Reference 11 for an excellent discussion.

Free-energy changes and the problem of intracellular organization of metabolism

Coupling of catabolism with anabolism (the metabolism) is the way through which "negentropy" or free energy is extracted from the medium. Evolution has selected the adenine nucleotides to fulfill this important task of coupling catabolism and anabolism (Fig. 1). A possible explanation is the rather high standard free-energy change ($\Delta G^{\circ} = 31.5$ kJ/mol) and high affinity of ATP and ADP for many enzymes and carriers (12). Cellular energetics is thus based on the reactions of ATP synthesis and use, $ADP + P_i \Leftrightarrow ATP + H_2O$. Taking water content as a constant and in excess (not changing in the reaction), the mass action ratio of the reaction of ATP synthesis is usually written as

$$\Gamma = \frac{[ATP]}{[ADP][P_i]} \tag{19}$$

By maintaining high mass-action ratio for the reaction of ATP synthesis, catabolic reactions supply also free energy for cellular work. According to Equations (14)–(16), free energy available in the cellular system is a function of the ratio of Γ to the equillbrium constant of the ATP synthesis, or

$$\Delta G_{ATP} = \Delta G^0_{ATP} + RT \ln \frac{[ATP]}{[ADP][P_i]} \tag{20}$$

This function is usually called phosphorylation potential (13, 14). The principal purpose of free-energy transformation associated to catabolic reactions is to keep a high value of the phosphorylation potential, which is mostly achieved through mitochondrial oxidative phosphorylation or photosynthesis in autotrophic organisms. The theory of phosphorylation potential for the analysis of the cellular life was first used by Veech et al. (13) and Kammermeier et al. (14).

However, now it has become clear that applying Equation (20) as well as any quantitative theory of physical chemistry to the real intracellular medium is not a simple task, because of the complex organization of cell structure and metabolism. It has become clear that it is not the global ATP content that is important, but the ATP and the free energy available in

microcompartments and macrocompartments that have to be accounted for, as it will be described below.

Macromolecular crowding, compartmentation, and vectorial metabolism

The first phenomenon to be taken into account in all cells is macromolecular crowding: The high concentrations of macromolecules in the cells (15–22) decrease the volume available for free diffusion of substrates and accordingly make it difficult to use correctly the laws of enzyme kinetics and equations that usually are applied in enzymatic studies carried out in diluted solutions (20, 22, 23). Cytoplasmic protein concentration may be as high as 200 to 300 mg/ml, which corresponds to a volume fraction of about 20–30% of intracellular medium occupied by these proteins (15, 16). In the mitochondria, the high density of enzymes and other proteins constitutes more than 60% of the matrix volume (17).

At first sight, this macromolecular crowding should cause a real chaos by making intracellular communication by diffusion of reaction intermediates difficult. This chaos and related problems are well described by Denis Noble in his recent book (24). In reality, however, macromolecular crowding yields new mechanisms based on specific protein–protein interactions: microcompartmentation, metabolic channeling, and functional coupling, and in this way to a fine organization and regulation of the metabolism. The origin of this order, the program, and source of these specific interactions and cellular organization are still a mystery hidden in undiscovered genetic laws, which include feedback control of gene expression, and in rules governing system level properties (24); their elucidation is the main challenge for cell sciences in the postgenomic era (24–27).

Heterogeneity of intracellular diffusion and metabolic channeling

Many new experimental techniques have been developed to study the molecular networks formed by protein–protein interactions (28). In the cells, the high protein density predominantly determines also the major characteristics of cellular environment, such as diffusion in heterogeneous compartments (23, 29–31). Distinct barriers to diffusion of solutes within the cells include binding and crowding. Whereas molecular crowding and sieving restrict the mobility of very large solutes, binding can severely restrict the mobility of smaller solutes (See Reference 30 and references therein). This phenomenon explains also the heterogeneity of the diffusion behavior of ATP in cells. Studies by using pulsed-gradient ^{31}P-NMR showed that the diffusion of ATP and phosphocreatine is anisotropic in muscle cells (31, 32). Recent mathematical modeling of the decreased affinity of mitochondria for exogenous ADP *in situ* in permeabilized cardiac cells also showed that the ADP or ATP diffusion in cells is heterogeneous. The apparent diffusion coefficient for ADP (and ATP) may be locally decreased (diffusion locally restricted) by an order, or even several orders, of magnitude (33). A similar limited diffusion of ATP in the subsarcolemmal area in cardiac cells was proposed by Terzic and Dzeja group (34, 35). Firm experimental evidence exists to suggest compartmentalization of ATP in cardiomyocytes (see the next section).

Because of molecular crowding and hindered diffusion, cells need to compartmentalize metabolic pathways to overcome diffusive barriers. Biochemical reactions can proceed successfully and can even be facilitated by metabolic channeling of intermediates caused by structural organization of enzyme systems into organized multienzyme complexes. Metabolite channeling directly transfers the intermediate from one enzyme to an adjacent enzyme without the need of free aqueous-phase diffusion (15, 18, 36–39). It is clear that the enhanced probability for intermediates to be transferred from one active site to the other by sequential enzymes requires stable or transient interactions between the relevant enzymes. Enzymes can associate physically in nondissociable, static multienzyme complexes, which are not random associations but an assembly of sequentially related enzymes.

Compartmentation phenomenon and vectorial metabolism

Thus, the principal ways and mechanisms of organization of cell metabolism are macrocompartmentation, microcompartmentation, metabolic channeling, and functional coupling. Macrocompartments are easy to understand, and they can be visualized by electron or confocal microscopy. They are compartments inside the organelles such as mitochondria or lysosomes, and they are insulated from the cytoplasm by membranes. The microcompartments are not usually visible by electron microscopy. However, now it is becoming clear that microcompartments related to multienzyme complexes and metabolic channeling, as described above, are the principal basis of organization and compartmentation of cellular metabolism. They are formed by specific protein–protein interactions within multienzyme complexes caused by the macromolecular crowding, anchoring of the glycolytic (40–43) and other enzymes to cytoskeleton, or membrane channels and transporters (43–48). Investigations carried out in Clegg's and Deutscher's laboratories have shown that mammalian cells behave as highly organized macromolecular assemblies that depend on the cytoskeleton (47, 48). Multienzyme complexes may be of different size and even include whole metabolic pathways—then they are called metabolons, according to the terminology introduced by Paul Srere in 1985 (49). Thus, there are glycolytic (41, 45), Krebs cycle metabolons (50), and many others (51, 52). New techniques such as FRET have been developed to study and visualize the microcompartmentation, of which a good example is the study of microcompartmentation of cyclic AMP (53, 54). Microcompartentation is sometimes taken to be synonymous of metabolic channeling (16, 39). However, metabolic channeling of the reaction intermediate between two enzymes (or a transporter and an enzyme) may occur *via* microcompartment or by direct transfer (55, 56). In both cases, it results in functional coupling (see below). Importantly, microcompartmentation may be of dynamic nature, which may finally result in coexistence of a whole set of organized metabolic networks (55).

These phenomena, in particular metabolic channeling, may be even older than life itself and related to its origin. Edwards

(57) and others have put forward the hypothesis according to which on the prebiotic earth, when no enzyme or metabolic complexes were initially present, archetypal catalytic complexes were formed at the mineral surface (for example, iron sulfide minerals), and biomolecules and catalysts would have been formed at specific sites relative to these complexes (57). The evolution of metabolic pathways in this case would have been dictated by the relative positions of substrates and catalysts, where only closely juxtaposed species would have been allowed to interact (57). It is now clear that the living cells are much more complicated and are much better organized than thought before (58, 59).

In the case of metabolic channeling of species *via* microcompartments with only a small number of molecules, the validity of introducing chemical potentials is questionable (6), whereas for macrocompartments and larger microdomains this classic theory remains useful. In the first case, application of thermodynamic activities instead of concentrations (18–20), and stochastic kinetics based on probabilities of different states of enzymes, will be essential (60, 61).

An important consequence of the organization of the enzymes into multienzyme complexes is vectorial metabolism and ligand conduction, which is a general principle proposed by Peter Mitchell after extensive enzymological studies and detailed characterization of mitochondrial proteins. One important example is the chemiosmotic coupling of energetic processes through the "protonic current" (62, 63). It brought together "transport and metabolism into one and the same chemiosmotic molecular level - biochemical process catalyzed by group-conducting or conformationally mobile group-translocating enzyme system" (63).

Vectorial metabolism, metabolic channeling, and functional coupling may well be explained and illustrated by the Maxwell's demon, which is a general and important philosophical concept in the history of physical sciences. Azzone and Mae-Wan Ho (64, 65) already in 1994 discussed the idea that in living cells, the Maxwell's demon is behind the Schrödinger's principle of negentropy. This metaphor is useful to explain general mechanisms that help the cell to keep entropy low through a very precise structural organization of all metabolic networks (66, 67). The principle of Maxwell's demon is realized in proper structural organization of the cellular metabolic systems. By controlling the direct supply of substrate to the enzyme and removing the product, the intelligence of Maxwell's demon helps to avoid unnecessary increase of entropy and helps to increase the efficiency of energy transduction. In Schrödinger's days, the fine structural organization of metabolism was unknown, but it clearly contributes to the foundations of the basic law that he discovered (7).

For analysis of the free-energy transduction in the cells with highly organized structure and high and changing energy demand, such as cardiac and skeletal muscle cells and brain cells, a system analysis is needed with main attention on the interaction between cellular components. This direction has been defined as molecular system bioenergetics (67), which is an important part of systems biology. Description of main results of this direction of research developed within several last decades is given in following sections.

Molecular System Bioenergetics of Cardiac and Brain Cells

Compartmentation of adenine nucleotides in cardiac cells

Application to cells of Equation (20) in its general form is complicated by the compartmentation of ATP and adenine nucleotides making the use of the easily measurable total ATP content very questionable and practically useless. For cardiac cells, macrocompartmentation of adenine nucleotides was demonstrated in Ruth Altschuld laboratory by using a controlled permeabilization technique (68). They found that 74% of total nucleotides are localized in the cytoplasm, about 20% are localized in mitochondria, and the remaining 6% are localized in cellular structures. A similar distribution was found in other cells (68). Important data on the possible compartmentation of ATP were obtained in studies of the metabolism of ischemic heart by Gudbjarnason et al. (69) and Neely et al. (70). They always observed very rapid decreases of contractile force in parallel with decrease in PCr concentration and complete stop of contraction in the presence of about 80–90% of intact ATP (see Fig. 3a). These basic, classic observations show that a very precise energy sensing mechanism present is in the cells, which responds mostly to the changes in PCr content. The interpretation of these experiments posed the following problem: why does the heart stop contracting in the absence of oxygen supply so quickly if there is still plenty of ATP in the cells? Many explanations that were proposed, such as pH changes, changes in the sensitivity of troponin to calcium, and so on, are not sufficient because these changes occur at longer time intervals (71, 72). The explanation proposed by Gudbjarnason et al. (69) highlights the importance of ATP compartmentation, only about 10% of which is in the functionally important pool(s) (69). Although the experiments described above showed almost constant ATP during a decrease of contraction force, more experiments with perfusion of heart during different hypoxic periods or perfusion with 2-deoxyglucose (a phosphate trap, decreasing the ATP content via adenylate kinase and 5′ nucleotidase reactions) (73–75) showed that the total content of ATP can be decreased by 70% (which corresponds to its amount present in cytoplasm) without significant effect on contraction, provided that total creatine content is not changed (Fig. 3b) (73, 74). Thus, the total content of ATP is dissociated from contractile force, which seems to depend only on a fraction (not more than 20–30%) of ATP. At the same time, inactivation of the creatine kinase system by inhibition of creatine kinase or replacing creatine with less active analogs significantly decreases the maximal work capacity of the muscle (76, 77). ATP compartmentation was confirmed in multiple indirect experiments performed on ischemic hearts that showed the importance of the so-called glycolytic ATP and in studies of energy supply to membrane ion pumps (78–81). Compartmentation of ATP has been demonstrated recently by imaging technique in several cell types (82).

Most important data that show the significance of compartmentation phenomenon for cardiac energy metabolism have

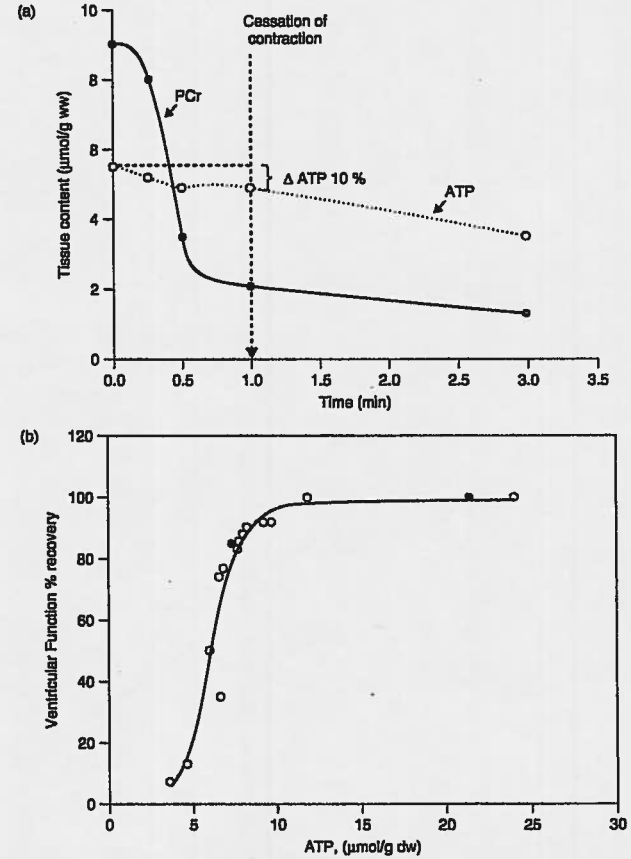
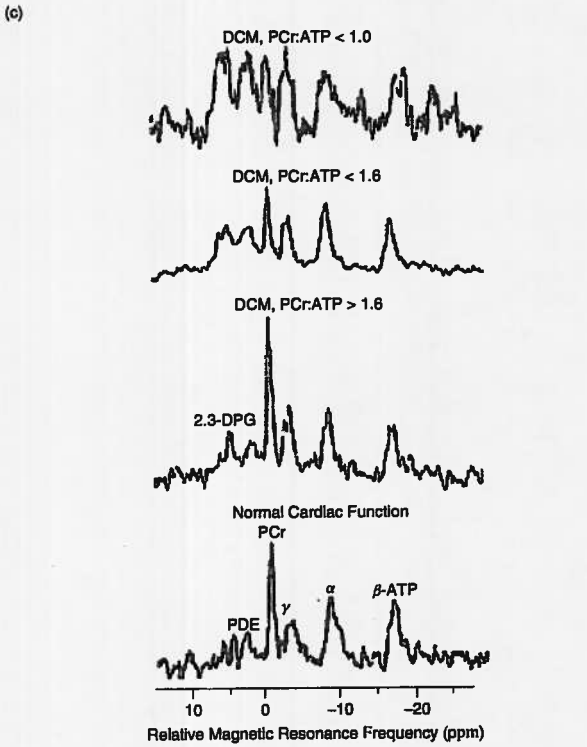


Figure 3 (a) The origin of the problem of the compartmentalization of adenine nucleotides and metabolic energy sensing in cardiac cells. The data show metabolic changes in the totally ischemic dog hearts 69. Within 1 minute, PCr content falls by 80% and contraction stops, but 90% of cellular ATP is still intact. The general problem is why the contraction stops when most ATP is not used up, and which mechanism allows sensing of the fall of PCr level as main source of cellular energy. For an explanation, see the text as well as Fig. (5). Data are redrawn from Reference 69; only the mean values of metabolites are shown. (b) Absence of any correlation between total ATP content in the cells and contractile function of heart muscle. Data are redrawn from References (73–75). ATP was changed by periods of hypoxic perfusion followed reperfusion with normal solutions 73, open circles, or by treatment of the hearts with 2-deoxyglucose (74, 75). (c) (d) Relationship between decreased phosphocreatine/ATP ratios in hearts of patients with dilated cardiomyopathy and increased mortality over study period. Panel C shows cardiac 31P-MR spectra (from bottom to top) in a healthy subject, a patient with dilated DCM and a normal PCr: ATP ratio (>1.6; 1.6 was the median of the ratio), a patient with DCM and a reduced PCr:ATP ratio (<1.6), and a patient with DCM and a severely reduced PCr:ATP ratio (<1.0). The patient with the severely reduced ratio died 7 days after undergoing magnetic resonance examination. *2,3-DPG*, 2,3-diphosphoglycerate; *PDE*, phosphodiesters. γ, α, and β denote phosphorus atoms of ATP. Panel D shows a Kaplan–Meier life-table analysis of mortality in two groups of patients with DCM: one with a higher PCr:ATP ratio and one with a lower ratio. Patients with an initially low ratio had an increased mortality over the study period (average follow-up, 2.5 years). Data are from Neubauer 83 with permission.

been collected by Neubauer's group (83); some of them are illustrated in Fig. 3c and 3d. By using ^{31}P-NMR spectroscopy in combination with imaging for investigation of cardiac muscle-energy metabolism in patients, the authors showed that in the patients with cardiac disease [such as dilated cardiomyopathy (DCM)], the decreased PCr/ATP ratio (lower than 1.6) is a very clear and strong diagnostic index of increased mortality. In the heart of patients with DCM, the ATP content remained the same as in healthy control patients, but PCr decreased by 70% compared with control. This finding



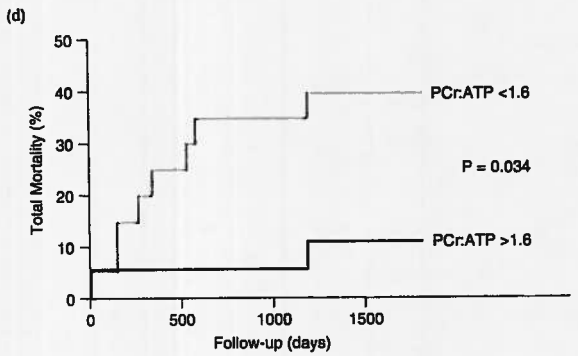


Figure 3 *(Continued)*

shows the vital importance of the phosphocreatine–creatine kinase energy-transfer network described below for the cardiac muscle normal function and life.

Unitary organization of energy metabolism and compartmentalized energy transfer in cardiac cells

Cardiac cells present a highly organized structure in which mitochondria are localized at the A-band level within the sarcomere limits (84, 85). Intermyofibrillar mitochondria are arranged in highly ordered crystal-like pattern in a muscle-specific manner with relatively small deviation in the distances that separate neighboring mitochondria (86, 87). Contrary to many other cells with less-developed intracellular structure, dynamic changes in mitochondrial position caused by their fission and fusion (88–90) are not found in adult and healthy cardiac and skeletal muscle cells because of their rigid intracellular structural organization, and mitochondria in these cells are morphologically heterogeneous. In this structurally organized medium, energy transfer between different subcellular microcompartments and macrocompartments (shortly called compartmentalized energy transfer) are of central importance. Existence of these rather complicated networks of energy transfer and signaling is a direct consequence of the compartmentalization of adenine nucleotides in the cells (33–35, 66, 67, 91–108). These networks are caused by significant heterogeneity and local restrictions in the diffusion of adenine nucleotides in cells as well as the necessity of rapid removal of ADP from the vicinity of MgATPases to avoid their inhibition by accumulating product MgADP. ATP is not only delivered by diffusion, but intracellular energy transfer is facilitated *via* networks that consist of phosphoryl-group transferring enzymes such as creatine kinase (CK), adenylate kinase (AK), and glycolytic phosphoryl-transferring enzymes (39, 91–109). Most important among them is the creatine kinase system. CK catalyzes the reversible reaction of adenine nucleotides transphosphorylation, the forward reaction of phosphocreatine (PCr) and MgADP synthesis, and the reverse reaction of creatine (Cr) and MgATP production:

$$MgATP^{2-} + Cr \leftrightarrow MgADP^{-} + PCr^{2-} + H^{+} \qquad (21)$$

Four CK isoforms, each with compartmentalized cellular location, exist in mammals. Specific mitochondrial CK isoenzymes (MtCK), called ubiquitous (uMtCK) and sarcomeric (sMtCK), are functionally coupled to oxidative phosphorylation and produce PCr from mitochondrial ATP. PCr in turn is used for local regeneration of ATP by the muscle cytoplasmic isoform of CK (M-CK), which drive myosin-ATPases or ion pump-ATPases (91–109). Recent studies in CK-deficient transgenic animals indicate that energy transfer and communication between ATP-generating and ATP-using sites within a muscle cell do not exclusively rely on the activity of CK, but they may rather include several additional intracellular phosphotransfer systems such as AK and glycolysis (106–108). AK-catalyzed reversible phosphotransfer between ADP, ATP, and AMP molecules may process cellular signals associated with ATP production and use (104, 108). Cluster organization and the high rate of unidirectional phosphoryl exchange in phosphotransfer systems promote ligand conduction and signal communication at cellular distances, which provides enhanced thermodynamic efficiency.

In the heart as well as in oxidative skeletal muscle, the intracellular energy transfer networks are structurally organized in the intracellular medium where macromolecules and organelles, which surround a regular mitochondrial lattice, are involved in multiple structural and functional interactions (67, 104, 110–112). **Figure 4** summarizes available information about such an organized and compartmentalized energy metabolism in cardiac cells. This scheme also illustrates the view that mitochondria in muscle cells are structurally organized into functional complexes with myofibrils and sarcoplasmic reticulum (67, 110–112). These complexes were called intracellular energetic units (ICEUs) and taken to represent the basic pattern of organization of muscle energy metabolism (66, 67, 110). No physical barriers exist between ICEUs; each mitochondrion (or several adjacent mitochondria) can be taken to be in the center of its own ICEU. This concept is consistent with very regular, crystal-like arrangement of mitochondria in cardiac cells (85–87) and describes the organized functional connections of mitochondria with their neighbors. ICEUs are analogous to calcium release units (CRUs), which are structurally organized sites of Ca^{2+} microdomains (Ca^{2+} sparks) that form a discrete, stochastic system of intracellular calcium signaling in cardiac cells (113, 114). The structural organization of ICEUs results in local confinement of adenine nucleotides and Cr/PCr couple in discrete dynamic energetic circuits between actomyosin ATPases and mitochondrial ATPsynthases (67, 100–104). Similar discrete microdomains in cardiomyocytes have been shown for cAMP in the range of approximately 1 μm, which exhibit high local concentrations (53, 115, 116).

This concept is supported by Weiss et al. (117), who presented a holistic view of cardiovascular metabolism, considering it from the perspective of physical network, in which various metabolic modules are spatially distributed throughout the interior of the cell to optimize ATP delivery to specific ATPases (117). In addition to mitochondrial module (which is represented ICEUs), the authors considered also a module consisting of glycolytic enzyme complexes serving for energy channeling to molecular complexes in sarcolemma and sarcoplasmic reticulum, and modules of calcium cycling [which Wang et al. (113) called CRUs]. These modules were analyzed from the abstract perspective of fundamental concepts in network theory (118, 119) and dynamic perspective of interactions between modules (117). Understanding the nature of these interactions within hierarchical modular structures is a main challenge of research of cardiac metabolism to gain deeper understanding of possible mechanisms of cardioprotection (117).

Central role of coupled mitochondrial creatine kinase in regulation of cardiac energetics

The central mechanism in compartmentalized energy transfer is the functional coupling between the mitochondrial ATP/ADP-translocase (ANT), and mitochondrial creatine kinase (MtCK).

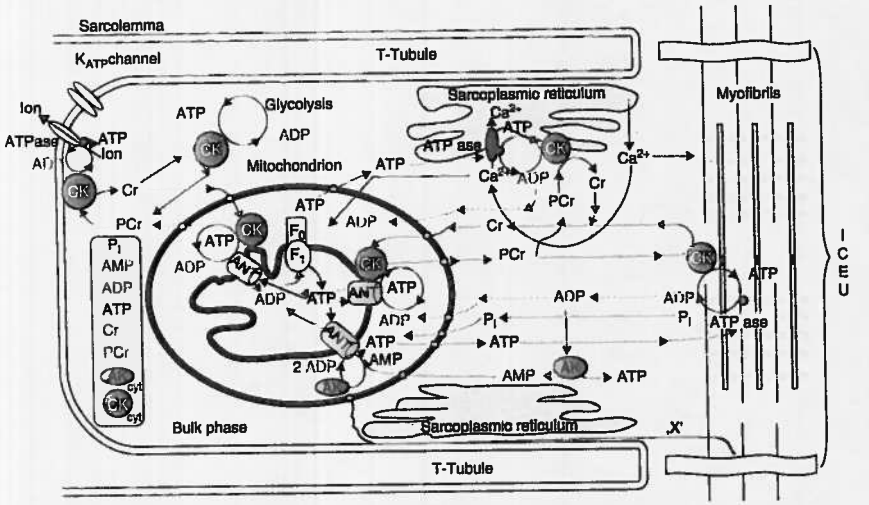


Figure 4 Organization of compartmentalized energy transfer and metabolism in cardiac cells by ICEU. The scheme shows the structural organization of the energy-transfer networks of coupled CK and AK reactions within an ICEU. By interaction with cytoskeletal elements, the mitochondria and SR are precisely fixed with respect to the structure of sarcomere of myofibrils between two Z-lines and correspondingly between two T-tubules. Calcium is released from SR into the space in ICEU in the vicinity of mitochondria and sarcomeres to activate contraction and mitochondrial dehydrogenases. Adenine nucleotides within ICEU do not equilibrate rapidly with adenine nucleotides in the bulk water phase. The mitochondria, SR, and MgATPase of myofibrils and ATP sensitive systems in sarcolemma are interconnected by metabolic channeling of reaction intermediates and energy transfer within ICEU by the creatine kinase–phosphocreatine and myokinase systems. The protein factors (still unknown and marked as "X"), most probably connected to cytoskeleton, fixes the position of mitochondria, and possibly controls the permeability of the VDAC channels for ADP and ATP. Adenine nucleotides within ICEU and bulk water phase may be connected by some more rapidly diffusing metabolites as Cr–PCr. Reproduced from Reference 104 with permission.

MtCK is localized on the outer surface of the mitochondrial inner membrane, in close vicinity of the ANT (120–122). The structure of the ANT was recently resolved at 2.2 Å (123), as well as structures of all creatine kinase isoenzymes (see References 102 and 103). The translocation of both ATP and ADP in the Mg^{2+}-free forms is related to the conformation changes of pore-forming monomers (123, 124). In the mitochondrial matrix side, ANT forms a supercomplex, a synthesome with ATP synthase F_0F_1 and Pi Carrier (125). ANT in the inner mitochondrial membrane forms tight complexes with negatively charged cardiolipin (1:6 ratio) (126, 127). It has been shown that positively charged MtCK is fixed to this cluster by electrostatic forces through three C-terminal lysines that interact strongly with the negatively charged cardiolipin in complex with ANT at the outer surface of the inner mitochondrial membrane (122, 127).

Kinetic evidence for functional coupling between MtCK and ANT

When oxidative phosphorylation is not activated, the MtCK reaction does not differ kinetically and thermodynamically from that catalyzed by other creatine kinase isoenzymes: The reaction favors the ATP production; ADP and phosphocreatine binding is more effective because of higher affinities than that of ATP or creatine, respectively, and the kinetic constants obey the Haldane relationship (128–132). However, under conditions of oxidative phosphorylation, the mitochondrial creatine kinase reaction is strongly shifted in direction of phosphocreatine (PCr) synthesis and may use all ATP produced in mitochondria for PCr production (66, 67, 128, 130, 131). Although the kinetic constants for creatine were not changed and were the same in both conditions, the oxidative phosphorylation had a specific effect on the kinetic parameters for adenine nucleotides (66, 67, 131). Complete kinetic analysis of the MtCK reaction under conditions of oxidative phosphorylation revealed that both two constants for MgATP dissociation from the MtCK.MgATP and MtCK.Cr.MgATP complexes, Kia and Ka, respectively, were decreased, among them most significantly Ka which was dramatically decreased by one order of magnitude (67, 131). The explanation is the direct transfer of ATP from ANT to MtCK because of their spatial proximity, which results also in an increased uptake of ADP by ANT from MtCK (reversed direct transfer). As a result, the turnover of adenine nucleotides is increased manifold at low external concentration of MgATP, which maintains high rates of oxidative phosphorylation and coupled phosphocreatine production in the presence of creatine. This outcome was confirmed in direct experiments in which MtCK was detached from the inner mitochondrial membrane into the intermembrane space by isotonic KCl solutions: In this case, the effect of the oxidative phosphorylation on the MtCK

kinetics was lost (132). Quantitative analysis of the experimental data on the coupled MtCK and ANT by a rather complicated mathematical model based on the analysis of the energy profile of the reaction gave evidence for the direct transfer of ATP from ANT to MtCK with significant changes of the energy level of complexes of ATP bound to ANT (133).

Thermodynamic evidence for functional coupling between MtCK and ANT

The creatine kinase reaction equilibrium is shifted in the direction of ATP production, the apparent $K'eq = Keq \times (H^+)$ in this direction,

$$K'_{eq} = ((ATP) \times (Cr))/((PCr) \times (ADP))$$

having a value for pH 7.0, 38°C, and free $(Mg^{2+}) = 1$ mM equal to ~170, and respectively, the standard free-energy change of the creatine kinase reaction in the direction of ATP synthesis $\Delta G^\circ = -13.4$ kJ/mol (134–136). In the reverse direction, the apparent K'_{eq} is respectively equal to 6×10^{-3}. In the experiments described in References 137 and 138, the reaction conditions were such that $\Gamma = ((PCr)(ADP))/((Cr)(ATP))$ in the medium is higher than K'_{eq}; therefore, according to the laws of chemical thermodynamics, the reaction of phoshocreatine production should have proceeded under these conditions in direction of lowering Γ to K'_{eq} by decreasing phosphocreatine concentration, as it could be predicted from Fig. 2. However, in the presence of oxidative phosphorylation, the concentration of PCr increases, and Γ moves uphill (along the curve in Fig. 2) away from equilibrium. There should be a strong force, a significant amount of free-energy change underlying this uphill movement, according to Fig. 2, which means that oxidative phosphorylation is controlling the MtCK reaction. Most probably, the control occurs by direct supply of mitochondrial ATP to the active center of MtCK by ANT, and rapid removal of ADP by the latter—the functional coupling mechanism. When the oxidative phosphorylation reaction is inhibited by oligomycin, the MtCK can fully obey the thermodynamic rules, and Γ decreases to the value of K'_{eq} 5 (137).

Historical note

Already in 1939, Belitzer and Tsybakova (139) observed in well-washed skeletal muscle homogenates a strong stimulation of respiration by Cr without addition of exogenous adenine nucleotides. This result was the earliest indication of the functional coupling of the MtCK with oxidative phospborylation. Much later, Kim and Lee (140) showed the same effect for isolated pig heart mitochondria and Dolder et al. (141) for liver mitochondria from transgenic mice expressing active MtCK. All these data show rapid recycling of endogenous ADP and ATP already present in mitochondria, because of functional coupling of MtCK with ANT.

Heterogeneity of ADP diffusion in permeabilized cells: importance of structural organization

Studies on the regulation of mitochondrial respiration in permeabilized muscle cells and fibers have rendered important information on the structure–function relationship of cellular energetic systems and the role of creatine kinases (142, 143). The principal advantage of the permeabilized cell technique is that it allows an investigation of system-level properties, which determine mechanisms of regulation of mitochondrial respiration in the intracellular milieu with all interactions between cell components intact (67). System-level properties are those not predictable on the basis properties of isoleted components and are dependent on interactions between them (25–27). Metabolic compartmentation described above and unitary organization of energy metabolism are clear and important examples of system-level properties.

In intact cardiac cells, mitochondria are arranged very regularly at the level of A-bands of sarcomeres (Fig. 5a) and are functionally coupled to multiple intracellular ATP-consuming processes by energy transfer and metabolic feedback signaling networks within highly organized energetic units (see above). However, recently, Claycomb et al. (144) described the cultured (continuously dividing) HL-1 cell line with differentiated cardiac phenotype. Growing HL-1 cells with a different serum leads to cells devoid of beating properties, which we assigned as NB HL-1 cells (145). Thus, the NB HL-1 cells represent an original phenotype that displays cardiac characteristics. Most remarkably, these cells are devoid of sarcomeric structures and possess randomly organized filamentous dynamic mitochondria (145). Unlike in adult rat cardiomyocytes, rapid movement, fission, and fusion of mitochondria characterize filamentous mitochondria in NB HL-1 cells (Fig. 5b) (146). Also, striking differences in the kinetics of respiration regulation by exogenous ADP are exhibited by these cells (Fig. 5c and 5d). In permeabilized adult cardiomyocytes, the apparent Km for exogenous ADP is very high, 360 ± 51 μM (Fig. 5c and 5d). This outcome has been reported previously (66, 67, 98, 101, 104, 105, 110–112, 142, 143), and it was explained by local restrictions of the ADP diffusion in the cells, which include limitation of its diffusion across the mitochondrial outer membrane (66, 67, 147). In permeabilized cardiomyocytes, treatment with trypsin resulted in dramatic changes in intracellular structure that were associated with three-fold decrease in apparent Km for ADP in regulation of respiration (148). In contrast to permeabilized cardiomyocytes, in NB HL-1 cells the apparent Km for exogenous ADP (25 ± 4 μM) was 14 times lower (Fig. 5d). The regulation of respiration by exogenous ADP in NB HL-1 cells is very close to that in isolated mitochondria (Fig. 5d). Although in normal adult cardiomyocytes creatine significantly activates the respiration and decreases the apparent Km for ADP, in HL-1 cells it exerted very little influence, if any, because of the downregulation of creatine kinase (146). These results show that in normal adult cardiomyocytes, intracellular local restrictions of diffusion of adenine nucleotides and metabolic feedback regulation of respiration *via* phosphotransfer networks are related to the complex structural organization of these cells. These local restrictions of diffusion of ADP and ATP are bypassed in cardiac cells by the creatine kinase system (67, 147).

The very informative experimental protocol to investigate the role of MtCK in regulation of mitochondrial respiration in the cells *in situ* and to reveal some important aspects of

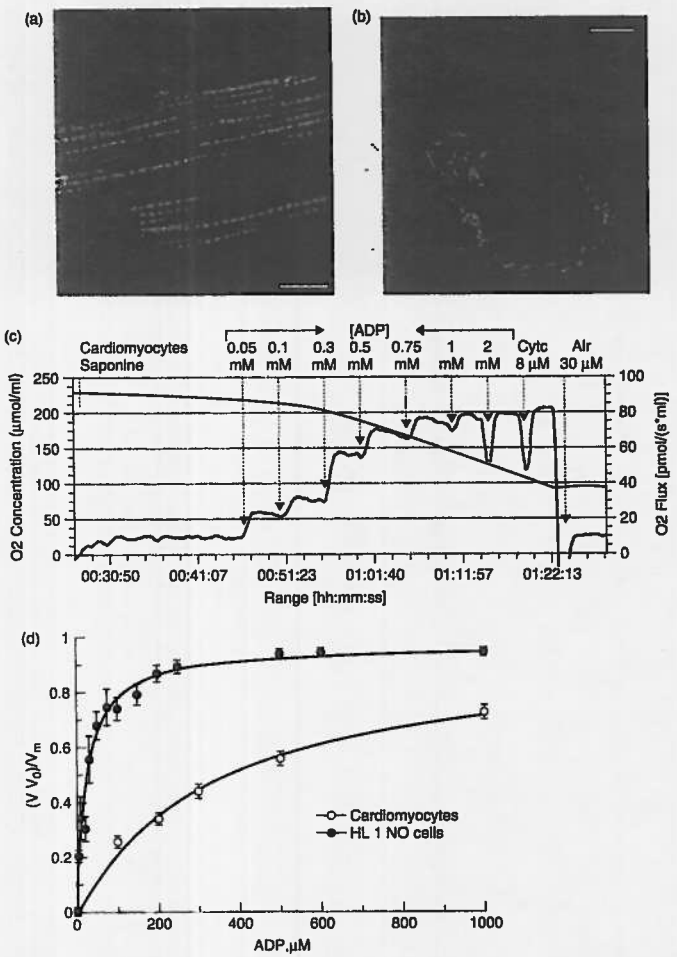


Figure 5 Confocal fluorescent images of mitochondria in adult isolated cardiomyocytes (a) and cultured nonbeating HL-1 cells (b). Scale bars, 10 µm. Mitochondrial arrangement was visualized by MitoTracker Deep Red (Molecular Probes, Eugene, USA) as described in Reference 146. (c) Recordings of the changes of the respiration rate of permeabilized cardiomyocytes in response to the addition of exogenous ADP in increasing final concentrations. Upper traces—oxygen concentration; lower traces—respiration rate. Cytochrome c was added to test the intactness of the outer mitochondrial membrane in the cells: Absence of the effect of cytochrome c shows the intactness of this membrane. Addition of atractyloside (Atr) returns the respiration to the initial State 2 level, this mechanism shows the intactness of the inner mitochondrial membrane. (d) Comparison of respiration kinetics as normalized respiration rates *versus* ADP in permeabilized adult cardiomyocytes and in permabilized HL-1 NB cells. Values of apparent Km for exogenous ADP were equal to 360 ± 51 µM for isolated and permeabilized cardiomyocytes (calculated from Fig. (5)c) and 25 ± 4 µM for HL-1 NB cells 146. Respiration rates were recorded using a two-channel high-resolution respirometer (Oroboros oxygraph-2k, Oroboros, Innsbruck, Austria), in respirometry medium Mitomed 146. For normalization, the respiration rates were expressed as fractions of the maximal rates, Vmax, found by analysis of experimental data in double-reciprocal plots.

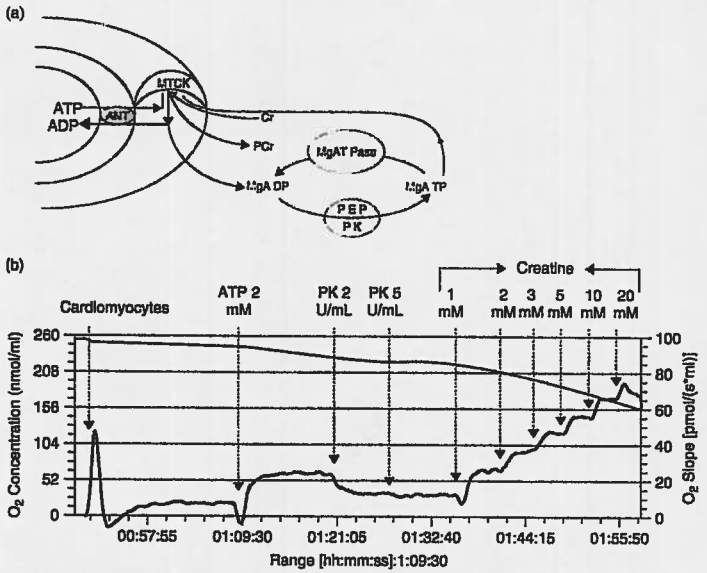


Figure 6 Complete protocol for investigation of the role and kinetics of MtCK-controlled respiration in the isolated and permeabilized cardiomyocytes or fibers. (a) Schematic presentation of the principle of study. It shows the competition between PK–PEP system and mitochondria for endogenous ADP in permeabilized cardiomyocytes and the strong control of respiration by the mitochondrial creatine kinase reaction. In addition, the PK–PEP system rephosphorylates all extramitochondrial MgADP produced by MgATPases and extramitochondrial MM–CK in myofibrils. Therefore, respiration rate is controlled exclusively by ADP produced by MtCK. (b) Oxygraph recordings of kinetics of respiration regulation in permeabilized cardiomyocytes recorded using a two-channel high-resolution respirometer (Oroboros oxygraph-2k, Oroboros, Innsbruck, Austria), in respirometry medium Mitomed 146. The respiration was activated by endogenous ADP after addition of 2-mM MgATP. The protein concentration was 0.0621 mg/ml. In the presence of 3-mM PEP, addition of PK in increasing amounts effectively removes this ADP, at 20 IU/ml of PK about 25–50% of initial ADP dependent respiration is observed. In the presence of this powerful ADP-consuming PEP–PK system, activation of MtCK reaction by stepwise addition of creatine rapidly increases the respiration up to maximal values: The acceptor control ratio is about 7 and close to that observed with saturating concentrations of ADP 146.

functional coupling between MtCK and ANT and system-level properties important for regulation of respiration in permeabilized cells is described in **Fig. 6**. In these experiments, endogenous ADP production is induced by addition of MgATP (2 mM) to activate the mitochondrial respiration in permeabilized cardiomyocytes. Then, the powerful competing pyruvate kinase–phosphoenolpyruvate (PK-PEP) system is added to trap the ADP (**Fig. 6a**), this decreasing the respiration to about 50–60% of initial value. By removing extramitochondrial ADP, the PK-PEP system also maintains constant concentration of MgATP, which is a substrate for MtCK. The addition of creatine rapidly activates respiration up to almost maximal level close to that for State 3, despite the presence of powerful ADP trapping system (**Fig. 6b**). That means that the ADP produced locally in the MtCK reaction is not at all available to the PEP-PK system. Earlier, Gellerich et al. (149) have used the same system in the experiments with isolated heart mitochondria to find that in this case the PEP-PK system inhibited more than 50% of creatine-stimulated respiration. The same degree of inhibition was seen in permeabilized cardiomyocytes after their treatment with trypsin (138). These data show that in the cardiac cells *in vivo* mitochondrial outer membrane permeability is limited and controlled by some cytoskeletal proteins (66, 67, 138, 150) and clearly show restrictions of the diffusion of adenine nucleotides within highly organized structure of ICEUs (**Fig. 4**). In any case, the limited permeability of the outer mitochondrial membrane for ADP strengthens the functional coupling between MtCK and ANT and increases the role of MtCK in communication between mitochondria and cytoplasm (101–105). Most interestingly, the data on **Fig. 6b** show that the respiration controlled by coupled MtCK in permeabilized cardiomyocytes *in situ* is very effectively regulated by creatine: Addition of creatine in the concentrations of 1–2 mM already results in very significant increase of respiration, the apparent Km (Cr) being about 1–1.5 mM. This observation is consistent with the view that in the muscle cells *in vivo* creatine is an effective feedback regulator of respiration, and inducing effective phosphocreatine production under these conditions allows to overcome (or bypass) the local restrictions of ADP and ATP diffusion. In permeabilized NB HL-1 cells, this effect of creatine on respiration was practically absent because of downregulation of MtCK (146).

Interestingly, a parameter that is very easy to measure in biopsy samples—the apparent Km for exogenous ADP, by reflecting the complexity of intracellular interactions and organization in oxidative muscle cells, has become an important diagnostic parameter in the studies of skeletal muscle bioenergetics and exercise physiology (150–153). Thus, this parameter has a clear practical and diagnostic value for muscle performance (152, 153). Identification of cell components responsible for specific organization of energy transfer systems and intracellular diffusion merits additional investigation. For elucidation of the nature of cytoskeletal components responsible for specific organization of both mitochondrial arrangement and complex metabolic signaling and intracellular energy transfer pathways, additional investigations on the proteome are needed.

The functional coupling mechanism is supported by structural and functional studies of MtCK performed by Wallimann and colleagues (100, 102, 103). It was also shown in mice with knock-out of MtCK that, as predicted by the theory described above, these hearts had lower levels of PCr and reduced postischemic recovery (154–156). An important role of the control by MtCK over ANT is to hinder the opening of the mitochondrial permeability transition pore (141) and cell death showing that because of the active functional coupling between MtCK and ANT, the MtCK induced ADP recycling strongly decreases the production of reactive oxygen species (ROS) in brain mitochondria (157). Taking into account that ROS production is now considered as a main reason of many age-related diseases and ageing itself (158, 159), the importance of these results is difficult to overestimate.

The principle of Maxwell's demon mentioned above may be taken to be well realized in compartmentalized energy transfer pathways, such as creatine kinase systems with structurally fixed (bound) creatine kinase isoenzymes that interact with the adjacent ATP to produce, transport, or consume systems as illustrated in Fig. 4. The Maxwell's demon metaphor may be used to describe the functional coupling between MtCK and ANT, as analyzed above. ANT that supplies the substrate ATP for MtCK and removes the product ADP from local microcompartments fulfils the intelligent role of the demon directing the MtCK reaction uphill from equilibrium, as described in Fig. 2. At the same time, MtCK by using ATP and supplying ADP for ANT fulfils the same role of the demon for ANT. For regulation of mitochondrial respiration in many cells with high-energy fluxes, this mechanism is key. Similar schemes may be used for description of the roles of other CK and AK isoenzymes in other subcellular compartments, which are shortly described below, and for the phenomena of metabolic channeling in general.

Myofibrillar and membrane-bound creatine kinases, energy sensing

The myofibrillar end of creatine–phosphocreatine cycle is represented by the MM isozyme of creatine kinase localized in the sarcomere and functionally coupled to the actomyosin MgATPase (94, 102, 103, 160). Within the contraction cycle, ADP release is a necessary step for new binding of MgATP, dissociation of actomyosin crossbridges, and for muscle relaxation, to start anew the contraction cycle (161–163). This step is often found to be the slowest in the contraction cycle and therefore is rate controlling. MgADP may compete with MgATP for the substrate site on myosin and inhibit crossbridge detachment by MgATP, the inhibition constant K_i being in the range of 200 μM both in MgATPase reaction and in sliding of fluorescent actin on myosin (163, 164). Thus, MgADP should be rapidly removed from actomyosin and the high local value of the MgATP/MgADP ratio along with the local phosphorylation potential, maintained. The MM CK is bound specifically to the M-line, and a significant portion of this isozyme is found in the I-band of sarcomeres (165–171). An increasing amount of evidence points out that this MM creatine kinase is involved intimately in the contraction cycle at the level of ADP release and ATP rebinding steps by the mechanism of functional coupling likely including myofibrillar microcompartments of adenine nucleotides (94, 96, 100–105). Most probably, this effective interaction occurs *via* small microcompartments of adenine nucleotides in myofibrils and is facilitated by anisotropic diffusion. When PCr is exhausted then, the rate of local regeneration of ATP in myofibrils decreases slowing the contraction cycle irrespective of high cytoplasmic ATP concentration (67, 102, 165, 170–172). In agreement with this interpretation, it has recently been shown that inhibition or knock-out of the CK system by genetic manipulations results in a very significant loss of work capacity (173). The rigor state known as ischemic contracture appears only after complete exhaustion of all ATP in the cell (79) and can be significantly delayed by local production of ATP by the myofibrilar glycolytic system or accelerated by inhibition of this process [so called "glycolytic ATP" effect (78–81)]. Under these conditions, the rate of glycolytic ATP production, along with its diffusion from the cytoplasm, are too slow to sustain contraction.

Other ATP-consuming systems are both in the membranes of sarcoplasmic reticulum (SR) and in the plasmalemma (sarcolemma). Their function is to maintain the ionic homeostasis and particularly, the regulation of the calcium cycle. The role of the MM CK connected to the membrane of the SR and functionally coupled to the Ca, MgATP- dependent ATPase (SERCA) is to rapidly rephosphorylate local MgADP produced in the Ca, MgATPase reaction, which maintains a high local value of phosphorylation potential to keep a high efficiency of calcium pumping and to avoid its reversal or inhibition because of accumulation of ADP (102, 174–179).

In the control of the excitation–contraction coupling in the heart, a principal step is the sarcolemmal membrane metabolic sensor complex (34, 35,104–106, 108, 180–189). Its main component is the sarcolemmal ATP sensitive K^+ (K_{ATP}) channel that acts as an alarm system to adjust cell electrical activity to the metabolic state of the cell (34, 180–183). The sarcolemmal MM CK creatine kinase rephosphorylates the local ADP to maintain a high ATP/ADP level in these microcompartments for coordination of membrane electrical activity with cellular metabolic status, notably with PCr levels (see Fig. 7a). The K_{ATP} channel was discovered by Noma (184), and it was found that this channel has high affinity for ATP, about 100 μM (184, 185). Nevertheless, the channel is opened in the presence of millimolar ATP, as observed from rapid membrane repolarization

and shortening of action potential in ischemic and hypoxic hearts or as shown directly in experiments with internally perfused cardiomyocytes (34, 35). This observation is explained by strong diffusional restriction and thus ATP compartmentation in the subsarcolemma and is linked to the cellular pool of PCr via CK reactions. Pool exhaustion in the first minutes of ischemia certainly contributes to the cessation of contraction caused by opening of the K_{ATP} channel and decreased calcium entry. This energy transfer and the control functions are shared by the whole system, which include the creatine kinase, the adenylate kinase and glycolytic systems, as it was observed in experiments that involve genetic manipulation (35, 104–108). Similar cell-membrane metabolic sensors may be also important in brain cells. The phosphotransfer relays communicate metabolic signals that originate in mitochondria or at cellular ATPases to metabolic sensors conveying information about "high" or "low" cellular energy, oxygen supply, or hormonal states (34, 35, 104–109, 176, 187, 188). CK and AK deficiencies or their altered activity ratio compromise metabolic signaling to K_{ATP} channels (34, 189). Decreases in CK activity, such as in CK knock-outs or failing hearts, can be partially compensated by the increase in glycolytic phosphotransfer to alleviate cardiomyocyte electrical instability (106–108).

In vivo kinetic evidence of energy transport by phosphotransfer networks

One of the main problems in cardiac energetics is to explain the linear increase of respiration rate with an increase of workload induced by elevated filling of left ventricle—which is the metabolic aspect of Frank-Starling law that governs the cardiac physiology (104, 190–194)). Increased left-ventricular filling induces length-dependent activation of sarcomeres and work performance under conditions when no changes in PCr and ATP contents are observed (metabolic stability) and calcium transients remain unchanged. This activity excludes any role of calcium in respiration regulation under these conditions (104, 195–204). The explanation of this phenomenon is given by the mechanisms of energy transfer and feedback regulation by phosphotransfer networks (104).

Key support for the current understanding of metabolic signaling networks in their full complexity have come with application of new methodologies of investigations of *in vivo* kinetics of the energy transfer (106–108, 205–208). High-energy phosphoryl fluxes through creatine kinase, adenylate kinase, and glycolytic phosphotransfer, captured with ^{18}O-assisted ^{31}P NMR, tightly correlate with the performance of the myocardium under various conditions of stress load (205–208), which implicates phosphotransfer reactions as indispensable routes that direct flow of high-energy phosphoryls between cellular ATPases and the ATP production machinery in mitochondria. This mechanism allows quantitative evaluation of cellular ATP turnover and the distribution of energy fluxes between different phosphotransfer pathways *in vivo*. This outcome supports the notion that the CK pathway carries the major part of energy flux out of mitochondria to the ATPases under normal physiological conditions in heart cells, in agreement with the mathematical modeling of the compartmentalized energy transfer (see below). About 10–15% of cellular high-energy phosphoryls can be carried by the adenylate kinase (AK)-glycolytic systems, whose contribution increases with muscle contraction and in failing hearts (104, 106–108, 205–208).

Mathematical modeling of metabolic feedback regulation of respiration

Because of the high complexity of processes involved, the mathematical modeling is increasingly important part of molecular system bioenergetics and is used very effectively to analyze the mechanisms of regulation of respiration and cellular-energy fluxes (209–218). For that, different models with distinct levels of detailization are used, depending on the purpose and point of view of authors. An increasing consensus exists between different groups that mathematical modelling supports the theory of metabolic feedback signaling from ATPases to mitochondria (104, 212, 217, 218), which is in agreement with experimental observations (219). The compartmentalized energy transfer models are aimed to describe quantitatively the results of *in vivo* studies of respiration regulation in the ICEUs under physiological conditions of the Frank-Starling law. These models are based on the concepts of ICEUs and include: kinetics of ATP hydrolysis by actomyosin ATPase during contraction cycle, diffusional exchange of metabolites between myofibril and mitochondrial compartments, VDAC-restricted diffusion of ATP and ADP across mitochondrial outer membrane, the mitochondrial synthesis of ATP by ATP synthase, ΔpH and ΔΨ controlled Pi and ADP transport into mitochondrial matrix, PCr production in the coupled mitochondrial CK reaction, and its use in cytoplasmic CK reaction. These events are considered in a system consisted of a myofibril with a radius of 1 μm, a mitochondria, and a thin layer of cytoplasm interposed between them (209, 210). The results of modeling show cyclic changes in the concentration of ADP in the core of myofibrils in ICEUs in a microcompartment that contains myofibrillar-bound MM-CK, where ADP is first produced by actomyosin MgATPase during the contraction cycle of crossbridges, and then rephosphorylated by the CK because of a nonequillibrium state of the CK reaction (209, 210). Interestingly, these calculated cyclic changes in PCr, ATP, and Cr, which are in the range of 5–10% of their cellular contents, are in good agreement with the multiple observations of the cyclic changes of these compounds in the contraction cycle published in the literature (220, 221). These changes in Cr, PCr, and total ATP, however, are close to the experimental errors of their detection, which gives an overall impression of metabolic stability (222–224). Changes in ADP and Pi concentrations are relatively much more significant because of very low initial values. Without CK, the changes of local ADP concentrations in these microcompartments will be much more dramatic (209, 210).

Within the whole contraction cycle, the rates of ADP and ATP cycling and thus the respiration in mitochondria coupled to the PCr production are increased with elevation of the workload. Increasing cyclic changes in the local ADP production in myofibrils are immediately displacing the myofibrillar MM-CK reaction in the direction of local ATP regeneration. The amplitude of displacement of CK from equilibrium is proportionally

increased with workload (209, 210). In this regard, CK, adenylate kinase, and other phosphotransfer isoenzymes in different intracellular compartments are "pushed" or "pulled" from the equilibrium in opposite directions, which depends on the activity of an associated process that drives steady-state high-energy phosphoryl flux (104).

The model quantitatively describes the observations by Williamson et al. (210, 222) on the dependence of the respiration rate on the workload.

An increase in the rate of ATP synthesis and of the coupled respiration (oxidative phosphorylation) is possible only if the supply of both ADP and Pi to mitochondria is increased, which makes both metabolites good candidates for metabolic feedback signals that control respiration (225). ADP is supplied by the metabolic signaling networks described above to overcome the possible restriction of its diffusion and to avoid the inhibition of MgATPases. Additionally, the CK reaction, if running in the direction of ATP regeneration, compensates for pH changes caused by MgATPase hydrolysis (98). Application of the metabolic control analysis to the mathematical model developed showed that in parallel to the cyclic changes in ADP, at least at low workloads, P_i flux to mitochondria plays an important regulatory role (218). This conclusion was confirmed experimentally (219) and recently by models by Beard (213). Thus, the feedback metabolic signal has a complex nature; several of its components such as P_i, ADP, and Cr may act in parallel and their relative contribution changes with workload (104). Thus, there seems to be a set of self-regulatory metabolic feedback signaling via phosphotransfer pathways and Pi fluxes. As a response to sarcomere stretch and cross-bridge cycling, this complex signal leads to increases in the respiration rate, coupled with mitochondrial PCr production, and energy flux via the CK-PCr system, while also maintaining metabolic stability (homeostasis) at elevated workload.

Synchronization of cell function with its energy and substrate supply

The existence of two interrelated systems that regulate mitochondrial respiration and energy fluxes in the cells is increasingly recognized (see **Fig. 7a**). The first system is composed of structurally organized enzymatic modules and networks of the CK-AK-glycolytic systems that communicate flux changes from cellular ATPases to mitochondrial oxidative phosphorylation in nonequilibrium steady state (67, 92–108). The second system consists of amplifying system based on cellular and mitochondrial calcium cycles, which adjust the capacity of substrate oxidation and energy-transducing processes to meet increasing cellular energy demands (67, 226–234). Moreover, the coupled CK, AK, and glycolytic reactions by communicating signals to the K_{ATP} channel, which is a sarcolemmal metabolic sensor, provide fine-tuned regulation of excitation-contraction and thus the calcium cycle within a cell (235, 236). **Figure 7a** shows such integration of energetic and ion-signaling systems, which provide the basis for "metabolic pacing," which synchronize electrical and mechanical activities with energy supply processes. These processes are fundamental for optimal heart function and for sustaining adequate force-frequency relationship during stress (104). During the cardiac cycle, an electrical impulse induced by action potential and membrane depolarization causes Ca^{2+} influx through L-type Ca^{2+} channels in the sarcolemma and T-tubules which trigger Ca^{2+} release from intracellular stores in the SR through ryanodine receptor-channels (RyR) and cardiomyocyte contraction (226–228). ATP consumed during myofibrillar contraction and Ca^{2+} pumping is rapidly replenished by CK, AK, and glycolytic phosphotransfer relays that maintain optimal local ATP/ADP ratios and free energy of ATP hydrolysis. Simultaneously, these systems generate and translate feedback signals to stimulate ATP production in mitochondria and to convey information to energy demand sensors, such as K_{ATP} channels and also AMPK. The amount of Ca^{2+} that enters a cell is controlled by the action potential duration regulated by K_{ATP} channels located in sarcolemma and T-tubules (226–228). Conversely, the activity of this metabolic sensor is regulated by phosphotransfer reaction and Ca^{2+} feedbacks. Ca^{2+} that enters the cell or released from intracellular stores activates substrate transport (recruitment of glucose transporters), dehydrogenase (DH) activity (such as pyruvate dehydrogenase, PDH, an entry point into the mitochondrial Krebs cycle), and glycogenolysis to meet incoming energy needs (229–234).

The first system—metabolic feedback signaling—explains quantitatively the metabolic aspect of the classical Frank-Starling law regarding regulation of cardiac function and respiration under conditions of metabolic stability and unchanged calcium transients (101, 104). This system is based on the compartmentalized energy transfer; a deficit of which explains the rapid fall of contractile force in the first minutes of total ischemia (67). The second system explains adrenergic modulation of cardiac cell function and energetics under stress (227–234). By regulating the sarcolemmal metabolic sensor-K_{ATP} channels, the CK-AK-glycolytic network affects the excitation-contraction process and thus the calcium cycle of the cell (235, 236). Both systems may be activated simultaneously, as it is observed in the case of positive inotropy induced by β-adrenergic agents, when Frank-Starling curves are shifted upward (104, 190). These two more or less independent systems that regulate cellular energetics have already been described separately by mathematical models (210, 237). The physiological mechanism of respiration regulation described above has the important advantage of ensuring effective control of free-energy conversion across the whole physiological range of workloads, without requiring a severe increase in cytoplasmic calcium and ADP concentrations. Thus, it avoids any danger of mitochondrial calcium overload that would open the mitochondrial permeability transition pore and thus lead to cell death (67, 102, 104). Functioning of the coupled MtCK–ANT system in mitochondria prevents from the ROS (oxygen free radicals) formation in mitochondrial respiratory chain and helps to avoid many problems related to ROS production, such as PTP opening, necrosis, apoptosis and rapid ageing (157). In this way, the CK–PCr network may contribute significantly in the positive effects of physical exercise on the human health: exercise-induced increased fluxes via this pathway increase the ADP–ATP turnover in the coupled MtCK–ANT reactions in mitochondria and keeps the ROS production low.

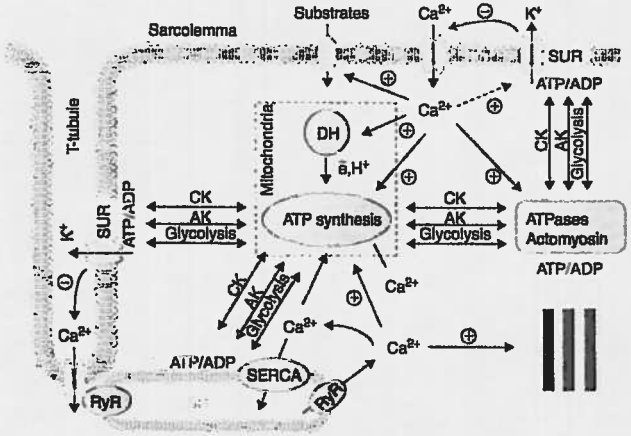


Figure 7 Mechanisms of regulation of integrated energy metabolism in cardiac cells. (a) Metabolic feedback signaling from MgATPases by phosphoransfer and glycolytic systems and their interaction with calcium signaling: cardiac excitation-contraction-energy coupling and synchronization of electrical and metabolic activities. Electrical pacing-induced action potential and membrane depolarization causes Ca^{2+} influx through L-type Ca^{2+} channels in sarcolemma and T-tubules that trigger Ca^{2+} release from intracellular stores in SR through RyR and cardiomyocyte contraction. Interplay between CK, AK, and glycolytic phosphotransfer relays, energetic modules (mitochondria and ATPases), metabolic sensors (K_{ATP} channel), and Ca^{2+} transients, generate metabolic pacing signals in synchrony with the electrical and functional activity to ensure cellular energetic homeostasis. Intracellular and mitochondrial Mg^{2+} activates metabolic enzymes and tunes Ca^{2+} signaling. After contraction, intracellular Ca^{2+} is sequestrated by SR Ca^{2+} ATPase (SERCA), by mitochondria, and removed from the cell by the Na^+/Ca^{2+} exchanger and a Ca^{2+} pump in sarcolemma (see details in the text). Modified from Reference 104 with permission. (b) The scheme of regulation of substrate supply for mitochondrial respiration and the mechanisms of feedback regulation of the fatty acid and glucose oxidation by workload elevation in oxidative muscle cells 238.

FFAs are taken up by a family of plasma-membrane proteins [fatty acid transporter protein (FATP1), fatty acid translocase (CD36) and in cytoplasm associated with fatty acid binding protein (FABP)]. FFAs are esterified to acyl-CoA via fatty acyl-CoA synthetase. The resulting acyl-CoA is then transported through the inner membrane of the mitochondrion, via the exchange of CoA for carnitine by carnitine-palmitoiltransferase 1 (CPT 1). Acylcarnitine is then transported by carnitine–acylcarnitine translocase into the mitochondrial matrix where a reversal exchange takes place through the action of carnitine-palmitoyltransferase 2 (CPT 2). Once inside, the mitochondrion acyl-CoA is a substrate for the beta-oxidative pathway, which results in acetyl-CoA production. Each round of beta-oxidation produces one mole of NADH, one mole of $FADH_2$, and one mole of acetyl-CoA. Acetyl-CoA enters the TCA cycle, where it is oxidized to CO_2 with the concomitant generation of three moles of NADH, one mole of $FADH_2$, and one mole of ATP.

Acetyl-CoA, which is formed in the mitochondrial matrix, can be transferred into the cytoplasm with participation of carnitine, carnitine acetyltransferases, and carnitine acetyltranslocase [carnitine acetylcarnitine carrier complex (CAC)].

Glucose (GLU) is taken up by glucose transporter-4 (GLUT-4) and enters the Embden-Meyerhof pathway, which converts glucose via a series of reactions into 2 molecules of pyruvate (PYR). Because of these reactions, a small amount of ATP and NADH are produced.

G6P, glucose 6-phosphate; *HK*, hexokinase; *PFK*, phosphofructokinase; *GLY*, glycogen; F1,6diP, fructose-1,6-bisphosphate; *GAPDH*, glyceralhehydephosphate dehydrogenase; *1,3DPG*, 1,3 diphosphoglycerate.

The redox potential of NADH is transferred into the mitochondrial matrix via the malate/aspartate shuttle.

OAA, oxaloacetate; *αKG*, alpha–ketoglutarate; *ASP*, aspartate.

Malate generated in the cytosol enters the matrix in exchange for αKG and can be used to produce matrix NADH. Matrix OAA is returned to the cytosol by conversion to ASP and exchange with Glut. Most metabolic energy derived from glucose can come from the entry of pyruvate into the citric-acid cycle and oxidative phosphorylation via the acetyl-CoA production. NADH and $FADH_2$ are oxidized in the respiratory chain (complexes I, II, III and IV). These pathways occur under aerobic conditions. Under anaerobic conditions, pyruvate can be converted to lactate.

Feedback regulation. A glucose-fatty acid cycle (Randle hypothesis): If glucose and FFA are both present, then FFA inhibit the transport of glucose across the plasma membrane, acyl-CoA oxidation increases the mitochondrial ratios of acetyl-CoA/CoA and of NADH/NAD^+, which inhibit the PDH complex, and increased citrate (produced in the TCA cycle) can inhibit PFK. These changes would slow down oxidation of glucose and PYR and increase G6P, which would inhibit HK, and decrease glucose transport. The MtCK catalyzes the direct use of intramitochondrially produced ATP for phosphorylation of cytosolic Cr, which produces ADP and PCr. ADP enters the matrix space to stimulate oxidative phosphorylation, whereas PCr is transferred via cytosolic Cr/PCr shuttle to MMCK functionally coupled to ATPases (actomyosin ATPase and ion pumps), which results in release of high free energy of ATP hydrolysis. If the workload increases, then ATP production and respiration are increased because of metabolic feedback signaling via CK system, this method leads to decrease of mitochondrial acetyl-CoA content, which is transferred into cytoplasm with participation of CAC. Acetyl-CoA carboxylase (ACC) is responsible for converting acetyl-CoA to Malonyl-CoA, which is a potent inhibitor of CPT-I, with the aim to avoid overloading the mitochondria with fatty acid oxidation intermediates, when the workload is decreased. Inactivation of ACC occurs via phosphorylation catalyzed by AMP-activated protein kinase (AMPK). Phosphorylation and inactivation of ACC leads to a decrease in the concentration of malonyl-CoA. A fall in malonyl-CoA levels disinhibits CPT1, which results in increased fatty-acid oxidation. Malonyl-CoA is also converted back into acetyl-CoA in the malonyl-CoA decarboxylase (MCD) reaction. Increase in the workload increases the rate of acetyl-CoA consumption and that automatically decreases the malonyl-CoA content. The regulation of ACC and MCD occurs under stress conditions when the AMP/ATP ratios are increased, but the regulation is unlikely to occur under normal workload condition of the heart. Thus, AMPK may be envisaged as a modulator, under situations of cellular stress, rather than as a master on-off switch of fatty-acid oxidation. Reproduced from Reference 238 with permission.

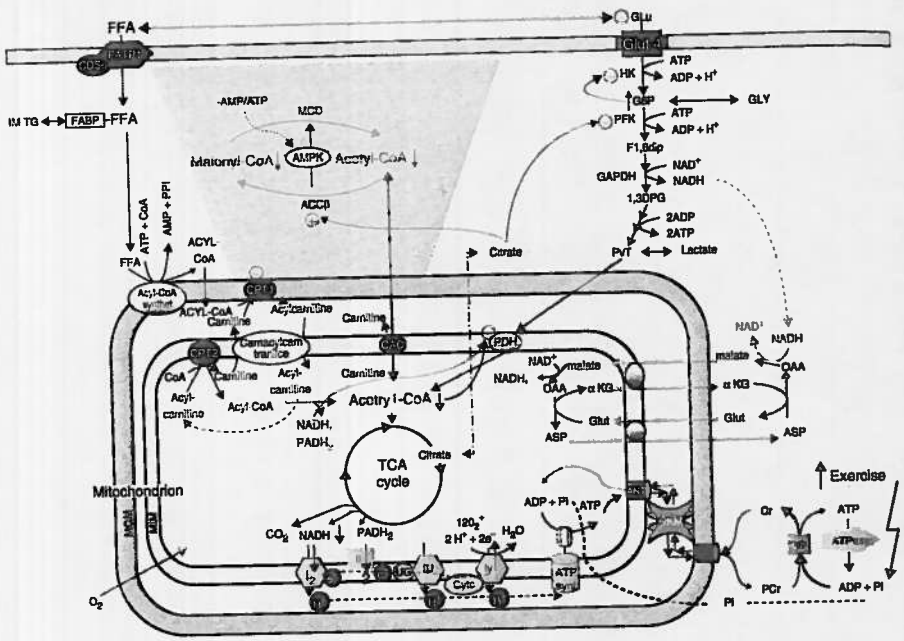


Figure 7 (*Continued*)

Effective cardiac work and fine metabolic regulation of respiration and energy fluxes need the organized and interconnected energy transfer and metabolic signaling systems, as well as regulation of substrate supply for the heart (238). In muscle cells, contractile function and cellular energetics are fueled by oxidation of carbohydrate substrates and fatty acids (239–245). The choice of substrates depends on their availability, and the rates of their use are very precisely regulated by multiple interactions between the intracellular compartmentalized and integrated bioenergetic systems of glycolysis, fatty acid oxidation, and the Krebs cycle in the mitochondrial matrix, which is linked directly to the activity of the respiratory chain and the phosphorylation process catalyzed by the ATP synthase complex (104, 238, 245). The rates of all these processes are geared to the workload, mostly by the mechanism of the feedback metabolic regulation described above (104, 238, 245). The network of reactions of the main substrate supply for mitochondrial respiration in muscle cells and their multiple interactions and feedback mechanisms of regulation are illustrated in Fig. 7b. A workload-dependent increase in the rate of the actomyosin ATPase reaction results in a release of increasing amounts of ADP and Pi; this metabolic signal is transmitted to the mitochondrial ANT by phosphotransfer networks under conditions of apparent metabolic stability as described above (104). Pi enters the mitochondria by a special phosphate carrier (3). These signals activate the ATP synthase and the use of the proton transmembrane electrochemical gradient ($\Delta\mu_{H+}$) for ATP synthesis in mitochondria (3, 104, 238). This process always leads to an increase in the electron transfer to oxygen via respiratory chain and NADH as well as $FADH_2$ oxidation. Independently from the substrate used, an increased workload always decreases the ratio of $NADH/NAD^+$ in the mitochondrial matrix (222, 238, 242). This decrease in the $NADH/NAD^+$ ratio increases the rates of the dehydrogenase reactions in the Krebs cycle and gears the latter to the rate of electron transfer via the respiratory chain (238–242, 245). It is the Krebs cycle, which is on the crossroads between the metabolic pathways of glucose and fatty acid oxidation, and its intermediates that play a very important role of feedback metabolic regulation of upstream pathways of substrate oxidation (238, 244, 245).

The integrated network shown in the Fig. 7b explains all the main experimental observations on the regulation of respiration and substrate use in the heart. Malonyl-CoA seems to be rather clearly at the end of this sequence of events, and the AMPK-signaling pathway apparently modifies only this last step of regulation under cellular stress conditions under which the (AMP)/(ATP) ratio increases significantly.

For oxidative skeletal muscle cells, the integrated regulatory mechanisms described in Fig. 7 for heart and the sequence of the reactions given above may be probably valid, as well, especially for some muscle types and under certain conditions (endurance, exercise, training, etc.) (246).

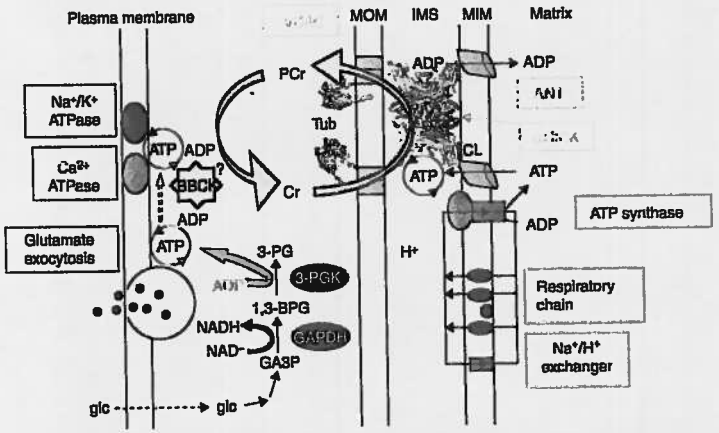


Figure 8 Creatine kinase in brain synaptosomes. Sites of ATP production (mitochondrial matrix) and sites of ATP consumption (ion transport across the plasma membrane and vesicle trafficking for neurotransmitter uptake and release, e.g., glutamate) are linked by an energy-transfer pathway represented by the phosphocreatine/creatine kinase system. uMtCK bound to mitochondrial inner membrane (MIM) via cardiolipin (black squares). ATP consumed by the energy-consuming reactions is reproduced locally by BBCK from PCr.
GA3P, glyceraldehyde-3-phosphate; *1,3-BPG*, 1,3 biphosphoglycerate; *3-PG*, 3-phosphoglycerate; *3-PGK*, 3-phosphoglycerate kinase; *VDAC*, voltage dependant anion channel; *MOM*, mitochondrial outer membrane; Tub–αβ heterodimer of tubulin that interacts with the VDAC channels and limits its permeability for adenine nucleotides. Modified from Reference 251 with permission.

Similar to the cardiac cells, brain cells exhibit high levels of CK activity, which is represented by uMtCK and cytosolic brain for BB CK (247–250). Detailed description of the state-of-the-art studies of brain energy metabolism is given in Reference 249. As a recent development in the field, however, a plethora of new data provide evidence for the importance of the CK system and of Cr metabolism for normal function of the brain. Furthermore, oral Cr supplementation shows some rather remarkable positive effects on brain function and neuroprotective properties for acute and chronic neurological diseases (for a review, see References 102 and 250). As for skeletal and cardiac muscle, the specific functional properties of neuronal tissue also require very high cellular-energy resources. The brain may spend up to 20% of the body's energy resources. A very high turnover of ATP is necessary to maintain electrical membrane potentials, as well as signaling activities of the central nervous system and peripheral nervous system. Rapid changes in ATP demands are occurring during physiological function of neurons, whereas cellular energy reserves are small. Because of widely distributed cellular processes and sites of high energy consumption that are localized at remote locations from the neuronal cell body (i.e., synapses), an effective coupling of ATP-generating and ATP-consuming processes is needed to maintain a sufficiently high energy transfer (247–250). The CK/PCr-circuit has been described to play a key role in the energy metabolism of the brain and neurons (Fig. 8). Hence, this system is supposed to play a pivotal role in neuronal ATP metabolism; consequently, Cr depletion in the brain is associated with disruption of neuronal function, changes in morphology, and clinical pathology (102, 250).

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Integrin Signaling

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Advanced Article

Article Contents

Integrins are a large family of heterodimeric receptors that mediate the adhesive behavior of cells. Most integrins bind to extracellular matrix (ECM) molecules, and they transmit signals that are critical in growth, development, tissue homeostasis, and host defense. A central feature of these receptors is their ability to transduce bidirectional signals into and out of the cell. In this article, we will give an overview of our current understanding of the structure and cellular signaling functions of integrins.

An amazing feature of life is that cells, the tiny units of an organism, can develop such elaborate molecular systems to sense all kinds of environmental information and translate it into various cellular responses to survive. The extracellular matrix (ECM) is composed of a complex mixture of polysaccharides and large fibrous proteins such as fibronectin, collagen, and laminin. ECM serves as a key environmental cue for cells in multicellular organisms, because many fundamental cellular processes, including proliferation, survival, migration, and differentiation, are regulated by the cells' adherence to the ECM and the composition of the ECM (1, 2). In the 1980s, integrins were recognized as the major metazoan receptors for the ECM (3). Ever since, intensive efforts have been made to unravel its complex functions as an important signal transducer. Importantly, the underlying structure–activity relationships have also started to be elucidated by resolving the high resolution three-dimensional (3-D) structure of integrins. It is now widely appreciated that integrins serve as a pivotal module to mediate a bidirectional signal transduction, namely outside-in and inside-out signaling pathways (4). Upon engagement of ECM ligands, integrins make transmembrane connections to cytoskeleton and regulate many intracellular responses through the outside-in signaling. Conversely, intracellular signaling pathways can modulate integrin-mediated cell adhesion to ECM via the inside-out signaling. In this article, we summarize recent progress in this area by discussing the bidirectional signaling of integrins.

Integrin Family (An Overview)

A functional integrin is a heterodimeric protein complex consisting of an α and a β subunit. So far, the mammalian integrin family consists of 8 β subunits and 18 α subunits, which are known to assemble into 24 distinct integrins (Fig. 1). The different combination of subunits accounts for heterodimer specificity, with certain integrins showing preference for particular ECM molecules (4–6). Each subunit has a large extracellular domain, a single transmembrane (TM) segment, and a short cytoplasmic tail (with the exception of β4). The *N*-terminal portion of the α and β subunits associate to form the headpiece, which contains the ligand-binding site, whereas the *C*-terminal segments traverse the plasma membrane and mediate interaction of the integrin with the cytoskeleton and other signaling molecules. Hence, the exterior and interior of a cell are physically linked by integrins, which allows the bidirectional transmission of mechanical and biochemical signals across the plasma membrane, and leads to a cooperative regulation of cellular functions, including adhesion, migration, growth, survival, and differentiation.