Chapter 11 Queries

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11
System Analysis of Cardiac Energetics–Excitation–Contraction Coupling: Integration of Mitochondrial Respiration, Phosphotransfer Pathways, Metabolic Pacing, and Substrate Supply in the Heart

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Abstract

Because of high and changing energy demands and perfect structural, metabolic, and functional organization of the cells (cardiomyocytes), heart muscle is a classical object of intense bioenergetic studies, and yet many unanswered questions remain. Recent progress in studies of cellular microcompartmentation, organized energetic units, and phosphotransfer networks, culminating in the concept of cardiac system bioenergetics, has significantly increased our understanding of how mitochondrial respiration and energy fluxes throughout cellular compartments are regulated, how energy is sensed, and how precise mechano-energetic coupling is maintained over a broad rage of functional activity. System analysis indicates that integration of cellular energetic systems with ion currents during action potential, Ca^{2+} signaling, and changes in sarcomere length during muscle extension constitute the mechanisms of the basic Frank–Starling law of the heart. The changes in energy demand are transmitted to mitochondria by the networks of metabolic signaling via phosphotransfer pathways to regulate ATP production and thus maintain the energy homeostasis of the cell. Because of the metabolic sensors at the sarcolemma, coupled phosphotransfer reactions provide a high-fidelity regulation of ion fluxes and excitation–contraction coupling. Here, we describe in quantitative terms the basic intracellular mechanisms of integration of energetics with calcium and magnesium signaling systems as a basis of metabolic pacing, synchronizing cellular electrical and mechanical activities with energy supply and substrate oxidation. Such analysis of cell energy metabolism as a whole functional unit at systemic level provides new insights about function of the most marvelous nature's created engine – the heart.

11.1
Introduction

Under normoxia, cardiac energy metabolism relies on aerobic oxidation of fatty acids and carbohydrate substrates in mitochondria, with the majority of ATP con-

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sumed by contractile machinery and ion pumps [1–3]. ATP is used during contractions with the production of ADP and inorganic phosphate in the actomyosin ATPase reaction [4–8]. In brain cells, aerobically produced ATP is used mostly to support ion gradients by Na^+/K^+-ATPase and for processing of neurotransmitters [9]. In cardiac cells, the specific contractile function is controlled and regulated by processes of excitation–contraction coupling [1, 10–12]. An active role in the regulation of cytoplasmic calcium can also be attributed to the mitochondrial calcium cycle [13–18]. These events have been found to be localized in the form of calcium sparks as visualized by confocal microscopy [18–20]. Normal muscle function depends on the fine interplay, or interaction, of energy metabolism and calcium metabolism inside the cells. Analysis of the integrated energy metabolism described in many chapters of this book, by applying the methods of molecular system bioenergetics, shows that highly organized phosphotransfer pathways play a central role in intracellular communication and signaling. This chapter 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.

11.2
Cardiac Energetics: The Frank-Starling Law and Its Metabolic Aspects

The heart maintains normal blood circulation under a wide range of workloads, a function governed by the Frank–Starling law [1, 21–25], originally described by Otto Frank [22] and Ernest Starling [23, 24]. This law states that cardiac performance increases with an increase in end-diastolic ventricular volume. In this way, the heart responds to increases in venous filling pressure [22–26]. In their original experiments, Starling and coworkers used heart–lung preparations and measured the rate of oxygen consumption, which was taken as a measure of "the total energy set free in the heart during its activity," following the definition of biological oxidation by Lavoisier and Laplace (see the Introduction to this volume) [24, 26]. Some of the results of these historical experiments are reproduced in Fig. 11.1. Evans, Starling, and their coworkers found that cardiac work (see Fig. 11.1A) increased linearly with an increase in left ventricular end-diastolic volume followed by an increase in respiration rates (Fig. 11.1B). They concluded that "any increase in the work demanded of the heart is met by corresponding increase in the oxygen consumption and in the amount of chemical changes taking place" [24]. This is the metabolic underpinning of the Frank–Starling law, and elucidation of its molecular and cellular mechanism is still a central question in cardiac cellular bioenergetics [27, 28]. By increasing the rate of left ventricular filling, Williamson et al. showed that the rate of respiration in isolated working hearts can be changed by more than an order of magnitude, about 15–20 times, from unloaded VO_2 of around 8–12 μmol min^{-1} g^{-1} dry weight to a maximal value of 170 μmol min^{-1} g^{-1} dry weight (see Fig. 11.2A) [29]. In these experiments, Williamson et al. also measured the redox state of the $NADH/NAD^+$ system in heart cells [29]. As illustrated in Fig. 11.2C, an increase in the workload

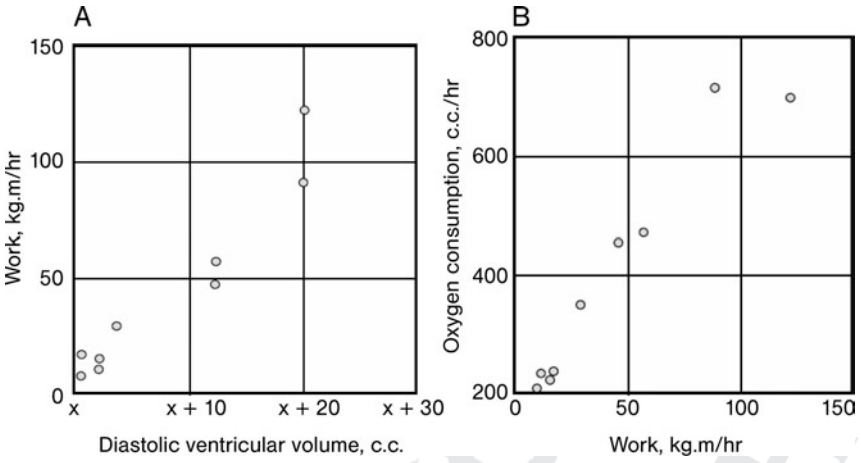

Fig. 11.1 Classical experiments showing the metabolic aspect of the Frank–Starling law of the heart, published by Starling and Visscher in 1926. Linear dependence of work (A) and oxygen consumption (B) upon left ventricular filling. (Reproduced from [24] with permission of the *Journal of Physiology*).

was accompanied by profound oxidation of NADH in these experiments. Another principal characteristic of heart energetics is that the linear increase in VO_2 with workload is observed in the absence of measurable changes in the ATP and phosphocreatine (PCr) cellular content (Fig. 11.2B) [30, 31]. This remarkable metabolic stability also has been called "metabolic homeostasis" [31]. In the face of large changes in muscle work and respiration, this observed metabolic stability or homeostasis underlying the Frank–Starling law is referred to as the "stability paradox" [32].

At present, there are sufficient experimental data, described in different chapters of this book, to make detailed conclusions about the nature of the mechanisms regulating cardiac cell respiration, energy fluxes, and substrate supply and thus to explain in molecular terms the metabolic basis of the Frank–Starling law of the heart [27, 28]. In mitochondria, the respiration rate is regulated by the availability of ADP for adenine nucleotide translocase (ANT) [33]. This is the classical respiratory control mechanism discovered by Lardy and Wellmann [34] and by Chance [35]. Jacobus et al. have found that even in the presence of ATP in high (physiological) concentrations, mitochondrial respiration follows the changes in ADP but not in the ratio of ADP to ATP [36]. In cells *in vivo* the main problem is the way in which signals are transmitted in the organized intracellular medium from MgATPases, which produce ADP and P_i, to mitochondria, which consume these substrates, to match ATP production to its demand. This signaling is controlled by multiple processes. It will be shown in this and related chapters that the integrated mechanisms of the system-level regulation of cardiac muscle energetics, respiration rate, and substrate supply under normal physiological conditions, governed by the Frank–Starling law, are described by the complex

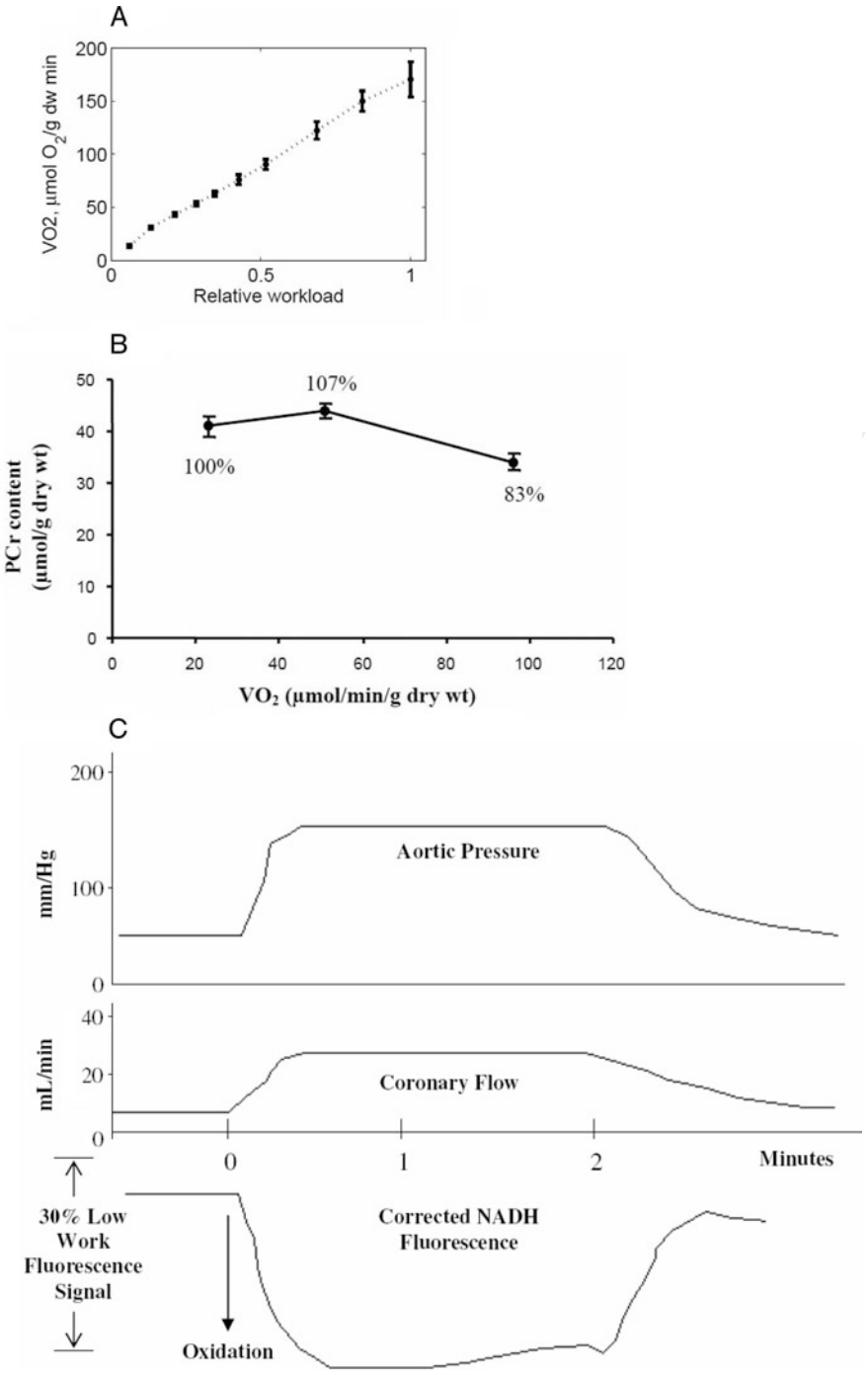

Fig. 11.2 (legend see p. 371)

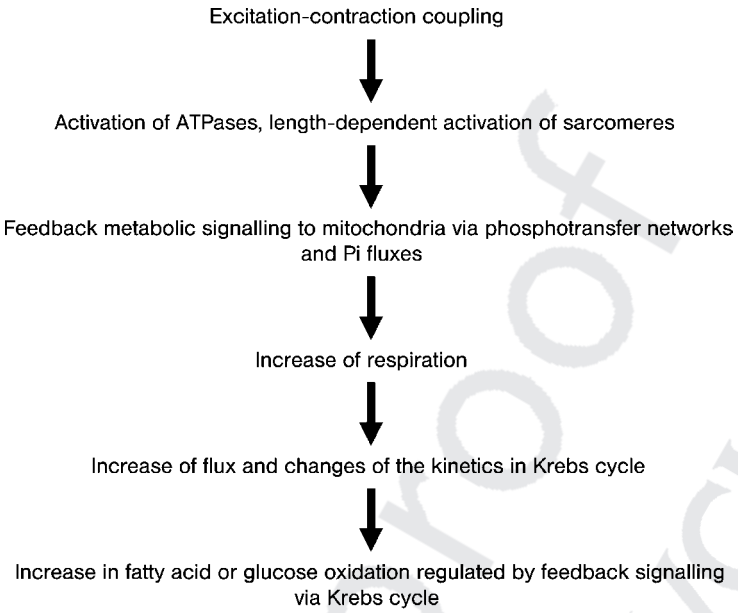

Fig. 11.3 Schematic representation of sequences of processes of workload-dependent regulation of cardiac energetics and mitochondrial respiration *in vivo*.

sequence of processes shown in Fig. 11.3. These sequences can be modified by changes in contraction frequency or hormonal stimulation, which are described in ▌Chapter 13▌. All steps described in Fig. 11.3 will be briefly described below.

11.3 Excitation–Contraction Coupling and Calcium Metabolism

11.3.1 Excitation–Contraction Coupling

The cardiac contraction is initiated and regulated by the process of excitation–contraction coupling. In 1883 Sidney Ringer discovered that contraction of iso-

Fig. 11.2 (A) Dependence of the rate of oxygen consumption by isolated perfused working rat heart on the work regulated according to the Frank–Starling law. Data are from the work of Williamson et al. [29]. The maximal workload corresponded to 0.6 kg m^{-1} (g d.w. min^{-1}) at the ventricular filling rate of 56 mL min^{-1} [29]. (B) The remarkable metabolic stability of the heart. (Reproduced from [28] with permission. (C) The workload dependence of oxidation of NADH in the heart. Redrawn from [29]).

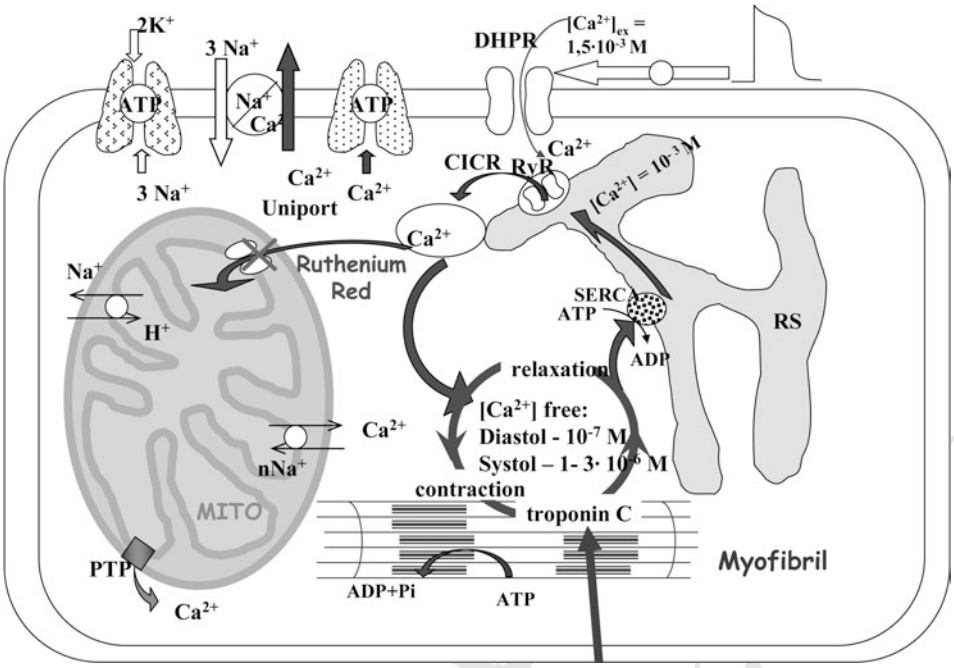

Fig. 11.4 Schematic representation of excitation–contraction coupling in cardiac cells. The arrow points to the importance of length-dependent activation of sarcomeres as a main regulatory mechanism under conditions of the Frank–Starling law. DHPR: dihydropyridine receptor; CICR: calcium-induced calcium release; SERCA: sarcoplasmic–endoplasmic reticulum calcium-dependent ATPase; RS: sarcoplasmic reticulum; Mito: mitochondrion; PTP: permeability transition pore.

lated perfused heart requires the presence of calcium in the perfusate [37, 38]. More than 100 years after these studies, an explanation for this phenomenon can now be given at almost atomic resolution (for reviews, see [10–12]). A simplified and general version of these events is given in Fig. 11.4. Calcium enters the cardiac cells via slow L-type calcium channels (also called dihydropyridine receptors [DHPRs]) in sarcolemma during the plateau phase of action potential. The amount of calcium entering the cell is dependent on the duration of this plateau phase, but it is not sufficient to directly activate the contraction [12]. This small calcium current, however, activates a massive release of calcium from its large pools in the sarcoplasmic reticulum via ryanodine-sensitive calcium release channels [10–12]. In skeletal muscle, calcium release from the sarcoplasmic reticulum is initiated by a sarcolemmal voltage sensor protein that is a homologue of the DHPR; therefore, contraction of skeletal muscle does not depend upon the extracellular calcium concentration [10, 39]. Calcium liberated from local intracellular stores by calcium-induced calcium release (CICR) mechanisms during

excitation–contraction coupling activates the contraction cycle by binding to troponin C in the troponin–tropomyosin complex of thin filaments [4–8]. Subsequent uptake of calcium by the Ca/MgATPase of the sarcoplasmatic reticulum terminates this calcium cycle.

The force developed by the myofibrils in the contractile cycle in heart cells is determined mostly by two parameters: duration and rate of calcium entry and release by a CICR mechanism (these depend on the duration of the action potential) and the length of sarcomeres, because of their length-dependent activation (arrow in Fig. 11.4). Under normal physiological conditions, when cardiac contraction is regulated by the Frank–Starling law, the latter mechanism is the central one (see below).

Direct measurements of the cyclic changes in calcium concentration (calcium transients) in the cytoplasm of cardiomyocytes became possible after application of fluorescent or bioluminescent calcium probes [40, 41]. However, the use of confocal microscopy and imaging techniques revealed the unique, discrete nature of calcium transients as consisting of localized changes within calcium release units (CRUs), structurally organized sites of Ca^{2+} microdomains (Ca^{2+} sparks) that form a discrete, stochastic system of intracellular calcium signaling in cardiac cells [19]. In these studies the theory of intracellular compartmentation of metabolites including calcium (see ▌Chapter 3▌) found its brilliant confirmation.

11.3.2 The Mitochondrial Calcium Cycle

The mitochondrial calcium cycle, whose elucidation began with the pioneering works of Lehninger, Hunter, and Carafoli (for a review, see Refs. [13–18]), includes a calcium entry-and-export system that

1. regulates calcium concentration in the mitochondrial matrix, where calcium is an important activator of the Krebs cycle dehydrogenases (such as pyruvate dehydrogenase [PDH], isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase [42]), thus increasing the capacity of oxidative phosphorylation [42–45];
2. allows active regulation of compartmentalized cytoplasmic calcium fluctuations and related signaling [14, 46]; and
3. protects mitochondria from calcium overload [15–17, 47].

Rizzuto and Pozzan have discovered a direct communication between the SR and mitochondria and calcium transfer from the SR into the mitochondrial intermembrane space [15, 16], evidencing the important role of mitochondria in the control of the cellular calcium cycle.

It is assumed by some authors that calcium signals can match the demand for ATP in the cell to its production in mitochondria and thus be entirely responsible for the control of respiration [18, 31, 48–52]. However, quantitative estimates of calcium effects in mitochondria are in conflict with the magnitude of changes in the respiratory rate *in vivo* [27, 28]. Both experimental studies of calcium effects on mitochondrial respiration *in vitro* [50] and mathematical modeling of mito-

chondrial metabolism [53] have shown that changes in calcium concentration can at most double mitochondrial respiration. The problem with this hypothesis is that there is already a high respiration rate at zero calcium [27, 28, 50, 53]. This degree of activation of mitochondrial respiration is far too small to explain the energy flux changes of more than an order of magnitude that are observed in muscle cells *in vivo* (see also below). In cells with small fluctuations in energy fluxes, direct regulation of mitochondrial activity by calcium may be sufficient [49], but for excitable cells with high and rapidly fluctuating energy fluxes – such as the heart, skeletal muscle, brain, and other cells – this is not the case. The calcium hypothesis of respiration regulation always predicts an increase in NADH production in response to the elevation of workload as an immediate mechanism of activation of the respiratory chain [31, 54]. However, in actual experiments the opposite effects are seen: an increase in the workload of the heart, including that by the Frank–Starling mechanism, always results in a very clear and significant decrease in the ratio of NADH:NAD^+ in the mitochondrial matrix, as is shown in Fig. 11.2C [3, 29, 55–58]. This may be explained only by a metabolic feedback regulatory mechanism (see also ▌Chapter 13▐). Interestingly, the nature of this mechanism of respiration regulation was elucidated mostly in cardiac physiological studies concerning the cellular basis of the Frank–Starling law and was found not to be directly related to the calcium cycle [27, 28]. The changes in the NADH/NAD^+ redox state are more complicated when contraction frequency is increased: in this case initial oxidation of NADH is followed by its reduction due to mitochondrial calcium accumulation [58].

11.4
Length-dependent Activation of Contractile System

It was discovered by Hibberd and Jewell and then confirmed by many others that the cellular mechanism behind the Frank–Starling law is the force–length relationship, a length-dependent activation of myofilaments that results from increased sensitivity of the thin filaments to calcium at greater sarcomere length [5, 21, 25, 59–65]. Intracellular calcium probes have revealed that practically no changes in the intracellular calcium transients accompany the stretching of sarcomeres sufficient to activate myofilaments [64, 65]. Only a slow increase in calcium transients after a length change was observed in the experiments of Allen and Kurihara [60]. However, it has clearly been shown in Suga's laboratory that the Frank–Starling mechanism does not affect intracellular calcium recirculation between the sarcoplasm and the sarcoplasmic reticulum [66]. The number of strongly bound cross-bridges and the force generated after calcium binding depend upon the length of sarcomeres [5–8, 21, 22, 63]. The mechanism of this length-dependent activation is complex. It includes changes in myofilament lattice spacing with possible involvement of titin [67], resulting in a decrease in the distance between actin and myosin filaments and an increased probability of

cross-bridge formation with an increase in sarcomere length [67, 68], positive cooperativity of cross-bridge binding to actin [63], and an increase in the affinity of the troponin complex for calcium, induced by strong binding of cross-bridges [5, 61–63]. Because of these mechanisms, sarcomere stretch at submaximal calcium concentrations in the cytoplasm results in an increase in the number of active cross-bridges, and thus increased release of the products of ATP hydrolysis, first P_i during the power stroke and then ADP [4–8, 61–63]. By this mechanism of length-dependent activation of sarcomeres, the steady-state rates of ATP consumption can change by an order of magnitude without any changes in the calcium cycle. This is also confirmed by mathematical modeling [69] (see also ▌Chapter 13▌). Under these conditions, the rate of ATP production is increased by a factor of 15–20 to match the rates of ATP consumption in cardiomyocytes, without the need of alteration of calcium signals. Theoretically, the feedback mechanism of this regulation should respond linearly to the ADP and P_i liberation within the contraction cycle. At the same time, MgADP, as a close structural analogue of MgATP, is an efficient, competitive inhibitor of ATPases, e.g., with a K_i close to 200 μM for filament sliding [70]. In addition, MgADP induces the actomyosin–ADP complex, which cooperatively promotes strong-binding cross-bridges such as the rigor complexes and increases myofilament Ca^{2+} sensitivity, thus masking the length-dependent activation [71]. The reversible ion pumps of the sarcoplasmic reticulum and sarcolemma are also very sensitive to inhibition by MgADP [72]. Therefore, accumulation of MgADP in the vicinity of any ATPase slows down the contraction cycle [70, 73] and impairs the Frank–Starling mechanism [63, 67, 71, 74].

Intracellular energy and metabolic signaling phosphotransfer networks within a defined cellular structural organization have the potential both (1) to protect cells from an excess of cytosolic free calcium and ADP and (2) to regulate respiratory ATP production in close correspondence to ATP consumption [27].

11.5 Integrated Phosphotransfer and Signaling Networks in Regulation of Cellular Energy Homeostasis

The CK-PCr circuit represents an efficient regulator of energy flux and uses metabolite channeling as a fine-tuning device for local ATP levels (see ▌Chapters 3 and 7▌). The significance of such a regulated channeling circuit operating at high total PCr and Cr pools lies in its high sensitivity towards ADP that prevents, especially in excitable cells, the accumulation of ADP and, consequently, AMP through the adenylate kinase reaction [75, 76], unless severe stress, such as hypoxia or ischemia, is imposed. In the latter case, AMP-activated protein kinase (AMPK) and other AMP-sensitive components would be activated by free AMP initiating signaling cascades that would turn on compensatory mechanisms for increasing energy supply and reducing energy consumption [77, 78].

11.5.1
Evidence for the Role of MtCK in Respiration Regulation in Permeabilized Cells *in Situ*

Studies of the regulation of mitochondrial respiration in permeabilized muscle cells and fibers have provided important information on the structure–function relationship and the role of creatine kinases in this process, as well as on calcium effects on the respiration [79–87].

First, Fig. 11.5A shows further that calcium in its physiological concentrations induces a decrease in apparent K_m for exogenous ADP in permeabilized cardio-

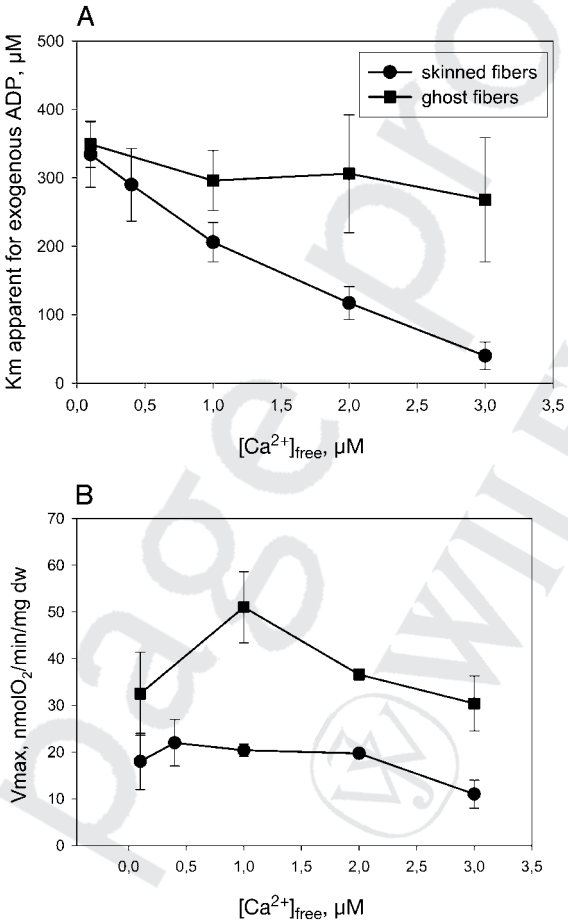

Fig. 11.5 Effects of the elevation of free calcium concentration in the medium on apparent K_m for exogenous ADP (A) and maximal rate of respiration (B) in skinned cardiac fibers and their ghost fibers (after extraction of myosin). (Redrawn from [86]).

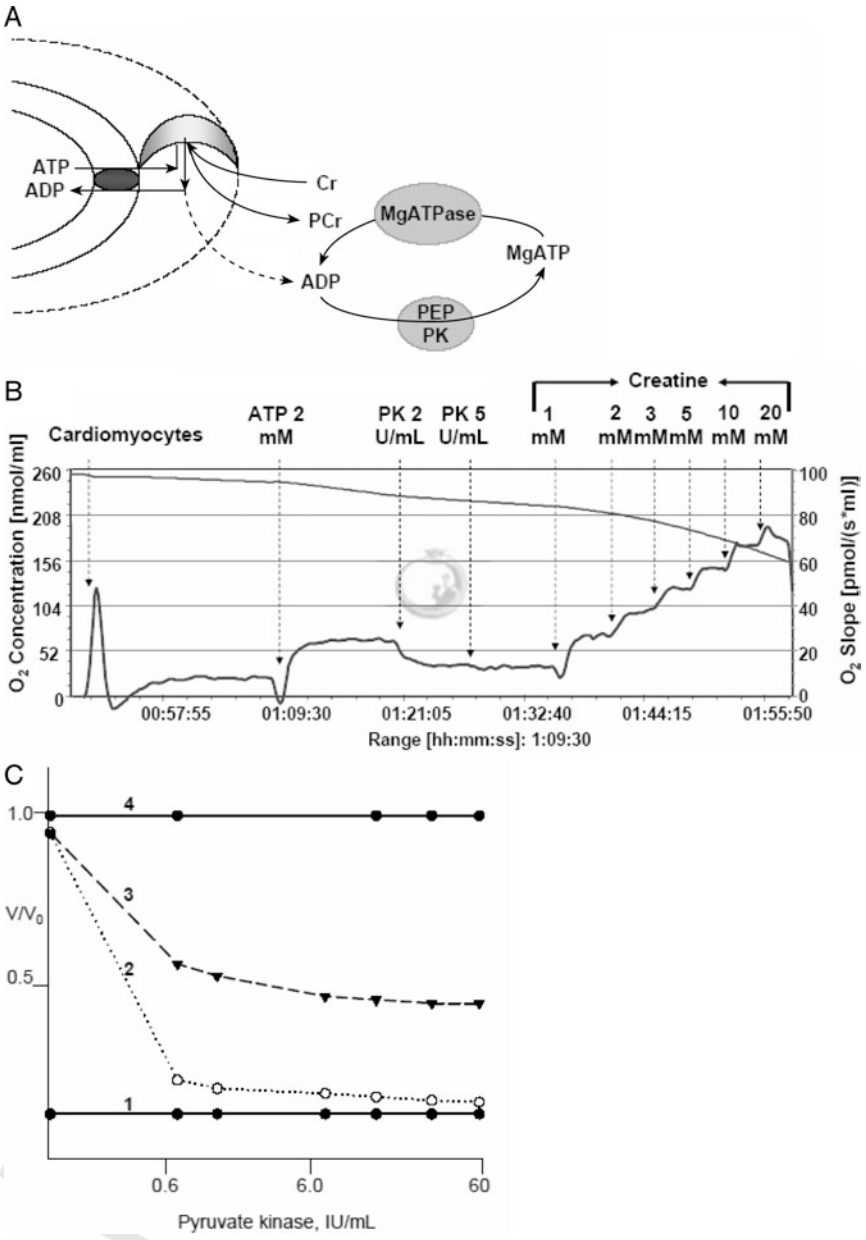

Fig. 11.6 (legend see p. 378)

myocytes due to supercontraction-related structural changes [86], because in ghost cardiomyocytes obtained after removal of myosin, no changes were observed in this parameter. However, in both cases, an increase in calcium concentration induced only a transitory increase in the maximal respiration (Fig. 11.5B). This quantitatively confirms the earlier results of Territo and Cortassa discussed above [50, 53] and conforms to the point of view that calcium is not a major direct regulator of respiration in cardiac cells but may regulate respiration via activation of Ca-dependent ATPases [88].

The permeabilized cell technique also allows us to demonstrate the role of MtCK in the regulation of mitochondrial respiration in the cells *in situ* and reveals some of the important aspects of functional coupling between MtCK and ANT discussed in ▌Chapter 3▌ (Fig. 11.6). In these experiments, endogenous ADP production was induced by addition of MgATP (2 mM) to activate mitochondrial respiration in permeabilized cardiomyocytes. Then the powerful competing pyruvate kinase–phosphoenolpyruvate system was added to trap the ADP (Fig. 11.6A), this decreasing the respiration to 25% of the initial value (in these experiments, the calcium concentration was zero and endogenous ADP production was activated mostly by MgATPases) (Fig. 11.6B). The addition of creatine, however, rapidly activated respiration up to almost maximal levels, despite the presence of a powerful ADP-trapping system (Fig. 11.6B). This means that the ADP produced locally in the MtCK reaction is not at all available to the PEP–PK system. Earlier,

Fig. 11.6 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 [87].
(A) Competition between the pyruvate kinase (PK)–phosphoenolpyruvate (PEP) system and mitochondria for endogenous ADP in permeabilized cardiomyocytes, by which the strong control of respiration by the mitochondrial creatine kinase reaction can be seen. (B) Oxygraph recordings. Respiration was activated by endogenous ADP after addition of 2 mM MgATP. The protein concentration was 0.0621 mg mL^{-1}. In the presence of 3 mM PEP, addition of PK in increasing amounts effectively removes this ADP; at 20 IU mL^{-1} of PK, about 25% of the initial ADP-dependent respiration is observed. In the presence of this powerful ADP-consuming PEP–PK system, activation of the MtCK reaction by stepwise addition of creatine rapidly increases the respiration up to maximal values: the acceptor control ratio is about 7 and is close to that seen with saturating concentrations of ADP [87].
(C) Comparison of the effects of the PEP–PK system of ADP trapping on the respiration of isolated heart mitochondria (1–3) and permeabilized cardiomyocytes (4). (1) State 2 respiration of isolated mitochondria; (2) respiration of isolated mitochondria activated by MgATP and the hexokinase–glucose system of regeneration of ADP in medium; (3) respiration of isolated heart mitochondria controlled by the MtCK reaction in the presence of creatine (20 mM); (4) respiration of permeabilized cardiomyocytes in the presence of MgATP (2 mM) and creatine (20 mM). In cardiomyocytes, ADP generated in the MtCK reaction is not accessible for the PEP–PK system, while in isolated heart mitochondria, about 50% of ADP produced by MtCK is used up by the PK–PEP system. This shows the very limited permeability of the outer mitochondrial membrane for ADP in permeabilized cells *in situ*. Curves 1–3 are redrawn from [89]. Curve 4: unpublished results.

Gellerich et al. used the same system in experiments with isolated heart mitochondria to find that in this case the PEP–PK system inhibited more than 50% of creatine-stimulated respiration (curve 3 in Fig. 11.6C) [89]. The same degree of inhibition was seen in permeabilized cardiomyocytes after their treatment with trypsin, in contrast to permeabilized intact cardiomyocytes in which the PK–PEP system was not at all able to reduce MtCK-stimulated respiration (curve 4 in Fig. 11.6C) [87]. These data favor the hypothesis that in cardiac cells *in vivo*, mitochondrial outer membrane permeability is limited and controlled by some cytoskeletal proteins [81–84] and clearly show the restrictions of the diffusion of adenine nucleotides within the highly organized structure of ICEUs (see ▌Chapter 3▐). 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 the cytoplasm [81, 82–87].

Thus, the results of studies on respiration regulation in permeabilized cardiac cells strongly support the metabolic feedback regulation of respiration via phosphotransfer networks.

11.5.2 *In Vivo* Kinetic Evidence

Further insights into and key support for the current understanding of metabolic signaling networks in their full complexity have come with the application of new methodologies in investigations of the *in vivo* kinetics of energy transfer [75–78, 90–93]. 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 (Fig. 11.7) [77, 91], implicating phosphotransfer reactions as indispensable routes that direct the flow of high-energy phosphoryls between cellular ATPases and the ATP production machinery in mitochondria. This new methodology allows quantitative evaluation of cellular ATP turnover and the distribution of energy fluxes between different phosphotransfer pathways *in vivo*. In the experiments with Langendorff-perfused hearts shown in Fig. 11.7, the creatine kinase flux increased linearly with cardiac performance (evaluated as the rate–pressure product) and reached a value close to 300 nmoles min^{-1} mg^{-1} of protein with the heart performing work at about 30,000 mmHg min^{-1}, corresponding to a rate of oxygen consumption of about 40 μmoles of O_2 per minute per gram dry weight [94]. Taking into account the CK flux in the equilibrium state (in arrested heart) (Fig. 11.7), protein content of tissue of about 150 mg g^{-1} wet weight, and a wet weight:dry weight ratio of 5, these data show a PCr:O_2 ratio close to 5. This supports the notion that the CK pathway carries the major part of the energy flux out of mitochondria to the ATPases under normal physiological conditions in heart cells, as described in ▌Chapters 3 and 7▐ and in agreement with the mathematical modeling of the compartmentalized energy transfer [27–95]. About 10–15% of cellular high-energy phosphoryls can be carried by the adenylate kinase

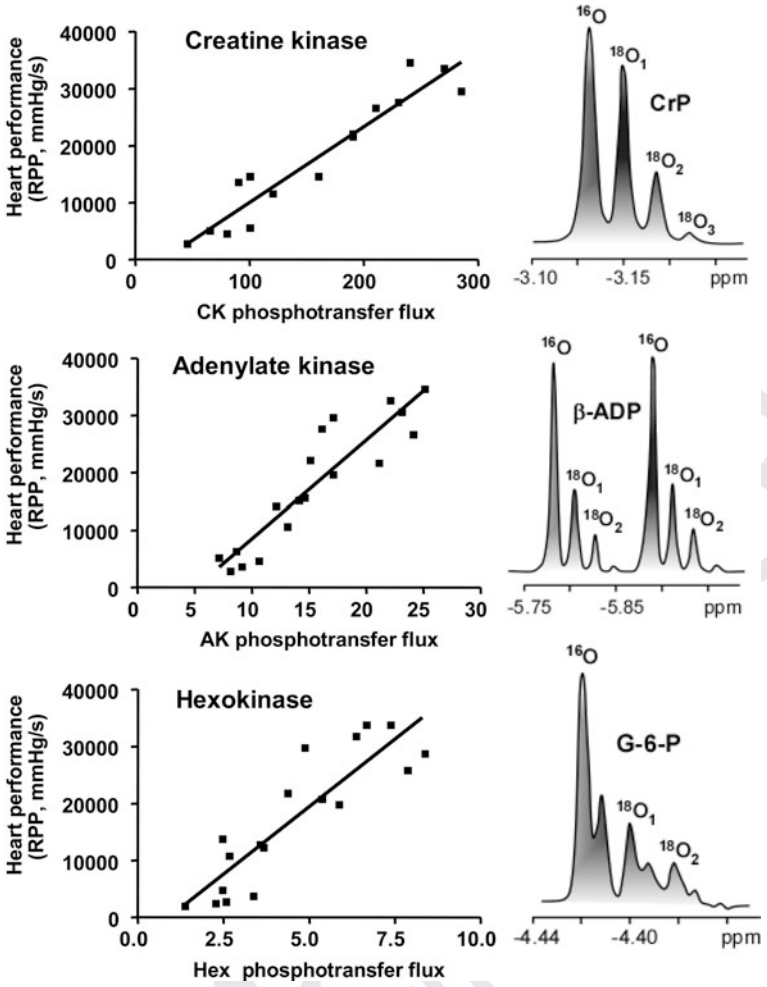

Fig. 11.7 Phosphotransfer networks mediate coupling between heart functional activity and ATP generation. Processing of high-energy phosphoryls through creatine kinase (CK), adenylate kinase (AK), and glycolytic (hexokinase) systems correlates linearly with heart functional activity (left panels). Phosphotransfer flux measurements obtained from ^{18}O-induced shift in ^{31}P-NMR spectra of phosphocreatine (CrP), β-ADP, and glucose-6-phosphate (G-6-P) (right panels) [77, 91]. Appearance of $^{18}O_1$, $^{18}O_2$, and $^{18}O_3$ peaks in corresponding phosphoryl-containing metabolites indicates phosphotransfer flux through the CK, AK, and hexokinase steps in the glycolytic system [77, 93]. Control hearts and contractile function modulated by ischemia–reperfusion are included in calculations [91]. RPP: rate–pressure product. Phosphotransfer flux is expressed as nmol min^{-1} mg^{-1} of protein of corresponding metabolite.

(AK)–glycolytic systems, whose contribution increases with muscle contraction and in failing hearts [27, 75–77, 90–93]. Such a distribution of contributions between CK, AK, and glycolytic systems is based on the assumption of parallel phosphotransfer pathways. However, in the cellular environment, these pathways are closely co-localized and interconnected, allowing high-energy phosphoryls to flow from one system to another [90–98]. The linear relationship between the creatine kinase flux and the workload (and thus the respiration rate) is also consistent with its measurements by the ^{31}P-NMR saturation transfer method [96, 99].

The high values of the CK fluxes and their linear relationship with workload confirm the central role of the CK system in the energo-mechanical coupling underlying the heart's Frank–Starling law.

11.5.3
Mathematical Modeling of Metabolic Feedback Regulation

Because of the high complexity of the processes involved, mathematical modeling is being increasingly and very effectively used to analyze the mechanisms of regulation of respiration and cellular energy fluxes [81, 82, 85, 94, 95, 100–108]. For this purpose, different models with distinct levels of detail are used, depending on the aim and point of view of the authors [82, 102, 107]. There is an increasing consensus among different groups that mathematical modeling supports the theory of metabolic feedback signaling from ATPases to mitochondria [95, 100, 102, 108], in agreement with experimental observations [109]. In the literature, different mathematical models of energy metabolism of muscle cells are available. The mathematical model of compartmentalized energy transfer was initially developed by Aliev and Saks [82, 95] and was modified further by Vendelin et al. [100]. These groups of models with similar structure may be called Vendelin–Aliev–Saks [102] or VAS models. Interesting models have been developed by Beard [102], by Cortassa et al. [101], and by Matsuoka and Noma (Kyoto model) [103], and then there are Korzeniewski's models of respiratory chain and "parallel activation" [104–106]. In the latter models, however, compartmentalized energy transfer is not yet analyzed in detail [106], as is done in VAS models [82].

Our VAS models (reviewed in [82]) quantitatively describe the results of *in vivo* studies of respiration regulation in ICEUs (see ▌Chapter 3▌) under physiological conditions of the Frank–Starling law. The latest version of one of these models is available at http://cens.ioc.ee/~markov/etransfer/current_model.pdf). These models are based on the concepts of ICEUs described in ▌Chapter 3▌ and include

- the kinetics of ATP hydrolysis by actomyosin ATPase during the contraction cycle,
- the diffusional exchange of metabolites between myofibril and mitochondrial compartments,
- VDAC-restricted diffusion of ATP and ADP across the mitochondrial outer membrane,
- the mitochondrial synthesis of ATP by ATP synthase,

- ΔpH- and ΔΨ-controlled P_i and ADP transport into the mitochondrial matrix, and
- PCr production in the coupled mitochondrial CK reaction and its utilization in the cytoplasmic CK reaction.

These events are considered in a system consisting of a myofibril with a radius of 1 μm, a mitochondrion, and a thin layer of cytoplasm interposed between them [81, 82, 94, 95]. The computations of diffusion and chemical events were first performed for every 0.1-μm segment of chosen diffusion path at each 0.01-ms time step [95]. This allowed the simulation of space-dependent changes throughout the entire cardiac cycle. The mitochondrial section of the model was first based on a simple kinetic scheme of mitochondrial ATP synthase with parameters allowing the description of experimental ADP and P_i dependences of oxidative phosphorylation in isolated mitochondria [95]; in the latest version, Korzeniewski's kinetic scheme [104] for the respiratory chain was adapted [100]. Mitochondrial oxidative phosphorylation is activated by ADP and P_i produced by ATP hydrolysis in the myofibrillar compartment. The kinetics of ATP hydrolysis by myosin in contracting muscle was predicted from dP/dt changes in isovolumic rat heart: a linear increase in the ATP hydrolysis rate up to 30 ms, followed by its linear decrease to zero at 60 ms into the contraction–relaxation cycle. The total duration of this cycle was taken to be 180 ms [95]. In mitochondria, the ATP/ADP translocase (ANT) and the P_i carrier (PiC) regulate the matrix concentrations of ATP, ADP, and P_i available for the ATP synthase. These carriers establish constant positive ADP and P_i gradients between the matrix and the mitochondrial intermembrane space. The model of cellular events considers CK compartmentation: the molecules of the cytoplasmic isoenzyme of CK (MM-CK), 69% of total activity, are taken to be freely distributed in the myofibrillar and cytoplasmic spaces. A remaining part of cellular CK, the mitochondrial isoenzyme of CK (MtCK), is localized in the mitochondrial compartment. The resulting close proximity of MtCK and ANT allows direct channeling of adenine nucleotides between their adjacent active centers; this channeling is the actual base for shifting the MtCK reaction toward the synthesis of PCr from translocase-supplied ATP even at high levels of ATP in the myoplasm of *in vivo* heart cells. In the modeling, we accounted for the limitation of the outer mitochondrial membrane for ADP in cardiac cells *in vivo*.

The results of the modeling show cyclic changes in the concentration of ADP in the core of myofibrils in ICEUs (Fig. 11.8A) in a microcompartment containing myofibrillar-bound MM-CK, where ADP is first produced by actomyosin MgATPase during the contraction cycle of cross-bridges and then rephosphorylated by CK due to the non-equilibrium state of the CK reaction [95, 100]. 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 [110, 111]. These changes in Cr, PCr, and total ATP are, however, close to the experimental errors of their detection, thus giving an

overall impression of metabolic stability [27, 28, 100]. Changes in ADP and P_i concentrations are relatively much more significant because of very low initial values (Fig. 11.8A). Without CK, the changes in local ADP concentrations in these microcompartments are much more dramatic [82, 94, 95].

Within the whole contraction cycle, these coupled reactions are in the steady state, in which the rates of ADP and ATP cycling, and thus the respiration in mitochondria coupled to PCr production, are increased with elevation of the workload. Increasing cyclic changes in the local ADP production in myofibrils immediately displace the myofibrillar MM-CK reaction in the direction of local ATP regeneration (Fig. 11.8B). The amplitude of displacement of CK from equilibrium is proportionally increased with workload (Fig. 11.8B,C) [100]. In this regard, CK, adenylate kinase, and other phosphotransfer isoenzymes in different intracellular compartments are ''pushed'' or ''pulled'' from the equilibrium in opposite directions, depending on the activity of an associated process that drives steady-state, high-energy phosphoryl flux [27, 83, 92, 112].

If myoplasmic CK is structurally organized and bound to the cytoskeleton, these cyclic changes may be channeled to mitochondria by a mechanism of so-called vectorial ligand conduction – spatially directed group translocation down-gradients of group potential – in accordance with the theories of metabolic networks [76, 82, 83, 90, 91, 113]. The stimulatory effect of these CK ligands (Cr and/or ADP) on mitochondrial respiration is amplified by the functional coupling between MtCK and ANT [28, 81, 82]. Therefore, MtCK always catalyzes the unidirectional PCr and ADP production from mitochondrial ATP and cytoplasmic creatine (Fig. 11.8C) [95, 100]. Local ADP produced in this reaction controls the rate of respiration. Figure 11.8E shows that the model quantitatively describes the observations of Williamson et al. [29] on the dependence of the respiration rate upon the workload.

Under conditions of metabolic stability, an increase in the rate of ATP synthesis and of the coupled respiration (oxidative phosphorylation) is possible only if the supply of ADP and P_i to mitochondria is increased, making both metabolites good candidates for metabolic feedback signals controlling respiration [27, 28, 114]. 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 due to MgATPase hydrolysis [98]. Application of 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 [108]. This conclusion has been confirmed experimentally [109] and, recently, by models [102]. 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 [27, 28, 76, 82, 92, 108]. Instead of the parallel activation of all ATP-producing and -utilizing systems proposed by Korzeniewski [106], there seems to be a self-regulatory metabolic feedback signaling via phosphotransfer pathways and P_i fluxes. In any case, as a response to sarcomere stretch and cross-bridge

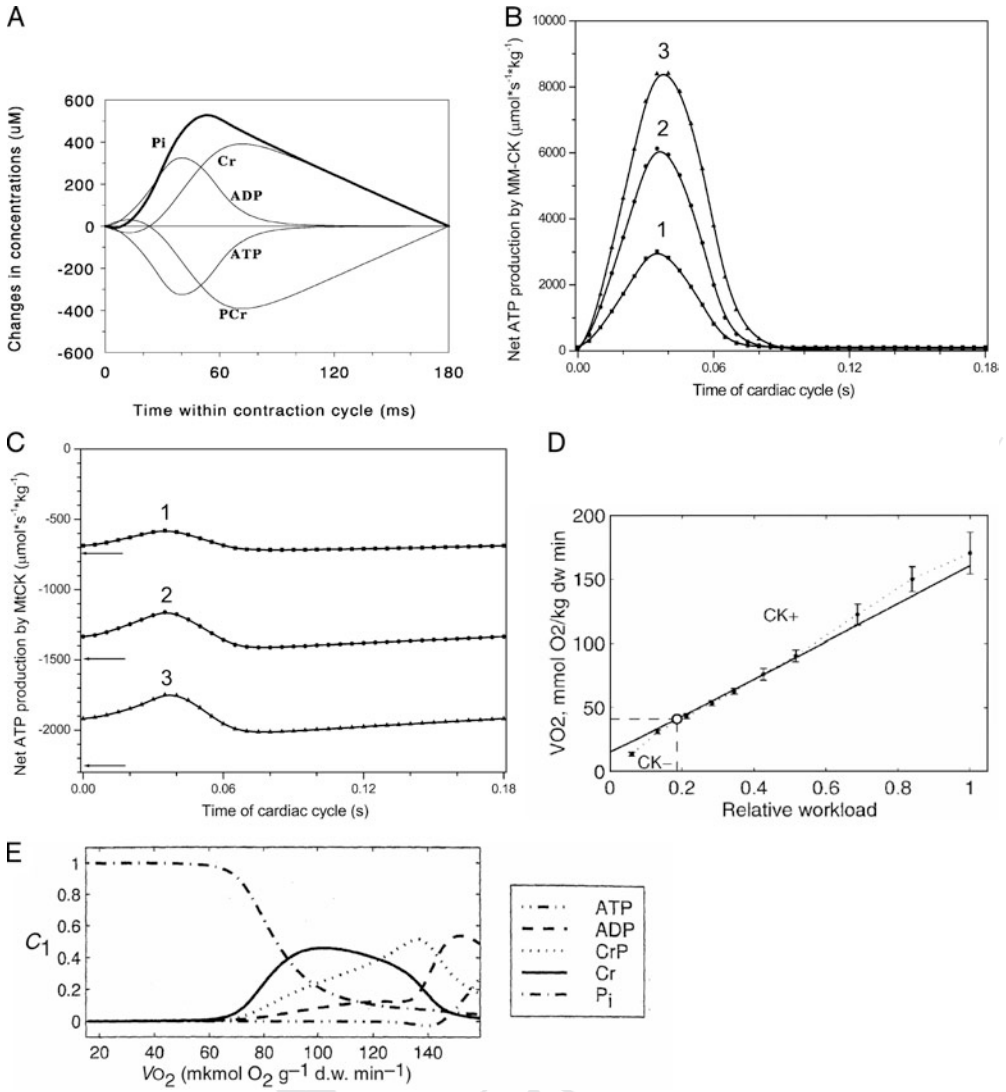

Fig. 11.8 (legend see p. 385)

cycling, this complex signal leads to increases in respiration rate, coupled mitochondrial PCr production, and energy flux via the CK–PCr system, while also maintaining metabolic stability (homeostasis) at elevated workload.

Thus, there is no need to search for some mysterious agent, such as the ''parallel activator'' of ATPases and the respiratory chain proposed by Korzeniewski [106]: the metabolic feedback mechanisms for regulating respiration via phospho-

transfer pathways and P_i fluxes are there, and they carry out the task of matching ATP production to its demand very effectively.

11.6 "Metabolic Pacing": Synchronization of Electrical and Mechanical Activities With Energy Supply

The existence of two interrelated systems regulating mitochondrial respiration and energy fluxes in the cells is increasingly being recognized (Fig. 11.9). The first system is composed of structurally organized enzymatic modules and networks of the CK–AK–glycolytic systems communicating flux changes from cellular ATPases to mitochondrial oxidative phosphorylation in non-equilibrium steady state [27, 28, 75, 92, 116], and the secondary amplifying system is based on cellular and mitochondrial calcium cycles, which adjust the capacity of substrate oxidation and energy-transducing processes to meet increasing cellular en-

Fig. 11.8 Mathematical modeling of the regulation of mitochondrial respiration in cardiac cells *in vivo* under physiological conditions controlled by the Frank–Starling mechanism.
(A) Phasic changes in metabolite concentrations in the core of myofibrils of ICEUs over the cardiac contraction cycle. Reproduced from [82] with permission.
(B) Workload dependence of the dynamics of net ATP production by MM-CK in myofibrils. The reaction rates for workloads of 750 (curve 1), 1500 (curve 2), and 2250 (curve 3) μmol ATP s^{-1} kg^{-1} are shown. Net rate of the reaction means displacement from the equilibrium position of the creatine kinase reaction (observed in the diastolic phase) as a result of local ADP production in the contraction cycle.
(C) Workload dependence of the dynamics of net PCr production by MtCK. Workload values are also indicated by the arrows on the respective curves. BandC [AQ4]: complete model of Dos Santos et al. [94] used in simulations for [82]. Mitochondrial $\Delta\Psi = -160$ mV; V_{max} of ATP synthase = 4269.3 μmol ATP s^{-1} kg^{-1}. PCr export by mitochondria is 90.9%, 89.1%, and 85.8% of total mitochondrial energy export for workloads of 750, 1500, and 2250 μmol ATP s^{-1} kg^{-1}, respectively. Due to the functional coupling between ANT and MtCK, the reaction always runs out of equilibrium in the direction of PCr synthesis [100], the steady-state values of the rates of this coupled reaction are increased by increasing the workload (cyclic changes in MgATP and MM-CK reactions in myofibrils), and there is metabolic signaling of these changes to mitochondria by the creatine kinase–phosphocreatine energy transfer system. Reproduced from [27] with permission.
(D) Computed (solid line) and experimental (points with standard deviations, from [29]) oxygen consumption rates of working cardiac muscle. Relative workload is the fraction of maximal workload applied; in computations this is the rate of ATP hydrolysis by actomyosin MgATPase. CK+ shows the calculations for the active creatine kinase system; CK− shows the calculations for the cells with inactivated creatine kinases (results shown within the square). The model includes the diffusion restriction of ADP through the mitochondrial outer membrane. Reproduced from [100, 27] with permission.
(E) Metabolic control analysis of the mathematical model of compartmentalized energy transfer. Flux control coefficients for different metabolites are shown. Reproduced from [108] with permission.

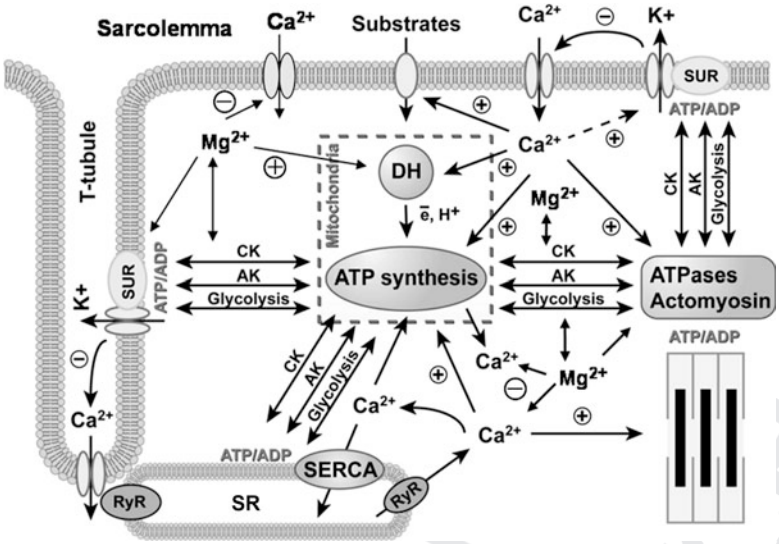

Fig. 11.9 Cardiac excitation–contraction energy coupling: synchronization of electrical and metabolic pacing. Electrical pacing–induced action potential and membrane depolarization cause Ca^{2+} influx through L-type Ca^{2+} channels in the sarcolemma and T-tubules, triggering Ca^{2+} release from intracellular stores in the sarcoplasmic reticulum (SR) through ryanodine receptor channels (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+} and Mg^{2+} transients generates 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 the SR Ca^{2+}-ATPase (SERCA) and by mitochondria and is removed from the cell by the Na^{+}/Ca^{2+} exchanger and a Ca^{2+} pump in the sarcolemma (see details in the text). (Modified from [27] with permission).

ergy demands [31, 42, 53]. Moreover, the coupled CK, AK, and glycolytic reactions by communicating signals to the K_{ATP} channel, a sarcolemmal metabolic sensor, provide fine-tuned regulation of excitation–contraction and thus of the calcium cycle within a cell [75, 115, 117–119] (see ▌Chapter 3▌). Such integration of energetic and ion signaling systems provides the basis for ''metabolic pacing,'' the synchronizing of electrical and mechanical activities with energy supply processes, which is fundamental for optimal heart function and for sustaining an adequate force–frequency relationship during stress [27, 91–93]. The significance of such a relationship is indicated by a number of studies demonstrating that inhibition of CK or abnormal regulation of K_{ATP} channels disturbs the heart force–frequency relationship and inotropic responses to stress challenges [117–125]. In this regard, one of the earliest signs of heart failure is the disturbed force–frequency relationship [121, 126] indicating developing defects in the cardiac energetic signaling system and mechano-energetic coupling [27, 73, 90, 116, 127].

In recent years, accumulating evidence indicates that phosphotransfer-governed nucleotide exchange and metabolic sensing are essential for processing cellular information and sustaining energy, ionic, and hormonal homeostasis [76, 128–130]. By synchronizing electrical, ionic, and metabolic oscillations, phosphotransfer reactions ensure the pacing of cellular energy metabolism to adjust ATP production strictly according to demands, thus facilitating muscle energy economy [27, 92, 112]. As presented in Fig. 11.9, during the cardiac cycle, an electrical impulse–induced action potential and membrane depolarization cause Ca^{2+} influx through L-type Ca^{2+} channels in the sarcolemma and T-tubules, triggering Ca^{2+} release from intracellular stores in the sarcoplasmic reticulum (SR) through ryanodine receptor channels (RyR) and cardiomyocyte contraction [10, 115]. ATP consumed during myofibrillar contraction and Ca^{2+} pumping is rapidly replenished by creatine kinase (CK), adenylate kinase (AK), and glycolytic phosphotransfer relays, which maintain optimal local ATP:ADP ratios and free energy of ATP hydrolysis [28, 92, 98, 116]. 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 AMPK [27, 28, 75, 76, 93, 117]. The amount of Ca^{2+} entering a cell is controlled by the action potential duration regulated by K_{ATP} channels located in the sarcolemma and T-tubules [117–120]. Conversely, the activity of this metabolic sensor is regulated by phosphotransfer reactions and Ca^{2+} feedback [119, 130]. Ca^{2+} entering 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 [31, 44, 45, 53]. Another player in metabolic pacing is intracellular Mg^{2+}, the most abundant divalent cation in the cell, which activates a large number of metabolic enzymes and regulates Ca^{2+} entry, release from intracellular stores, and interaction with binding proteins (Fig. 11.19 [AQ1]) [131–136]. Intracellular free Mg^{2+} levels are regulated by adenylate kinase, and other phosphotransfer enzymes govern equilibrium of adenine nucleotide species, the Na^{2+}/Mg^{2+} exchanger, TRPM7, and the Mrs2 family of cation channels [131–135]. Ca^{2+} entering the cell and ATP consumption–induced metabolic oscillations can trigger parallel Mg^{2+} oscillations, which could affect dehydrogenases and a number of other critical Mg^{2+}-sensitive cellular components [132]. Previous and more recent studies indicate that Mg^{2+} indeed could serve as a feedback signal from the adenine nucleotide pool to ATP-generating processes, simultaneously tuning Ca^{2+} signals and reducing Ca^{2+} accumulation in mitochondria [134–136]. Both Ca^{2+} and Mg^{2+} signals can activate the PDH complex and promote substrate oxidation in heart mitochondria [125]. Such simultaneous recruitment of different steps in the cellular energetic system and ligand conduction–type handling of substrates in sequential phosphotransfer systems can increase metabolic flux many times without apparent changes in substrate concentrations [75, 76, 82, 112, 113].

The first system – metabolic feedback signaling – quantitatively explains the metabolic aspect of the classical Frank–Starling law regarding regulation of car-

diac function and respiration under conditions of metabolic stability and unchanged calcium transients. This system is based on compartmentalized energy transfer, a deficit in which explains the rapid fall of contractile force in the first minutes of total ischemia (see ▌Chapter 3▐). The second system explains adrenergic modulation of cardiac cell function and energetics under stress [14, 53, 137]. 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 (Fig. 11.9). Both systems may be activated simultaneously, as is observed in the case of positive inotropy induced by β-adrenergic agents, when Frank–Starling curves are shifted upward [1, 24]. These two more or less independent systems regulating cellular energetics have been described separately by mathematical models [100, 137]. Integration of all this information into quantitative models of whole-cell functioning and metabolism is an interesting and challenging perspective of molecular system bioenergetics.

Thus, both phosphotransfer-mediated metabolic feedback and Ca^{2+}/Mg^{2+} signaling are important components in regulated signal transmission and coordinated dehydrogenase activation, producing an optimal mitochondrial response to increased cellular energy demands.

11.7
Metabolic Channeling Is Needed for Protection of the Cell from Functional Failure, Deleterious Effects of Calcium Overload, and Overproduction of Free Radicals

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. It thus avoids any danger of mitochondrial calcium overload that would open the mitochondrial permeability transition pore and thus lead to cell death [47, 52]. The functioning of the coupled MtCK–ANT system in mitochondria prevents reactive oxygen species (ROS, oxygen free radicals) formation in the mitochondrial respiratory chain (see Fig. 3.9 in ▌Chapter 3▐) and helps to avoid many problems related to ROS production, such as PTP opening, necrosis, apoptosis, and rapid aging [138, 139]. In this way, the CK–PCr network may significantly contribute to the positive effects of physical exercise on human health: exercise-induced increases in fluxes via this pathway increase ADP–ATP turnover in coupled MtCK–ANT reactions in mitochondria and keep ROS production low.

Because of the central importance of this efficient and protective metabolic feedback signaling system, its alteration in pathological states of the cell contributes significantly to the development of cardiac failure, resulting from a decrease in total creatine content [140, 141], from alterations in CK expression [122, 123, 142], or from changes in the coupling of CK in mitochondria and with MgATPases [143] (for a review, see [144–146]. Ingwall et al. have shown that the product of CK activity and total creatine content, CK × [Cr], decreases by a factor of 3–6 in cardiomyopathy and in failing human hearts [140]. Figure 11.10 shows some

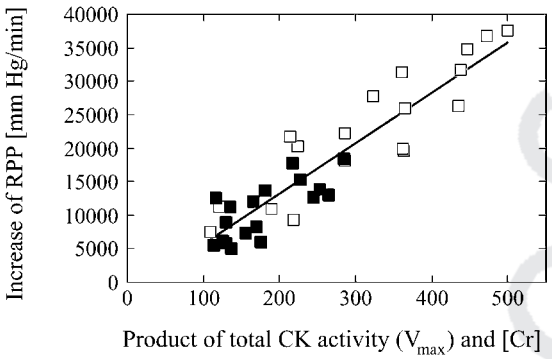

Fig. 11.10 Relationship between total creatine content, creatine kinase activity, and cardiac function in perfused rat hearts. RPP: rate–pressure product. (Reproduced from [146] with permission).

interesting data summarized by Wyss and Kaddurah-Daouk [146] on the relationship between creatine content, CK activity, and contractile performance. These results are consistent with earlier data showing that replacement of creatine by its much less productive analogue guanidino propionic acid (GPA), given to rats in diet, decreases by more than half the maximal work capacity and, correspondingly, the rate of respiration [147]. Also, significant washout of creatine during long periods of perfusion of frog hearts has been shown to lead to a hypodynamic state with a two- to threefold decrease in contractile force, which can be completely reversed by uptake of creatine [148, 149]. It was shown in Neubauer's laboratory that hearts with undetectable levels of creatine and phosphocreatine due to knock-out of a key enzyme of creatine biosynthesis [123] lost the ability to respond to inotropic stimulation even at low workload, and showed markedly impaired recovery of heart function during ischemia–reperfusion (see also [122, 141, 142]). Momken et al. found recently that double M-CK and MtCK knockout mice showed only 10% of work capacity in voluntary exercise in running wheels, as compared with that of normal wild-type mice [150].

Thus, effective cardiac work and fine metabolic regulation of respiration and energy fluxes require the organized and interconnected energy transfer and metabolic signaling systems. Direct transfer of ATP and ADP between mitochondria and different cellular compartments is not able to fulfill this important task efficiently.

11.8
Molecular System Analysis of Integrated Mechanisms of Regulation of Fatty Acid and Glucose Oxidation

Molecular systems analysis as a method is also useful for elucidation of the mechanisms of regulation of substrate supply for the heart [151]. In muscle cells, contractile function and cellular energetics are fuelled by oxidation of carbohy-

drate substrates and fatty acids [1–3, 27, 28, 55, 151–154]. The choice of substrates depends upon their availability [1–3, 55, 127, 152–155], and the rates of their utilization 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, linked directly to the activity of the respiratory chain and the phosphorylation process catalyzed by the ATP synthase complex [1–3, 127, 151–156]. The rates of all these processes are geared to the workload, mostly by the mechanism of feedback metabolic regulation [3, 55, 151, 156].

In 1931 Clark et al. showed in isolated frog heart that the oxidation of carbohydrates explains not more than 40% of oxygen uptake [157]. In 1954, Bing and coworkers demonstrated, by using coronary sinus catheterization, the absolute requirement of the human heart for free fatty acids (FFA) as fuel [152, 158]. The mechanisms of regulation of substrate uptake have been intensively studied by Opie et al., Neely, Morgan, Williamson, Randle, and many others [1–3, 27, 28, 127, 152–156, 159–165]. In general, the results of all these studies show that in heart, in the presence of both carbohydrate substrates and FFA, about 60–90% of the oxygen consumed is used for oxidation of FFAs and that the rates of both oxygen consumption and fatty acid oxidation increase linearly with the elevation of the workload [1–3, 139, 158, 159]. In cardiac muscle, all these important changes occur at unchanged global levels of ATP, phosphocreatine (PCr), ADP, and AMP [30–32, 159, 160].

The network of reactions of substrate supply for mitochondrial respiration in muscle cells and their multiple interactions and feedback mechanisms of regulation are illustrated in Fig. 11.11. This network emerged from 70 years of research on muscle energy metabolism. The choice of substrates for oxidation depends on their availability, and if glucose and FFAs are both present, FFAs strongly inhibit the transport of glucose across the plasma membrane in both heart and skeletal muscle [3, 153–155]. At relatively low workloads, mitochondrial acetyl-CoA and NADH produced by beta-oxidation tend to inhibit the pyruvate dehydrogenase complex in the mitochondrial inner membrane. Citrate, whose production is increased in the Krebs cycle after transport across the inner mitochondrial membrane into the cytoplasm, inhibits PFK [2, 3, 55, 164]. All these regulatory mechanisms explain the preference for FFAs for respiration in oxidative muscle cells. An important limitation of aerobic glycolysis is the necessity to maintain the rather low NADH:NAD^+ ratio in the cytoplasm needed for the high steady-state flux through the GAPDH reaction step that is achieved by transfer of reducing equivalents into the mitochondrial matrix by the malate–aspartate shuttle [55, 165]. However, this shuttle becomes the rate-limiting step at even medium workloads [165]. The fatty acid pathway is free of this kind of limitation. During contraction, the regulation of all reactions of substrate supply starts with an increase in the workload (see Fig. 11.3), which under normal physiological conditions is usually governed by the Frank–Starling law [27, 28]. The sequence of regulatory signals from cellular ATPases, such as the actomyosin ATPase for muscle contraction, to the mitochondrial matrix, which controls fatty acid oxidation during

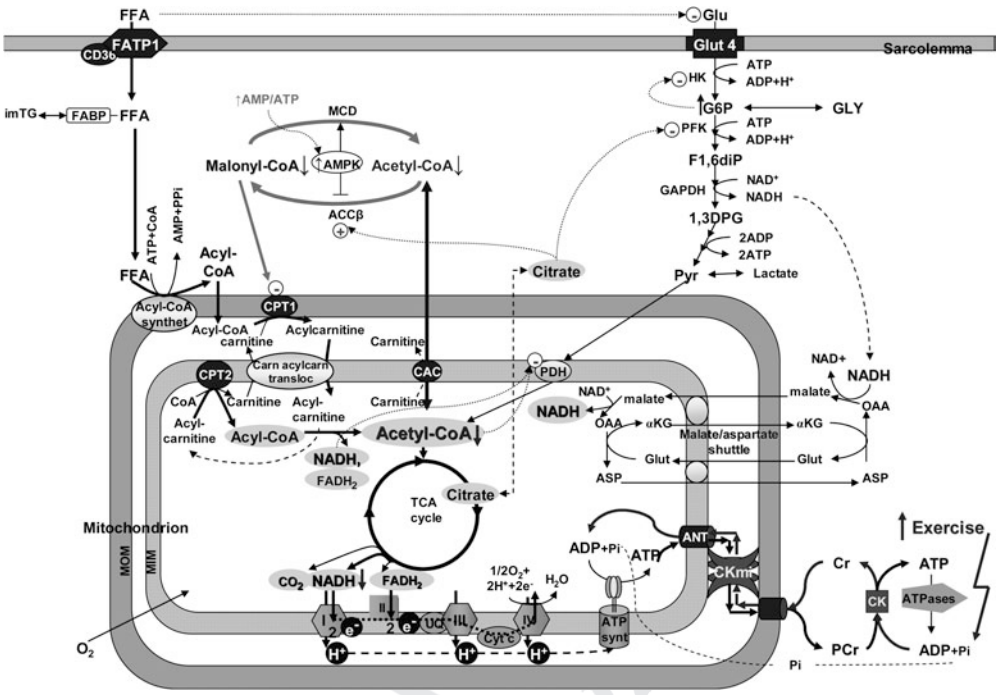

Fig. 11.11 The scheme of substrate supply for mitochondrial respiration and the mechanisms of feedback regulation of fatty acid and glucose oxidation during workload elevation in oxidative muscle cells: central role of TCA cycle intermediates. FFAs are taken up by a family of plasma membrane proteins (fatty acid transporter protein [FATP1], fatty acid translocase [CD36], and fatty acid binding protein [FABP]) and 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 palmitoyltransferase I (CPT I). Acylcarnitine is then transported by carnitine–acylcarnitine translocase into the mitochondrial matrix, where a reversal exchange takes place through the action of carnitine palmitoyltransferase II (CPT II). Once inside, the mitochondrion acyl-CoA is a substrate for the beta-oxidative pathway, resulting 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 further 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 the participation of carnitine, carnitine acetyltransferases, and carnitine acetyltranslocase (carnitine acetylcarnitine carrier complex, CAC). Glucose (GLU) is taken up by glucose transporter-4 (GLUT4) and enters the Embden–Meyerhof pathway, which converts glucose via a series of reactions into two molecules of pyruvate (PYR). As a result of these reactions, a small amount of ATP and NADH is produced. G6P: glucose 6-phosphate; HK: hexokinase; PFK: phosphofructokinase; GLY: glycogen; F1,6diP: fructose-1,6-bisphosphate; GAPDH: glyceraldehyde phosphate dehydrogenase; 1,3DPG: 1,3 diphosphoglycerate. The redox potential of NADH is transferred into the mitochondrial matrix via the malate–aspartate shuttle. OAA: oxaloacetate; Glut: glutamate; 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

workload changes, is shown in Figs. 11.3 and 11.11. A workload-dependent increase in the rate of the actomyosin ATPase reaction results in the release of increasing amounts of ADP and P_i; this metabolic signal is transmitted to the mitochondrial adenine nucleotide translocase (ANT) by phosphotransfer networks under conditions of apparent metabolic stability as described above [27]. P_i enters the mitochondria by a special phosphate carrier [27, 33, 109]. These signals activate the ATP synthase and the utilization of the proton transmembrane electrochemical gradient ($\Delta\mu_{H+}$) for ATP synthesis in mitochondria [1–3, 27, 33, 56–58, 165]. This process always leads to an increase in electron transfer to oxygen via the respiratory chain and NADH and $FADH_2$ oxidation. Independent of the substrate used, an increased workload always decreases the NADH:NAD^+ ratio in the mitochondrial matrix (see Fig. 11.2) [27, 28, 55–58]. This decrease in the

Fig. 11.11 (cont.)
OAA is returned to the cytosol by conversion to ASP and exchange with Glut. Most of the metabolic energy derived from glucose can come from the entry of pyruvate into the citric acid cycle and oxidative phosphorylation via acetyl-CoA production. NADH and $FADH_2$ are oxidized in the respiratory chain (complexes I–IV). These pathways occur under aerobic conditions. Under anaerobic conditions, pyruvate can be converted to lactate. *Feedback regulation.* Glucose–fatty acid cycle (Randle hypothesis): if glucose and FFAs are both present, FFAs 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 pyruvate dehydrogenase (PDH) complex, and increased citrate (produced in the TCA cycle) can inhibit phosphofructokinase (PFK). These changes would slow down oxidation of glucose and pyruvate and increase glucose-6-phosphate, which would inhibit hexokinase (HK) and decrease glucose transport. The mitochondrial creatine kinase (MtCK) catalyzes the direct transphosphorylation of intramitochondrially produced ATP and cytosolic creatine (Cr) into ADP and phosphocreatine (PCr). ADP enters the matrix space to stimulate oxidative phosphorylation, while PCr is transferred via the cytosolic Cr–PCr shuttle to functional coupling of CK to ATPases (actomyosin ATPase and ion pumps), resulting in the release of high free energy of ATP hydrolysis. If the workload increases, ATP production and respiration are increased due to feedback signaling via the creatine kinase (CK) system, leading to a decrease in mitochondrial acetyl-CoA content, which is transferred into the cytoplasm with the participation of the carnitine acetyl carrier (CAC). Acetyl-CoA carboxylase (ACC) is responsible for converting acetyl-CoA to malonyl-CoA, a potent inhibitor of CPT I, with the aim of avoiding 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 lead to a decrease in the concentration of malonyl-CoA. A fall in malonyl-CoA levels disinhibits CPT I, resulting in increased fatty acid oxidation. Malonyl-CoA is also converted back into acetyl-CoA in the malonyl-CoA decarboxylase (MCD) reaction. An increase in the workload increases the rate of acetyl-CoA consumption, and this automatically decreases the malonyl-CoA content. Regulation of ACC and MCD occurs under stress conditions when the AMP:ATP ratios are increased but is unlikely to occur under normal workload conditions 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 with permission from [151]).

NADH:NAD^+ ratio, on the other hand, 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 [3, 55]. 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 in feedback metabolic regulation of upstream pathways of substrate oxidation. There is a mitochondrial matrix pool of important intermediates of this cycle, which plays a role in feedback regulation of substrate oxidation [55]. One of the results of the activation of the reactions of the Krebs cycle is the strong decrease in the level of acetyl-CoA in the mitochondrial matrix at high fluxes through this cycle when the rate-controlling steps are shifted towards the acyl-carnitine transporter and beta-oxidation of fatty acids [159]. Acetyl-CoA, which is formed in the mitochondrial matrix space, is transferred into the cytoplasm with the participation of carnitine, carnitine acetyltransferases, and carnitine acetyltranslocase (carnitine acetyl carnitine carrier, CAC in Fig. 11.11). These reactions seem to be in rapid equilibrium [161–163, 166–171]. In the cytoplasm, acetyl-CoA is used to produce malonyl-CoA through the acetyl-CoA carboxylase (ACC) reaction, which is the malonyl-CoA decarboxylase (MCD) reaction converted back into acetyl-CoA [161–163, 168–172]. Malonyl-CoA is an effective inhibitor of carnitine palmitoyltransferase I (CPT I) and thus of the transfer of acyl groups into mitochondria for beta-oxidation (for reviews, see [2, 172, 173]). ACC is inhibited upon its phosphorylation by AMP-activated protein kinase (AMPK) [2, 173–177]. This reaction sequence served as the basis for a hypothesis that was advocated in numerous recent works, according to which malonyl-CoA is a key regulator of fatty acid oxidation [153, 154, 161, 172–177]. However, as an alternative, it seems much more reasonable to consider malonyl-CoA as a regular negative (inhibitory) metabolic feedback signal, as described elsewhere [1, 27]. The key regulator of respiration rate and energy fluxes, then, is the actual energy demand, the workload, and this also concerns fatty acid oxidation (see Fig. 11.3). Indeed, analysis of the changes in the intermediates of fatty acid oxidation showed that the workload-dependent increase in palmitate uptake and oxidation is accompanied by a significant decrease in acetyl-coenzyme A content [159]. This reflects the kinetics of the reactions in the Krebs cycle in response to an increase in the rate of respiration and NADH oxidation relative to acyl-carnitine transport into mitochondria and beta-oxidation [3]. A decrease in the content of acetyl-CoA leaves ACC in the cytosol with much less substrate, thus resulting in a significant decrease in malonyl-CoA in the presence of active MCD. ACC in the cytoplasm has a rather low affinity for acetyl-CoA [163, 169], and a decrease in this substrate due to changes in the kinetics of reactions in the Krebs cycle, as well as in acyl-carnitine transport and beta-oxidation, naturally results in decreased malonyl-CoA production [161, 171]. Therefore, malonyl-CoA decreases rapidly because of its decarboxylation by MCD back into acetyl-CoA, which is consumed in the Krebs cycle in the mitochondrial matrix [161, 171]. This ▌AQ2▌ then releases CPT I automatically from any inhibition, and FFA oxidation proceeds at a rate necessary for the energy supply of contraction and ion pumps. An additional interesting possibility, worthy of detailed studies, is the probable

compartmentation of AMPK at the sites of AMP production close to the acyl-CoA synthetase. This may be an additional local mechanism of keeping the level of malonyl-CoA low and releasing CPT I from inhibition during fatty acid oxidation.

Thus, the decrease in malonyl-CoA is not the reason for but rather the consequence of the increases in workload and fatty acid oxidation. If at some point the workload is decreased again and less energy is needed, the reaction sequence described is reversed (see Fig. 11.11), and malonyl-CoA may increase to fulfill its role as a negative feedback regulator of FFA oxidation, as was correctly pointed out by Opie [1], by inhibiting CPT I to safeguard mitochondria from overload by acyl-CoA, which has detergent properties, and also may degrade to FFA and, via the uncoupling protein (UCP), may uncouple oxidative phosphorylation [3, 177, 178].

The integrated network shown in Fig. 11.11 explains all of the main experimental observations on the regulation of respiration and substrate utilization in the heart. Malonyl-CoA clearly seems to be 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. 11.11 for heart and the sequence of the reactions given above are probably valid as well, especially for certain muscle types under certain conditions (endurance exercise training, etc.) [179, 180]. However, it should be emphasized that the mechanisms of regulation of skeletal muscle energy metabolism generally are different from those for the heart and are very different between distinct muscle fiber types, as described in ▌Chapter 17▌ (see also [179, 180]).

11.9 Concluding Remarks and Future Directions

In this chapter we have demonstrated the advantages of molecular systems analysis to address the problems of regulation of energetics of the cell as a whole integrated system. This is opposite to the reductionist approach, which tends to use only the results of studies of the isolated system, such as mitochondria, or to focus on small segments of the whole system. Thus, the effects of calcium on the Krebs cycle dehydrogenases and isolated mitochondria led to overestimation of the role of this divalent cation in respiration regulation in the cell, in particular when the experimental data on the whole heart were not accounted for [54]. The conclusion of the key role of malonyl-CoA in the regulation of fatty acid oxidation was made in studies of only one fragment of the system (see gray area in Fig. 11.11). While justified in separate experiments, these conclusions are no longer valid for explaining regulatory mechanisms in the integrated system *in vivo*, when interactions between the parts of the system lead to completely new and unexpected mechanisms, the elucidation of which needs a molecular systems approach. As we have shown in this book, molecular system bioenergetics, a

new approach to studying the integrated cellular systems of energy metabolism, is very helpful in the study and identification of cellular mechanisms of such complex phenomena as the acute contractile failure of the heart in the first minutes of total ischemia (see ▌Chapter 3▌), regulation of respiration under the conditions of the Frank–Starling law, and the mechanisms of substrate supply to the heart.

These achievements and conclusions are in concord with and are supported by recent work published by Weiss et al. [181]. These authors presented a holistic view of cardiovascular metabolism, considering it from the perspective of a physical network in which various metabolic modules are spatially distributed throughout the interior of the cell to optimize ATP delivery to specific ATPases [181]. In addition to the mitochondrial module (which is represented in our works by ICEUs; see ▌Chapter 3▌), the authors also considered a module consisting of glycolytic enzyme complexes that provide energy channeling to molecular complexes in the sarcolemma and sarcoplasmic reticulum and modules of calcium cycling (which Wang et al. called calcium release units [19]). These modules were further analyzed from the abstract perspective of fundamental concepts in network theory [182, 183] and the dynamic perspective of interactions between modules [181]. The authors emphasized that understanding the nature of these interactions within hierarchical modular structures is a major challenge of research into cardiac metabolism to gain a deeper understanding of possible mechanisms of cardioprotection [181].

Thus, in this new field of molecular system bioenergetics, structural, functional, genomic, and computational analysis of enzymatic clusters and networks is yet another future challenge in the area of cellular energetics. One of the most intriguing questions concerns the nature of local restrictions on the diffusion of adenine nucleotides in a highly structured cytosol despite the relatively high rates of their diffusion in the intracellular bulk water phase. These diffusion restrictions result in compartmentation of adenine nucleotides and kinetic and thermodynamic inefficiency of energy-dependent processes, which may explain the necessity of energy transfer and metabolic signaling networks. Future developments to verify predictions made by theoretical considerations and by increasingly complex mathematical models should include new experimental methods allowing detection of small changes in the transduced metabolic signals (Cr, ADP, AMP, P_i) in critical cellular compartments and new means of independent manipulation of energetic signals. These advances would provide a broader and more molecular understanding of the regulation of cellular energetics and metabolic signaling, a rapidly growing area in cellular systems biology.

Finally, to understand the complex network of cellular regulatory systems, it seems essential to include all layers of regulation in a systems biology approach, including, e.g., classical metabolic regulation together with more recent advances in cellular signaling cascades. It is clear, however, that further quantitative analyses of all these metabolic interactions in the network of substrate supply and utilization, by both experiments and mathematical modeling, are challenging and urgent tasks for molecular system bioenergetics in the future.

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