Published in final edited form as:

Wiley Interdiscip Rev Syst Biol Med. 2012 November ; 4(6): 599–613. doi:10.1002/wsbm.1188.

Mitochondrial network energetics in the heart

Miguel A. Aon and

Johns Hopkins University, Division of Cardiology, 720 Rutland Avenue, Ross Bldg. 1059, Baltimore, MD 21205 USA maon1@jhmi.edu

Sonia Cortassa

Johns Hopkins University, Division of Cardiology, 720 Rutland Avenue, Ross Bldg. 1059, Baltimore, MD 21205 USA

Abstract

At the core of eukaryotic aerobic life, mitochondria function like “hubs” in the web of energetic and redox processes in cells. In the heart, these networks - extending beyond the complex connectivity of biochemical circuit diagrams and apparent morphology - exhibit collective dynamics spanning several spatio-temporal levels of organization, from the cell, to the tissue, and the organ. The network function of mitochondria, i.e. *mitochondrial network energetics*, represents an advantageous behaviour. Its coordinated action, under normal physiology, provides robustness despite failure in a few nodes, and improves energy supply toward a swiftly changing demand. Extensive diffuse loops, encompassing mitochondrialcytoplasmic reaction/transport networks, control and regulate energy supply and demand in the heart. Under severe energy crises, the network behaviour of mitochondria and associated glycolytic and other metabolic networks collapse, thereby triggering fatal arrhythmias.

“...the study of complex systems should be the exclusive purview of no one but the responsibility of everyone: Each scientist, mathematician, or researcher unfurls the mysteries of nature and humankind in small, deliberate steps.”

Foote, R. 2007, quoted in Lloyd and Rossi, 2008.

In the midst of a scientific period that emphasizes integrative over molecular-based approaches, Systems Biology emerges as a discipline aiming at systems-level understanding of complex biological phenomena. Modern Systems Biology is deeply rooted in Integrative Physiology (1), from which it inherits its non-reductionist, systemic, traits. Systems Biology focuses on interrelationships among multiple rather than isolated components, and on collective instead of isolated processes behavior. In this historical context, and in parallel with several other disciplines, the emphasis in Medicine is shifting towards studying the *properties of networks*, and how these control the behavior of cells and organisms in health and disease (2-7).

Cardiovascular research has been amongst the fields to most rapidly adopt and develop the network approach (4, 7-11). A recent volume on heart function emphasizes the spatial and temporal organization of networks as mass-energy transducers and information (signaling) carriers (6, 12).

The single most distinguishing feature of complex behavior in systems is the appearance of emergent properties, which manifest themselves as novel, and unexpected, macroscopic spatio-temporal patterns (i.e. they cannot be anticipated from the behavior of the isolated components of such a system) (13-16). Emergence in complex systems arises from self-organizing principles resulting from nonlinear mechanisms and the continuous exchange of energy, matter, and information with the environment (13, 17-20). As such, emergent

complex behavior does not result from the existence of a central controller, and can be better described as heterarchical (15, 21) or control without hierarchy (22, 23). The multiple spatio-temporal layers exhibited by biological systems display scale-free dynamics allowing for simultaneous (sub)cellular, tissue, and organ modulation of timekeeping (13, 16, 24). Under crises, such as those arising as a consequence of a heart attack, catastrophic arrhythmias can happen as a result of the scaling properties, strong interconnectedness between levels of organization, and non-linear interactions among electrical, mechanical, and energetic, processes (14, 25-29).

1. NETWORKS EVERYWHERE

Biological networks have been characterized from four standpoints: *i*) architectural (structural morphology), *ii*) topological (connectivity properties), *iii*) dynamical (4) and *iv*) molecular interactions i.e. the network of interacting metabolites, proteins, and nucleic acids, levels (30).

From a biological perspective, the term network can refer to spatial (structural and topological) as well as temporal (different dynamics) aspects of function. In this wider sense, networks span all levels of organization, from the molecular, through the organellesubcellular, cellular, tissue, organ-organism, socio-economic, to the ecological (31, 32).

At the cellular level, *dynamic organization* (17) encompasses the architectural, topological, and molecular views of network analysis, accounting for both the autonomous dynamics exhibited by their components (nodes) and their defined interactions (connectivity), based on kinetic and thermodynamic principles (17-19). The collective dynamics exhibited by networks result in self-organized spatio-temporal behavior.

The networks approach was introduced in the realm of biochemistry by Metabolic Control Analysis (MCA). Developed in the second half of the past century, MCA represents an experimental approach with mathematical bases founded on the kinetics of enzymatic and transport networks in cells and tissues (33-35). MCA deals with networks of reactions of any topology and complexity to quantifying the control exerted by each process on systemic and local levels. As a quantitative methodological approach, MCA employs control, elasticity, and response coefficients.

Metabolic Flux Analysis (MFA), also called Flux Balance Analysis (FBA), represents another methodological approach to the study of reaction networks. Developed in the 1990s MFA is based on stoichiometric modeling and accounts for mass-energy relationships among metabolic network components (36). Different algorithmic tools have been developed over the years and applied to the understanding of metabolism in *S. cerevisiae* (37-39), *E. coli* (40, 41), plant (42) and mammalian cells (36, 43).

With respect to more traditional studies in metabolism, main contributions of MCA and MFA are their ability to handle entire metabolic pathways in a quantitative manner. Methodologically, MCA allows the quantification of control and regulation of metabolic and transport networks, whereas MFA offers a more rigorous assessment of the biochemical bases of physiological behavior in cells. MCA and MFA have pioneered the vision of networks as applied to metabolism in cellular and organ systems. They also paved the way to Systems Biology approaches from the beginning of the 21st century, when genomic information from multiple organisms became available (39).

2. MITOCHONDRIAL NETWORKS

At the convergence of most anabolic and catabolic pathways, mitochondria house core reactions of the metabolic “hub”, due to its multiple links to other pathways. The tricarboxylic acid cycle acts as either an input (source) or an output (sink) for metabolic components (4). In heart muscle, mitochondrial morphology appears as a network in the form of a regular lattice, spanning the whole myocardial syncytium like a power grid (3, 44, 45). In other cells like neurons or hepatocytes, more reticulate and, apparently, randomly organized mitochondrial networks exhibit striking morpho-dynamics driven by fusion-fission processes. This is an active research area where the impact on mitochondrial energetics and the corresponding consequences for cell physiology contributed by the balance between fusion and fission is being actively investigated (46, 47). Nonetheless, the mitochondrial network concept extends beyond the complex connectivity of biochemical circuit diagrams and the morphological appearance of the mitochondrial network.

In cardiac muscle, it was shown that mitochondria are dynamically organized as a network of coupled oscillators, which exhibits highly correlated, coordinated behavior. The collective mitochondrial oscillatory dynamics in key energetic and redox variables - $\Delta\psi_m$, NADH, and reactive oxygen species (ROS) - has been characterized as scale-free, i.e. it does not show a single characteristic but rather a multiplicity of periods spanning a wide range of frequencies of at least three orders of magnitude (from milliseconds to several minutes) (25, 48, 49). Nonlinear mechanisms govern the network dynamics, underlying stable, unstable, and oscillatory modes of mitochondrial bioenergetics, and its emergent macroscopic behavior as well (25). Recent evidence points to the fundamental importance of the complex spatiotemporal aspects of mitochondrial function to physiological signaling (48, 50-55), and the scaling of network instability (i.e. escalation of failures) to whole-cell and whole-organ (patho)physiological responses (56-62).

Complex nonlinear interactions between oxidative phosphorylation, ROS balance (production and scavenging) with the dynamics of inner membrane anion channels (28, 29, 63), and communication among mitochondria through ROS (48, 51), comprise the mechanistic core of the scale-free dynamics of mitochondrial energetics observed (64-66). It has been demonstrated that the crucial ROS signaling molecules and NADH also exhibit scale-free dynamics (49). Time-based synchronization and coordination of cellular networks of reactions appears to involve a mechanistic redox core whose rhythmic organization results in cycles of at least NAD(P)H, glutathione, ROS, and protein-thiols (24, 64-66). Mechanistically, we postulate that the scale-free dynamics observed arises from the ability of the redox core to produce oscillatory periods spanning from milliseconds to several minutes (64, 65). At this stage, a non-deterministic mechanism at the origin of scale-free dynamics cannot be ruled out (67). Rhythmic organization implicating redox balance appears to have been widely conserved in evolution from yeast to the mammalian heart (15, 64, 68).

2.1 Spatiotemporal organization in the heart

Functional integrity of the heart is dependent on bioenergetic, electrical, and mechanical components. The cardiac electrical activity recorded macroscopically in an electrocardiogram (ECG) during the heart contraction cycle constitutes the easiest observable output of the underlying sequence of electro-mechanical processes. The characteristics of a normal ECG and their relationship with each phase of the action potential (AP), ionic currents, ATP-consuming pumps, and mitochondrial energetic variables, are shown in Figure 1 during the AP. Using the ECME model, we calculated the time course of the state of electrical, mechanical, and energetic processes during a single beat of a cardiomyocyte (Fig. 1). This calculation reveals the multiple temporal and spatial

scales involved resulting from nested networks (networks within networks) of interacting dynamic systems (Fig. 2) (39, 69). Spatially, several compartments (sarcoplasmic reticulum, mitochondria, dyad, sarcolemma, bulk cytoplasm) are involved. Temporally, after voltage-gated Na^+ -channels in the sarcolemma are activated, the inward Na^+ -current induces a rapid depolarization of the cell membrane. This facilitates voltage-dependent opening of L-type Ca^{2+} -channels, and the resulting Ca^{2+} influx triggers the opening of the ryanodine receptor (RyR2 subtype), eliciting a vast release of Ca^{2+} from the sarcoplasmic reticulum (70, 71). Increased binding of cytosolic Ca^{2+} to troponin C of the myofilaments induces contraction of the cardiomyocyte. Figure 1 also shows that the electro-mechanical-energetic processes involved exhibit distinct relaxation times: mitochondrial energy drives the electrical and contractile machineries of the heart cell on a slower time scale (few seconds) compared with the electrical processes (milliseconds), which are followed by a calcium transient and an ATP-fueled mechanical cycle: increasing to a maximum (systole, which corresponds to the ejection of blood through aortic and pulmonary valves) and relaxation to a minimum (diastole, coinciding with passive refilling of the ventricles). During diastole, Ca^{2+} is either pumped back into the sarcoplasmic reticulum or transported out of the myocyte through the $\text{Na}^+ / \text{Ca}^{2+}$ exchanger.

The heart pumps roughly 75 gallons of blood per hour for about 100 years (61). Over 90% of heart metabolism is aerobic (72), and it accounts for nearly 10% of the O_2 consumption of the body at rest (73). Mitochondria provide the bulk of the ATP needed for cardiac muscle contraction (about two thirds) and sarcolemmal and sarcoplasmic ion transport (one third), responsible for the electrical activity of the cardiac cell (72). Thus, in the heart, oxidative phosphorylation represents the dominant source of energy for matching metabolic and contractile demand.

As the power grid of the heart, mitochondria constitute an extensive subcellular network within the myocardial syncytium (3). In electron micrographs, the mitochondrial network, which occupies ~30% of the heart cell volume, appears wrapped by the sarcoplasmic reticulum and in close vicinity with the myofilaments and t-tubules (74). At the level of the whole organism, the ATP turnover rate in an average person is estimated to be 9×10^{20} molecules per second (equating to a staggering 65 kg of ATP per day) (75), hence the compact, efficient and robust organization of the engine of oxygen delivery to all the tissues and organs of the human body. This may be realized through microdomains of compartmentalized Ca^{2+} release (76-81) and energy transfer (12, 82). During maximal workload, the whole ATP pool in the heart is turned over in a few seconds while ~2% of that pool is consumed in each heart beat (83). From a temporal perspective, these numbers underscore the highly tuned response in energy supply by the mitochondrial network for the energy demand of electro-mechanical events operating in the millisecond range (e.g. AP, calcium transients) (see Fig. 1).

The tight match between energy supply and demand in the heart can be more readily fulfilled by the highly synchronized and robust action of mitochondrial networks. The changing metabolic demand as workload requires both constancy and flexibility in mitochondrial functionality by, first, providing a steady supply of ATP to drive contraction, and second, to be able to adapt the rate of energy provision to energy demands.

3. NORMAL PHYSIOLOGY

3.1 Control and regulation of cardiomyocyte function

Because mitochondria as the main energy suppliers both modulate, and are in turn themselves modulated by the network of mechanical-electrical processes, the whole system becomes extremely complex: the resulting heterarchy is shown (Fig. 2).

Traditionally, the major processes (e.g., mitochondrial energetics, electrophysiology) have been studied in isolation. Consequently, it has been difficult to examine, in an intact system, the overall control of mitochondrial respiration; for instance, to investigate if the mechanisms controlling mitochondrial energetics differ depending on whether the muscle is at rest or at work. It is only recently that we have been able to quantify, for the first time, the control and regulation of mitochondrial respiration in the ECME model, a computational model integrating both excitation-contraction (EC) coupling and mitochondrial energetics (ME) (84, 85). We utilized a generalized matrix method (86) of metabolic control analysis (34, 35) to calculate the structure of control and regulation of the integrated metabolic and transport network of the cardiomyocyte (84) and isolated mitochondria (85).

The calculations with the ECME model were performed under resting and working conditions, when the contractile force is close to its maximum, and the energy-consuming pumps are nearly at maximal work during the contraction cycle. Although this procedure is rather artificial for a continuous beating heart, this is precisely the usefulness and advantage of a computational model; i.e. it allows you to gain insights into complex processes.

The significance of the results so obtained is validated by the ability of the ECME model to simulate: *i*) oscillations in mitochondrial membrane potential, NADH, glutathione, and ROS (63, 87), *ii*) the dynamics of mitochondrial NADH, Ca^{2+} , and ADP during changes in supply and demand in the heart (26, 88), and *iii*) the dynamics of the sarcolemmal membrane potential during mitochondrial oscillations in whole hearts undergoing arrhythmias (3, 28, 29, 56, 87, 89). Some of the main lessons learned during this modeling procedure are worth highlighting.

Firstly, although a large portion of the control of respiration was shared among several mitochondrial processes, not all the control of mitochondrial function resides within mitochondria. Quantitatively, the most rate-controlling steps were the rates of respiration itself and of ATP synthase and their associated rates of proton pumping through the respiratory and ATP synthase complexes, and the adenine nucleotide translocator. This control pattern was mostly due to the control exerted by those processes on the mitochondrial membrane potential ($\Delta\psi_m$) which is a main effector of the respiratory flux. However, under resting or working conditions, the control of respiration is also exerted by cytoplasmic and sarcolemmal membrane-linked processes, e.g. the myofibrillar and Na/K ATPases. This is especially true under working conditions, when the interaction between cytoplasmic and mitochondrial processes is quantitatively more important. A conspicuous example is the control over respiration exerted by the myofibrillar ATPase, whose degree of share of control appears to be significant only during working conditions. This result underscores the demand-led control of mitochondrial respiration when the energy supply is maximally required.

Similarly, the control of the ATP synthesis flux not only resides in the mitochondrion, but also extends to cytoplasmic processes. In this case, a rather surprising result emerged from our calculations. Counter intuitively, the myofibrillar ATPase controlled negatively the rate of mitochondrial ATP synthesis when one expects rather the contrary, i.e. that an ATP-consuming reaction controls positively ATP synthesis: the higher the enzyme activity the higher the stimulus for energy provision. The interpretation of this unexpected result rendered the discovery of a new concept: *control by diffuse loops*. We defined “control by diffuse loops” as the control that a process A exerts over process C or D without an apparent direct mechanistic link between them (84). In a diffuse control loop there is at least one intermediary process between A and C. Applied to the case of myofibrillar ATPase (AMATPase), the control through a diffuse loop will proceed as follows: a decrease in cytoplasmic ATP, ATPi, brought about by an increase in AM-ATPase, will in turn cause a

decrease in the activity of the SERCA pump (negatively controlled by AM-ATPase). Thus, AM-ATPase has a positive control on the concentration of cytoplasmic Ca^{2+} that increases as a result of the decrease in SERCA activity. The diffuse control loop continues as an increase of Ca^{2+} uniporter flux, transporting more Ca^{2+} into the mitochondria (also reflected by the large positive control of AM-ATPase on the uniporter). The effect of the Ca^{2+} uniporter to dissipate $\Delta\psi_m$ overrides the small positive control of the adenine nucleotide translocator brought about by the increase in ADP produced by the AM-ATPase. The net effect is a decrease of flux through the mitochondrial ATP synthase (84).

The uncovering of the existence of control by diffuse loops throws new light into the understanding of the secondary effects of pharmacological agents. The action of these agents on a complex network of reactions brings about changes in processes without direct mechanistic links between them. Figure 4 describes several examples of diffuse loops exhibiting different lengths as given by the number of intermediary steps involved.

3.2 Metabolic control of energy supply and demand in the heart

Understanding the mechanisms underlying the matching between energy supply and demand in cardiac or skeletal muscle has been a quest for more than four decades, and is still controversial (24, 85, 90, 91). The classical “respiratory control” hypothesis postulates that ADP is the intermediary signaling mitochondria to increase energy supply in response to higher energy demand. Later, the regulatory action of Ca^{2+} in modulating mitochondrial energy supply through activation of the dehydrogenases (isocitrate- and α -ketoglutarate) from the tricarboxylic acid cycle was discovered (92, 93). Following the recognition of the apparent constancy of adenine nucleotides in the heart (82, 94, 95), the existence of a “mitochondrial interactosome” (MI) was postulated (82). MI is composed by ATP synthase, adenine nucleotide translocase, phosphate carrier, mitochondrial creatine kinase and the voltage dependent anion channel (VDAC) located in the outer membrane in interaction with β -tubulin. Experimental support for the existence of an MI was provided by the work of Tepp et al. (2011) (96). Metabolic channeling at the level of the respiratory chain was found when control analysis was applied under conditions in which the organization of the internal milieu is preserved, such as in permeabilized cardiomyocytes. When respiration was activated with substrates of the creatine kinase system (MgATP, creatine, although not with ADP), metabolic channeling happened as detected by flux control coefficients larger than one (96). Summation of flux control coefficients larger than one is expected under channeling of substrates through organized macromolecular complexes (97, 98) lending support, although indirect, to the existence of an MI.

In vivo control analysis, using nuclear magnetic resonance, as applied to cardiac muscle contraction in response to hypoxia or various pharmacological agents has also been used (99). The strength of this approach resides in the non-invasive *in vivo* quantitation of energy metabolism under fully functional conditions. The study of the regulation of internal energy metabolism by the processes participating in either energy supply or consumption was carried out with hearts of mice adapted to conditions of hypoxia (chronic hypoxia) or controls subjected to partial ischemic conditions. The magnitude of the elasticity coefficient for phosphocreatine increased with chronic hypoxia leading to a decrease in the control coefficients for energy supply in contrast to controls in which reduced oxygen availability revealed large control coefficients for energy supply. These results may be interpreted as chronic hypoxia resulting in increased oxidative phosphorylation enabling improved performance under high energy demand (100).

The quantitative work performed with permeabilized cells or muscle preparations provided *in situ* or *in vivo* information, respectively, concerning the control of oxidative

phosphorylation under conditions that preserve spatial organization. In both cases, under fully integrated functional conditions, new mechanisms and concepts could be unveiled.

3.3 Control by diffuse loops in the heart: effects of *Digitalis*, a case study

The stimulation of contractile activity (inotropic) action of the therapeutic glycoside *Digitalis* has been extensively studied, being well understood at present (101). The importance of understanding the mechanism of action of *Digitalis* is the insight that it may provide concerning the consequences of disease processes altering ion distribution across the heart cell membrane. From our perspective, which focuses on the control and regulation of cardiomyocyte function, the mechanism of action of *Digitalis* serves a double purpose: *i*) as an example of a diffuse loop elicited from inhibition of the sarcolemmal NaK ATPase (NKA) and resulting in a positive control of contraction in the heart (Fig. 4), and *ii*) as a validation test of the structure of control of the ECME model.

Mechanistically speaking, the glycoside *Digitalis* binds to and inhibits the NKA resulting in an increase in cellular Ca^{2+} which is responsible for its positive inotropic action and its toxicity as well. According to the calculations performed on the overall cardiomyocyte function, the positive and negative signs in the diffuse loop shown in Figures 3 and 4 represent the type of control (positive or negative) that each edge (e.g. NKA) in the network has on other nodes or edges in the loop (an enzyme or channel represents an edge linking at least two nodes, i.e. substrate and product of the enzymatic reaction, or ion_{in} and ion_{out} in the case of a channel). A positive control means that, e.g. an increase in the activity of edge A controlling edge or node B will augment the activity of the latter, whereas negative control means that an increase in edge A will decrease the activity of edge or node B. For instance, since NKA has a negative control on cytoplasmic calcium, $\text{Ca}^{2+}_{\text{i}}$, inhibiting the pump will result in a positive control over $\text{Ca}^{2+}_{\text{i}}$ that, consequently, will increase. In turn, the increase in $\text{Ca}^{2+}_{\text{i}}$ level up regulates the activity of sarcoplasmic reticulum ATPase (SERCA) (due to higher response coefficient toward $\text{Ca}^{2+}_{\text{i}}$ by the enzyme) which exerts a positive control over contractility (compare loops in Figs. 3, bottom, and Fig. 4, top). Overall, then, the positive inotropic effect of *Digitalis* is recapitulated by the structure of control exhibited by the computational model.

Additionally, the “ Na^{+} pump lag” theory proposes that once the glycoside has bound to and inhibited NKA, intracellular Na^{+} (Na^{+}_{i}) increases in proportion to the new balance between influx and diminished extrusion, such that the accumulation of Na^{+} causes a secondary increase in Ca^{2+} via $\text{Na}^{+}/\text{Ca}^{2+}_{\text{i}}$ exchanger (NCX). When Na^{+}_{i} is increased during NKA inhibition, Ca^{2+} efflux is diminished even at resting potential where forward-mode exchange would ordinarily remove the Ca^{2+} that entered during systole ((101), and refs. therein). In addition, Ca^{2+} influx via the exchanger in the reverse mode at potentials positive to NCX is actually promoted by the same increase in Na^{+}_{i} resulting from pump inhibition thus causing positive inotropy and the development of SR Ca^{2+} overload toxicity (102). Presumably, toxicity develops and a negative inotropic effect occurs as the capacity of SR Ca^{2+} storage is exceeded following excessive pump inhibition and Na^{+} accumulation, leading to delayed after depolarization and arrhythmias (101, 103, 104).

The structure of control of our computational model shows that inhibiting NKA results in an increase of both cytoplasmic ATP, ATP_{i} , and $\text{Ca}^{2+}_{\text{i}}$, which exert negative and positive regulation (response coefficients), respectively, on mitochondrial respiration. According to our calculations, ATP_{i} appears as a major regulator of mitochondrial respiration in cardiomyocytes under working conditions, when compared with ADP and Ca^{2+} (84). Quantitatively, this result can be explained by the large elasticity that the ANT displays toward ATP_{i} , consistent with its role as the mediator of ADP/ATP exchange between mitochondria and the cytoplasm (105).

The open question is: during NKA inhibition, that controls respiration through an extensive diffuse loop, increasing both ATP_i and Ca^{2+}_i , which one predominates, the negative effect of ATP_i or the positive one by Ca^{2+}_i ? (Fig. 4). The answer to this important question is possibly due to the O_2 consumption that can be measured coupled to NKA inhibition, which is an important ATP consumer. In fact, ouabain, a widely used cardiotonic steroid to inhibit NKA, has been shown to cause secondary effects on cellular O_2 consumption. This effect has been used to estimate the contribution of NKA to ATP consumption in a large range of tissues (73, 106). In the heart, addition of cardiac glycosides to block NKA have repeatedly been found to stimulate respiration, an effect that could not be only accounted for by an increase in contractility but may well possibly be due to the increase in Ca^{2+}_i elicited by the activation of a diffuse loop.

Interestingly, very recent data confirm the existence of the extensive diffuse loop schematized in Figure 4. NKA inhibition with ouabain, in the presence of 100 nM isoproterenol, increased Ca^{2+}_i and Na^+_i but decreased mitochondrial Ca^{2+} , Ca^{2+}_m , which was associated with net mitochondrial NADH oxidation (107). Consistent with its positive inotropic effects, application of ouabain led to an increase of left ventricular developed pressure, and the rates of contraction and relaxation were also improved. Associated with the increased cardiac function, cardiac O_2 consumption augmented upon administration of ouabain and isoproterenol. After 10-min treatment of ouabain, whole-heart O_2 consumption increased by 18% and isoproterenol further increased O_2 consumption 25% above baseline (107). In the same work, the authors showed that the impairment in mitochondrial NADH production induced by ouabain in isolated myocytes, as a consequence of cytoplasmic Na^+ loading, could be prevented by an inhibitor of the mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchanger. Thus the increase in respiration observed would suggest that the positive response of respiration to Ca^{2+} predominated rather than the negative one produced by ATP_i (Fig. 4). However, the existence of control by diffuse loops in the heart, suggested by our calculations and the data reported, preclude a definitive answer to the question about the response of mitochondrial respiration to Ca^{2+} and ATP_i when NKA is inhibited (Fig. 4). On the one hand, the ATP levels were not measured (107), and on the other hand, the effects of Ca^{2+} are difficult to disentangle from ATP_i in the presence of diffuse loops, since the increase in AM-ATPase activity on activation of contractility decreases ATP_i exerting a negative control on SERCA thus increasing cytoplasmic Ca^{2+} (see Fig. 4 and section 3.1 above).

4. PATHOPHYSIOLOGICAL BEHAVIOR

4.1 Escalation of failures across multiple spatiotemporal scales under critical conditions

In normal health, biochemical and physiological functions exhibit a delicate balance that under stress is unleashed and becomes manifest as a crisis. When stressed, many complex systems become extremely sensitive to small perturbations exhibiting an exceptionally large susceptibility to external factors. Under these conditions, strong correlation among different parts of the system becomes apparent (51, 108), and novel macroscopic behavior emerges as has been shown in physical, social, financial and biological networks (31, 32, 51, 109).

Existing and emerging theoretical and experimental evidence suggest that the global response of cells appears to depend on the coordinated action of thousands of mitochondria arranged in regular lattices as in the heart (2, 4). The network organization of mitochondria in cardiomyocytes represents the most highly organized example (25, 52). The reticular, random-like, networks seen in yeast may perform in a similar manner (4, 49, 52, 65, 66). The idea that mitochondria may function as a coordinated network of oscillators emerged from studies in living cardiomyocytes subjected to metabolic stress (25, 87, 110). The network behavior of mitochondria depends on local as well as global coordination in the cell (51), and ROS-induced ROS release is responsible (3, 28, 111, 112).

The scaling of instability of the mitochondrial network to the whole-cell and whole-organ levels has been shown to underlie the electrophysiological and contractile dysfunction associated with cardiac disease (3, 56, 58, 59, 87). The initial collapse of the mitochondrial network $\Delta\psi_m$ initiates a cascade of events including activation of sarcolemmal K_{ATP} channels, alteration of the electrical excitability of the cardiomyocyte, and ultimately cardiac arrhythmias. This sequence of events has been inferred from experiments and simulated by the ECME model (26). Further development of this model accounts both for mitochondrial RIRR, and also the link between the mitochondrial energy state and electrical excitability mediated by the sarcolemmal K_{ATP} current (ECME-RIRR model) (29). Whole-cell model simulations performed with the ECME-RIRR model demonstrated that increasing the fraction of electron transport diverted from the respiratory chain to ROS production triggers limit-cycle oscillations of mitochondrial membrane potential, $\Delta\psi_m$, redox status (NADH, GSH), and mitochondrial respiration through the activation of the ROS-sensitive inner membrane anion channel (IMAC) (29, 113). Oscillatory changes in cytosolic ATP concentration, sarcolemmal K_{ATP} current, and action potential duration (APD) were initiated by increasing oxidative stress from the mitochondria. The decrease in the ATP/ADP ratio (sustained by a relatively larger increase in ADP and a modest decrease in ATP) produced the expected activation of the K_{ATP} current during $\Delta\psi_m$ depolarization, causing a significant shortening of the cardiac APD (29). Further APD shortening to the point where the cell becomes electrical inexcitable, was obtained with increase of the K_{ATP} current, and the K_{ATP} channel density in the sarcolemmal membrane.

Studies performed in isolated cardiomyocytes showed that the collapse of $\Delta\psi_m$, elicited by oxidative stress, is followed by sustained, low frequency, high amplitude, oscillations of $\Delta\psi_m$, and mitochondrial redox status (NADH, ROS, GSH). The abrupt collapse in $\Delta\psi_m$ was preceded by the gradual increase of ROS in the network, more specifically, when 60% of the mitochondria accumulated ROS to a threshold level. We referred to the state of the mitochondrial network just before depolarization as the point of *mitochondrial criticality* (50, 51). In the critical state, a small perturbation anywhere in the network can lead to the propagation of a $\Delta\psi_m$ depolarization wave (59, 114). Recently, arrhythmias mechanistically mediated by ROS-induced ROS release were demonstrated in intact hearts subjected to oxidative stress by acute addition of high H_2O_2 concentrations (115).

Recent computational and experimental studies further support the interpretation that ROS, and more specifically the superoxide anion, propagates by reaction-diffusion among mitochondria in the network, giving rise to the observed emergent macroscopic properties such as widespread $\Delta\psi_m$ depolarization and ROS waves. The implementation of the mitochondrial oscillator computational model into Reaction-Diffusion (RD) in one- and two-dimensional mitochondrial network models (28), allowed the first mechanistic assessment of RIRR. In a 2D network composed of 500 mitochondria, model simulations revealed $\Delta\psi_m$ depolarization waves similar to those observed when isolated guinea pig cardiomyocytes are subjected to a localized laser flash (51, 87), antioxidant depletion (25, 63, 114). The sensitivity of the propagation rate of the depolarization wave to $O_2^{\cdot-}$ diffusion, production, and scavenging in the RD-RIRR model is similar to that observed experimentally. These results indicate that local gradients of cytoplasmic $O_2^{\cdot-}$, determined by diffusion and scavenger capacity, play a significant role in the rate of propagation of the $\Delta\psi_m$ depolarization and repolarization waves (28). The results obtained with the RD-RIRR model illustrate how local neighbor-neighbor interactions (1–2 μm distance) can lead to long distance (> 100 μm in cells, and > 4000 μm in whole hearts) spatiotemporal patterns in cells (51, 87, 116) and in whole hearts (3, 59, 62, 89, 117).

CONCLUSIONS AND A PROSPECTIVE FOR FUTURE STUDIES

The emerging field of Systems Biology reflects the major shift between the *analytical* and *integrative* periods that Biology, as a discipline, is undergoing. This major transition, as did many other examples that took place at the turn of centuries, represents a hinge in the accelerating evolution and breadth of the scientific enterprise. The field of cardiac physiology reflects this transition, and as a result witnesses the blooming of new topics. An emerging central theme is the approach to the complexities of heart physiology through the study of the properties of networks. Central to this approach is the utilization of a combined experimental-theoretical strategy, which triggers an iterative, mutually beneficial, verification-validation loop of both experiment and model.

The ability to simulate multiple complex physiological responses simultaneously in cardiomyocyte function, at several temporal and spatial scales (26, 29, 118), in conjunction with high throughput technologies, has a great future for its potential ability to produce unprecedented insights into the inner working mechanisms of heart physiology, under health and disease. Profound insights into the rational design of new therapeutic approaches, is coming from the utilization of quantitative computational tools, such as metabolic control analysis, to calculate the basic control and regulatory properties of extended networks. Frequently, these networks comprise simultaneously occurring processes of differing nature, i.e. metabolic, transport, electro-mechanical, and cardiobiology provides a prime example of the successful utilization of computational models. Affecting these complex networks with pharmacological agents, such as the case of cardiotonic glycosides discussed in this work, triggers diffuse control loops with consequences that go beyond the expected targets, i.e. so-called “secondary” or “collateral” effects. These considerations suggest that we will need extreme caution and courage to accept that our interventions, targeted as they are, to some specific nodes of complex reaction networks, may well go beyond expectations. A major reason for caution comes from the fact, widely demonstrated in metabolic systems, that *control is delocalized* (i.e. distributed throughout several nodes) instead of concentrated at bottlenecks (i.e. in major limiting steps) (33).

While under normal physiological conditions the availability of energy is fine tuned to match changes in energy demand, under stress this is not the case. The power grid of the heart, represented by mitochondrial network energetics, along with its remarkable non-linear properties together with the essentially non-linear performance of the whole organ, set the stage for the appearance of critical phenomena and bifurcations leading to self-organized, emergent, behavior. An amazing example of the latter is given by the existence, at bifurcation points (i.e. mitochondrial criticality) (51), of emergent macroscopic self-organized behavior escalating from the subcellular to the whole heart, eventually leading to the death of the organism. The demonstration of the involvement of mitochondrial oscillations in reperfusion-related arrhythmias after ischemic injury (56, 62), and of their reversion with 4-chlorodiazepam, a pharmacological agent that antagonizes an inner membrane mitochondrial channel, thereby blunting oscillations, and stabilizing the action potential in several experimental animals (56, 58, 59) are strong proof of the involvement of mitochondrial network energetics. Under metabolically stressful conditions such as those encountered during a heart attack, these crucial pieces of evidence together, highlight the key role of arbiter of life and death played by the mitochondrial function.

Acknowledgments

This work was supported by NIH grants R01-HL091923-01 and R21HL106054.

References

1. Noble, D.; Boyd, CAR. The challenge of integrative physiology.. In: Boyd CARaN, D., editor. *The Logic of Life The Challenge of Integrative Physiology*. Oxford University Press; New York: 1993. p. 1-13.
2. Aon MA. From isolated to networked: a paradigmatic shift in mitochondrial physiology. *Frontiers in Physiology*. 2010; 1(20) doi: 10.3389/fphys.2010.00020.
3. Aon MA, Cortassa S, Akar FG, Brown DA, Zhou L, O'Rourke B. From mitochondrial dynamics to arrhythmias. *The international journal of biochemistry & cell biology*. 2009; 41(10):1940–1948.
4. Aon, MA.; Cortassa, S.; O'Rourke, B. *On the network properties of mitochondria*. Wiley-VCH2007a;
5. Barabasi AL, Oltvai ZN. Network biology: understanding the cell's functional organization. *Nat Rev Genet*. 2004; 5(2):101–113. [PubMed: 14735121]
6. Saks, V. Introduction: From the discovery of biological oxidation to Molecular System Bioenergetics.. In: Saks, V., editor. *Molecular System Bioenergetics Energy for Life*. Wiley-VCH, Verlag GmbH & Co. KGaA; Weinheim: 2007. p. 1-8.
7. Weiss JN, Yang L, Qu Z. Systems biology approaches to metabolic and cardiovascular disorders: network perspectives of cardiovascular metabolism. *Journal of lipid research*. 2006; 47(11):2355–2366. [PubMed: 16946414]
8. Dzeja PP, Hoyer K, Tian R, Zhang S, Nemutlu E, et al. Rearrangement of energetic and substrate utilization networks compensate for chronic myocardial creatine kinase deficiency. *The Journal of physiology*. 2011; 589(Pt 21):5193–5211. [PubMed: 21878522]
9. Dzeja PP, Terzic A. Phosphotransfer networks and cellular energetics. *J Exp Biol*. 2003; 206(Pt 12): 2039–2047. [PubMed: 12756286]
10. Ingwall JS. Transgenesis and cardiac energetics: new insights into cardiac metabolism. *Journal of molecular and cellular cardiology*. 2004; 37(3):613–623. [PubMed: 15350834]
11. Lusis AJ, Weiss JN. Cardiovascular networks: systems-based approaches to cardiovascular disease. *Circulation*. 2010; 121(1):157–170. [PubMed: 20048233]
12. Saks, V.; Monge, C.; Anmann, T.; Dzeja, PP. Integrated and organized cellular energetic systems: Theories of cell energetics, compartmentation, and metabolic channeling.. In: Saks, V., editor. *Molecular System Bioenergetics: Energy for Life*. Wiley-VCH Verlag GmbH & Co. KGaA; Weinheim: 2007. p. 59-109.
13. Aon, MA.; Cortassa, S. Chaotic dynamics, noise and fractal space in biochemistry.. In: Meyers, R., editor. *Encyclopedia of Complexity and Systems Science*. Springer; New York: 2009.
14. Aon, MA.; Cortassa, S.; Lloyd, D. Chaos in Biochemistry and Physiology.. In: Meyers, R., editor. *Encyclopedia of Molecular Cell Biology and Molecular Medicine: Systems Biology*. Wiley-VCH; Weinheim: 2011.
15. Lloyd, D.; Rossi, EL. Epilogue: A new vision of life.. In: Lloyd DaR, EL., editor. *Ultradian Rhythms from Molecules to Mind*. Springer Science+Business Media B.V.; New York: 2008. p. 431-439.
16. Qu Z, Garfinkel A, Weiss JN, Nivala M. Multi-scale modeling in biology: how to bridge the gaps between scales? *Prog Biophys Mol Biol*. 2011; 107(1):21–31. [PubMed: 21704063]
17. Aon, MA.; Cortassa, S. *Fundamentals as applied to cellular systems*. Chapman & Hall; London: 1997. *Dynamic biological organization*..
18. Lloyd D, Aon MA, Cortassa S. Why homeodynamics, not homeostasis? *ScientificWorldJournal*. 2001; 1:133–145. [PubMed: 12805697]
19. Nicolis, G.; Prigogine, I. *Self-organization in nonequilibrium systems : from dissipative structures to order through fluctuations*. Wiley; New York: 1977.
20. Yang JH, Yang L, Qu Z, Weiss JN. Glycolytic oscillations in isolated rabbit ventricular myocytes. *The Journal of biological chemistry*. 2008; 283(52):36321–36327. [PubMed: 18948270]
21. Yates, FE. *Self-Organizing Systems*.. In: Boyd, CAR.; Noble, D., editors. *The Logic of Life The Challenge of Integrative Physiology*. Oxford University Press; New York: 1993. p. 189-218.
22. Fox Keller, E. *The Century of the Gene*. Harvard University Press; Cambridge, USA: 2000.

23. Gordon DM. Control without hierarchy. *Nature*. 2007; 446(8):143–144. [PubMed: 17344838]
24. Sasidharan K, Tomita M, Aon M, Lloyd D, Murray DB. Time-structure of the yeast metabolism in vivo. *Advances in experimental medicine and biology*. 2012; 736:359–379. [PubMed: 22161340]
25. Aon MA, Cortassa S, O'Rourke B. Mitochondrial oscillations in physiology and pathophysiology. *Advances in experimental medicine and biology*. 2008b; 641:98–117. [PubMed: 18783175]
26. Cortassa S, Aon MA, O'Rourke B, Jacques R, Tseng HJ, et al. A computational model integrating electrophysiology, contraction, and mitochondrial bioenergetics in the ventricular myocyte. *Biophysical journal*. 2006; 91(4):1564–1589. [PubMed: 16679365]
27. Ivanov PC, Nunes Amaral LA, Goldberger AL, Havlin S, Rosenblum MG, et al. From 1/f noise to multifractal cascades in heartbeat dynamics. *Chaos*. 2001; 11(3):641–652. [PubMed: 12779503]
28. Zhou L, Aon MA, Almas T, Cortassa S, Winslow RL, O'Rourke B. A Reaction-Diffusion Model of ROS-Induced ROS Release in a Mitochondrial Network. *PLoS computational biology*. 2010; 6(1):e1000657. [PubMed: 20126535]
29. Zhou L, Cortassa S, Wei AC, Aon MA, Winslow RL, O'Rourke B. Modeling cardiac action potential shortening driven by oxidative stress-induced mitochondrial oscillations in guinea pig cardiomyocytes. *Biophysical journal*. 2009; 97(7):1843–1852. [PubMed: 19804714]
30. Xia Y, Yu H, Jansen R, Sringhaus M, Baxter S, et al. Analyzing cellular biochemistry in terms of molecular networks. *Annual review of biochemistry*. 2004; 73:1051–1087.
31. Barabasi AL. Scale-free networks: a decade and beyond. *Science (New York, NY)*. 2009; 325(5939):412–413.
32. Bascompte J. Disentangling the web of life. *Science (New York, NY)*. 2009; 325(5939):416–419.
33. Fell DA. Metabolic control analysis: a survey of its theoretical and experimental development. *The Biochemical journal*. 1992; 286(Pt 2):313–330. [PubMed: 1530563]
34. Heinrich R, Rapoport TA. A linear steady-state treatment of enzymatic chains. General properties, control and effector strength. *European journal of biochemistry / FEBS*. 1974; 42(1):89–95. [PubMed: 4830198]
35. Kacser H, Burns JA. The control of flux. *Symposia of the Society for Experimental Biology*. 1973; 27:65–104. [PubMed: 4148886]
36. Savinell JM, Palsson BO. Optimal selection of metabolic fluxes for in vivo measurement. II. Application to *Escherichia coli* and hybridoma cell metabolism. *J Theor Biol*. 1992; 155(2):215–242. [PubMed: 1453698]
37. Aon JC, Cortassa S. Involvement of nitrogen metabolism in the triggering of ethanol fermentation in aerobic chemostat cultures of *Saccharomyces cerevisiae*. *Metab Eng*. 2001; 3(3):250–264. [PubMed: 11461147]
38. Cortassa S, Aon JC, Aon MA. Fluxes of carbon, phosphorylation, and redox intermediates during growth of *saccharomyces cerevisiae* on different carbon sources. *Biotechnol Bioeng*. 1995; 47(2):193–208. [PubMed: 18623393]
39. Cortassa, S.; Aon, MA.; Iglesias, AA.; Aon, JC.; Lloyd, D. An introduction to Metabolic and Cellular Engineering. 2nd Ed.. World Scientific; Singapore: 2012.
40. Palsson, BO. Properties of reconstructed networks. Cambridge University Press; New York: 2009. Systems Biology..
41. Varma A, Palsson BO. Stoichiometric flux balance models quantitatively predict growth and metabolic by-product secretion in wild-type *Escherichia coli* W3110. *Appl Environ Microbiol*. 1994; 60(10):3724–3731. [PubMed: 7986045]
42. Boyle NR, Morgan JA. Flux balance analysis of primary metabolism in *Chlamydomonas reinhardtii*. *BMC Syst Biol*. 2009; 3:4. [PubMed: 19128495]
43. Zupke C, Stephanopoulos G. Intracellular flux analysis in hybridomas using mass balances and in vitro (13)C nmr. *Biotechnol Bioeng*. 1995; 45(4):292–303. [PubMed: 18623182]
44. Amchenkova AA, Bakeeva LE, Chentsov YS, Skulachev VP, Zorov DB. Coupling membranes as energy-transmitting cables. I. Filamentous mitochondria in fibroblasts and mitochondrial clusters in cardiomyocytes. *J Cell Biol*. 1988; 107(2):481–495. [PubMed: 3417757]
45. Saks VA, Kaambre T, Sikk P, Eimre M, Orlova E, et al. Intracellular energetic units in red muscle cells. *The Biochemical journal*. 2001; 356(Pt 2):643–657. [PubMed: 11368796]

46. Jezek P, Plecita-Hlavata L. Mitochondrial reticulum network dynamics in relation to oxidative stress, redox regulation, and hypoxia. *The international journal of biochemistry & cell biology*. 2009; 41(10):1790–1804.
47. Kuznetsov AV, Hermann M, Saks V, Hengster P, Margreiter R. The cell-type specificity of mitochondrial dynamics. *The international journal of biochemistry & cell biology*. 2009; 41(10): 1928–1939.
48. Aon MA, Cortassa S, O'Rourke B. The fundamental organization of cardiac mitochondria as a network of coupled oscillators. *Biophysical journal*. 2006b; 91(11):4317–4327. [PubMed: 16980364]
49. Aon, MA.; Cortassa, S.; O'Rourke, B. Is there a mitochondrial clock?. In: Lloyd, D.; Rossi, EL., editors. *Uladian Rhythms from Molecules to Mind A New Vision of Life*. Springer-Verlag; New York: 2008c. p. 129-144.
50. Aon MA, Cortassa S, Akar FG, O'Rourke B. Mitochondrial criticality: a new concept at the turning point of life or death. *Biochimica et biophysica acta*. 2006a; 1762(2):232–240. [PubMed: 16242921]
51. Aon MA, Cortassa S, O'Rourke B. Percolation and criticality in a mitochondrial network. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101(13): 4447–4452. [PubMed: 15070738]
52. Beraud N, Pelloux S, Usson Y, Kuznetsov AV, Ronot X, et al. Mitochondrial dynamics in heart cells: very low amplitude high frequency fluctuations in adult cardiomyocytes and flow motion in non beating HL-1 cells. *Journal of bioenergetics and biomembranes*. 2009; 41(2):195–214. [PubMed: 19399598]
53. Dzeja, PP.; Chung, S.; Terzic, A. Integration of adenylate kinase and glycolytic and glycogenolytic circuits in cellular energetics.. In: Saks, V., editor. *Molecular System Bioenergetics: Energy for Life*. Wiley-VCH Verlag GmbH & Co. KGaA; Weinheim: 2007. p. 265-301.
54. Kurz FT, Aon MA, O'Rourke B, Armondas AA. Spatio-temporal oscillations of individual mitochondria in cardiac myocytes reveal modulation of synchronized mitochondrial clusters. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107(32): 14315–14320. [PubMed: 20656937]
55. Wallimann, T.; Tokarska-Schlattner, M.; Neumann, D.; Epand, RM.; Epand, RF., et al. The phosphocreatine circuit: Molecular and cellular physiology of creatine kinases, sensitivity to free radicals, and enhancement by creatine supplementation.. In: Saks, V., editor. *Molecular System Bioenergetics: Energy for Life*. Wiley-VCH Verlag GmbH & Co. KGaA; Weinheim: 2007. p. 195-264.
56. Akar FG, Aon MA, Tomaselli GF, O'Rourke B. The mitochondrial origin of postischemic arrhythmias. *The Journal of clinical investigation*. 2005; 115(12):3527–3535. [PubMed: 16284648]
57. Akar FG, O'Rourke B. Mitochondria are sources of metabolic sink and arrhythmias. *Pharmacology & therapeutics*. 2011; 131(3):287–294. [PubMed: 21513732]
58. Brown DA, Aon MA, Akar FG, Liu T, Sorrairain N, O'Rourke B. Effects of 4'-chlorodiazepam on cellular excitation-contraction coupling and ischaemia-reperfusion injury in rabbit heart. *Cardiovascular research*. 2008; 79(1):141–149. [PubMed: 18304929]
59. Brown DA, Aon MA, Frasier CR, Sloan RC, Maloney AH, et al. Cardiac arrhythmias induced by glutathione oxidation can be inhibited by preventing mitochondrial depolarization. *Journal of molecular and cellular cardiology*. 2010; 48(4):673–679. [PubMed: 19962380]
60. Brown DA, O'Rourke B. Cardiac mitochondria and arrhythmias. *Cardiovascular research*. 2010; 88(2):241–249. [PubMed: 20621924]
61. O'Rourke B, Cortassa S, Aon MA. Mitochondrial ion channels: gatekeepers of life and death. *Physiology (Bethesda, Md)*. 2005; 20:303–315.
62. Slodzinski MK, Aon MA, O'Rourke B. Glutathione oxidation as a trigger of mitochondrial depolarization and oscillation in intact hearts. *Journal of molecular and cellular cardiology*. 2008; 45(5):650–660. [PubMed: 18760283]
63. Cortassa S, Aon MA, Winslow RL, O'Rourke B. A mitochondrial oscillator dependent on reactive oxygen species. *Biophysical journal*. 2004; 87(3):2060–2073. [PubMed: 15345581]

64. Aon MA, Roussel MR, Cortassa S, O'Rourke B, Murray DB, et al. The scale-free dynamics of eukaryotic cells. *PLoS ONE*. 2008a; 3(11):e3624. [PubMed: 18982073]
65. Lloyd D, Cortassa S, O'Rourke B, Aon MA. What yeast and cardiomyocytes share: ultradian oscillatory redox mechanisms of cellular coherence and survival. *Integr Biol (Camb)*. 2012; 4(1): 65–74. [PubMed: 22143867]
66. Lloyd D, Murray DB. Redox rhythmicity: clocks at the core of temporal coherence. *Bioessays*. 2007; 29(5):465–473. [PubMed: 17450596]
67. Nivala M, Korge P, Weiss JN, Qu Z. Linking flickering to waves and whole-cell oscillations in a mitochondrial network model. *Biophysical journal*. 2011; 101(9):2102–2111. [PubMed: 22067147]
68. Lloyd, D.; Murray, DB.; Klevecz, RR.; Wolf, J.; Kuriyama, H. The ultradian clock (40min) in yeast.. In: Lloyd DaR, EL., editor. *Ultradian Rhythms from Molecules to Mind*. Springer Science +Business Media B.V.; New York: 2008. p. 11-42.
69. Aon, MA.; Cortassa, S.; Lloyd, D. Chaos in Biochemistry and Physiology.. In: Meyers, RA., editor. *Advances in Molecular Biology and Medicine: Systems Biology*. Wiley-VCH Verlag GmbH & Co. KGaA; Weinheim: 2012. p. 239-275.
70. Bers, DM. *Excitation-contraction coupling and cardiac contractile force*. Kluwer Academic Publishers; Dordrecht: 2001.
71. Dedkova EN, Blatter LA. Mitochondrial Ca²⁺ and the heart. *Cell Calcium*. 2008; 44(1):77–91. [PubMed: 18178248]
72. Solaini G, Harris DA. Biochemical dysfunction in heart mitochondria exposed to ischaemia and reperfusion. *The Biochemical journal*. 2005; 390(Pt 2):377–394. [PubMed: 16108756]
73. Rolfe DF, Brown GC. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiological reviews*. 1997; 77(3):731–758. [PubMed: 9234964]
74. Katz, AM. *Physiology of the heart*. Raven Press; New York: 1992. Structure of the heart and cardiac muscle..
75. Lane, N. *Mitochondria and the meaning of life*. Oxford University Press; NY: 2005. Power, Sex, Suicide..
76. Dedkova EN, Blatter LA. Measuring mitochondrial function in intact cardiac myocytes. *Journal of molecular and cellular cardiology*. 2012; 52(1):48–61. [PubMed: 21964191]
77. Gunter TE, Sheu SS. Characteristics and possible functions of mitochondrial Ca(2+) transport mechanisms. *Biochimica et biophysica acta*. 2009; 1787(11):1291–1308. [PubMed: 19161975]
78. Kohlhaas M, Liu T, Knopp A, Zeller T, Ong MF, et al. Elevated cytosolic Na⁺ increases mitochondrial formation of reactive oxygen species in failing cardiac myocytes. *Circulation*. 2010; 121(14):1606–1613. [PubMed: 20351235]
79. Maack C, Cortassa S, Aon MA, Ganesan AN, Liu T, O'Rourke B. Elevated cytosolic Na⁺ decreases mitochondrial Ca²⁺ uptake during excitation-contraction coupling and impairs energetic adaptation in cardiac myocytes. *Circulation research*. 2006; 99(2):172–182. [PubMed: 16778127]
80. Maack C, O'Rourke B. Excitation-contraction coupling and mitochondrial energetics. *Basic research in cardiology*. 2007; 102(5):369–392. [PubMed: 17657400]
81. Rizzuto R, Pozzan T. Microdomains of intracellular Ca²⁺: molecular determinants and functional consequences. *Physiological reviews*. 2006; 86(1):369–408. [PubMed: 16371601]
82. Saks V, Kuznetsov AV, Gonzalez-Granillo M, Tepp K, Timohhina N, et al. Intracellular Energetic Units regulate metabolism in cardiac cells. *Journal of molecular and cellular cardiology*. 2012; 52(2):419–436. [PubMed: 21816155]
83. Harris DA, Das AM. Control of mitochondrial ATP synthesis in the heart. *The Biochemical journal*. 1991; 280(Pt 3):561–573. [PubMed: 1837214]
84. Cortassa S, O'Rourke B, Winslow RL, Aon MA. Control and regulation of mitochondrial energetics in an integrated model of cardiomyocyte function. *Biophysical journal*. 2009a; 96(6): 2466–2478. [PubMed: 19289071]
85. Cortassa S, O'Rourke B, Winslow RL, Aon MA. Control and regulation of integrated mitochondrial function in metabolic and transport networks. *International journal of molecular sciences*. 2009b; 10(4):1500–1513. [PubMed: 19468321]

86. Reder C. Metabolic control theory: a structural approach. *J Theor Biol.* 1988; 135(2):175–201. [PubMed: 3267767]
87. Aon MA, Cortassa S, Marban E, O'Rourke B. Synchronized whole cell oscillations in mitochondrial metabolism triggered by a local release of reactive oxygen species in cardiac myocytes. *The Journal of biological chemistry.* 2003; 278(45):44735–44744. [PubMed: 12930841]
88. Cortassa S, Aon MA, Marban E, Winslow RL, O'Rourke B. An integrated model of cardiac mitochondrial energy metabolism and calcium dynamics. *Biophysical journal.* 2003; 84(4):2734–2755. [PubMed: 12668482]
89. Lyon AR, Joudrey PJ, Jin D, Nass RD, Aon MA, et al. Optical imaging of mitochondrial function uncovers actively propagating waves of mitochondrial membrane potential collapse across intact heart. *Journal of molecular and cellular cardiology.* 2010; 49(4):565–575. [PubMed: 20624394]
90. Balaban RS. Domestication of the cardiac mitochondrion for energy conversion. *Journal of molecular and cellular cardiology.* 2009; 46(6):832–841. [PubMed: 19265699]
91. Liu T, O'Rourke B. Regulation of mitochondrial Ca^{2+} and its effects on energetics and redox balance in normal and failing heart. *Journal of bioenergetics and biomembranes.* 2009; 41(2):127–132. [PubMed: 19390955]
92. McCormack JG, Denton RM. Role of Ca^{2+} ions in the regulation of intramitochondrial metabolism in rat heart. Evidence from studies with isolated mitochondria that adrenaline activates the pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase complexes by increasing the intramitochondrial concentration of Ca^{2+} . *The Biochemical journal.* 1984; 218(1):235–247. [PubMed: 6424656]
93. McCormack JG, Halestrap AP, Denton RM. Role of calcium ions in regulation of mammalian intramitochondrial metabolism. *Physiological reviews.* 1990; 70(2):391–425. [PubMed: 2157230]
94. Balaban RS, Kantor HL, Katz LA, Briggs RW. Relation between work and phosphate metabolite in the in vivo paced mammalian heart. *Science (New York, NY).* 1986; 232(4754):1121–1123.
95. Neely JR, Denton RM, England PJ, Randle PJ. The effects of increased heart work on the tricarboxylate cycle and its interactions with glycolysis in the perfused rat heart. *The Biochemical journal.* 1972; 128(1):147–159. [PubMed: 5085551]
96. Tepp K, Shevchuk I, Chekulayev V, Timohhina N, Kuznetsov AV, et al. High efficiency of energy flux controls within mitochondrial interactosome in cardiac intracellular energetic units. *Biochimica et biophysica acta.* 2011; 1807(12):1549–1561. [PubMed: 21872567]
97. Kholodenko BN, Westerhoff HV. Metabolic channelling and control of the flux. *FEBS letters.* 1993; 320(1):71–74. [PubMed: 8462680]
98. van Dam K, van der Vlag J, Kholodenko BN, Westerhoff HV. The sum of the control coefficients of all enzymes on the flux through a group-transfer pathway can be as high as two. *European journal of biochemistry / FEBS.* 1993; 212(3):791–799. [PubMed: 8462550]
99. Dirolez P, Deschodt-Arsac V, Raffard G, Simon C, Dos Santos P, et al. Modular regulation analysis of heart contraction: application to in situ demonstration of a direct mitochondrial activation by calcium in beating heart. *Am J Physiol Regul Integr Comp Physiol.* 2007; 293(1):R13–19. [PubMed: 16971375]
100. Calmettes G, Deschodt-Arsac V, Gouspillou G, Miraux S, Muller B, et al. Improved energy supply regulation in chronic hypoxic mouse counteracts hypoxia-induced altered cardiac energetics. *PLoS ONE.* 2010; 5(2):e9306. [PubMed: 20174637]
101. Wasserstrom JA, Aistrup GL. Digitalis: new actions for an old drug. *American journal of physiology.* 2005; 289(5):H1781–1793. [PubMed: 16219807]
102. Levi AJ. A role for sodium/calcium exchange in the action potential shortening caused by strophanthidin in guinea pig ventricular myocytes. *Cardiovascular research.* 1993; 27(3):471–481. [PubMed: 8387887]
103. Ferrier GR. Digitalis arrhythmias: role of oscillatory afterpotentials. *Progress in cardiovascular diseases.* 1977; 19(6):459–474. [PubMed: 67613]
104. Kass RS, Lederer WJ, Tsien RW, Weingart R. Role of calcium ions in transient inward currents and aftercontractions induced by strophanthidin in cardiac Purkinje fibres. *The Journal of physiology.* 1978; 281:187–208. [PubMed: 702368]

105. Klingenberg M. The ADP and ATP transport in mitochondria and its carrier. *Biochimica et biophysica acta*. 2008; 1778(10):1978–2021. [PubMed: 18510943]
106. Clausen T, Van Hardeveld C, Everts ME. Significance of cation transport in control of energy metabolism and thermogenesis. *Physiological reviews*. 1991; 71(3):733–774. [PubMed: 2057526]
107. Liu T, Brown DA, O'Rourke B. Role of mitochondrial dysfunction in cardiac glycoside toxicity. *Journal of molecular and cellular cardiology*. 2010; 49(5):728–736. [PubMed: 20620145]
108. Sornette, D. Chaos, fractals, selforganization and disorder: Concepts and tools. Springer; Berlin, Heidelberg: 2000. Critical phenomena in natural sciences..
109. Vespignani A. Predicting the behavior of techno-social systems. *Science (New York, NY)*. 2009; 325(5939):425–428.
110. Romashko DN, Marban E, O'Rourke B. Subcellular metabolic transients and mitochondrial redox waves in heart cells. *Proceedings of the National Academy of Sciences of the United States of America*. 1998; 95(4):1618–1623. [PubMed: 9465065]
111. Zorov DB, Filburn CR, Klotz LO, Zweier JL, Sollott SJ. Reactive oxygen species (ROS)-induced ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes. *J Exp Med*. 2000; 192(7):1001–1014. [PubMed: 11015441]
112. Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial ROS-induced ROS release: An update and review. *Biochimica et biophysica acta*. 2006; 1757(5-6):509–517. [PubMed: 16829228]
113. Zhou L, Aon MA, Liu T, O'Rourke B. Dynamic modulation of Ca(2+) sparks by mitochondrial oscillations in isolated guinea pig cardiomyocytes under oxidative stress. *Journal of molecular and cellular cardiology*. 2011
114. Aon MA, Cortassa S, Maack C, O'Rourke B. Sequential opening of mitochondrial ion channels as a function of glutathione redox thiol status. *The Journal of biological chemistry*. 2007b; 282(30): 21889–21900. [PubMed: 17540766]
115. Biary N, Xie C, Kauffman J, Akar FG. Biophysical properties and functional consequences of reactive oxygen species (ROS)-induced ROS release in intact myocardium. *The Journal of physiology*. 2011; 589(Pt 21):5167–5179. [PubMed: 21825030]
116. Brady NR, Hamacher-Brady A, Westerhoff HV, Gottlieb RA. A wave of reactive oxygen species (ROS)-induced ROS release in a sea of excitable mitochondria. *Antioxidants & redox signaling*. 2006; 8(9-10):1651–1665. [PubMed: 16987019]
117. Jeong EM, Liu M, Sturdy M, Gao G, Varghese ST, et al. Metabolic stress, reactive oxygen species, and arrhythmia. *Journal of molecular and cellular cardiology*. 2011
118. Jo H, Noma A, Matsuoka S. Calcium-mediated coupling between mitochondrial substrate dehydrogenation and cardiac workload in single guinea-pig ventricular myocytes. *Journal of molecular and cellular cardiology*. 2006; 40(3):394–404. [PubMed: 16480740]

Figure 1. Time course of the main variables reflecting the state of electrical, mechanical, and energetic processes during a single cardiomyocyte beat as computed by the ECME model

When the ECME model is stimulated every 2 seconds, the depolarization and repolarization phases of the action potential (AP) takes place during the first 200 ms (A). During the AP, Na^+ channels are activated initiating the depolarization phase (C, left axis), which enables gating of L-type Ca^{2+} channels (B, left axis) followed by Ca^{2+} release from internal stores in the sarcoplasmic reticulum (SR) via ryanodine receptors, J_{rel} (B, right axis). Ca^{2+} is then recaptured into the SR via SERCA activity (E, left axis) or transported out of the myocyte through the $\text{Na}^+\text{Ca}^{2+}$ exchanger, I_{NaCa} (B right axis). The potassium currents I_{Ks} , I_{K1} and I_{KP} (C, right axis) participate in the repolarization of the membrane potential to its resting, -80 mV (diastolic) level. The Ca^{2+} transient in the cytoplasm (D, left axis) triggers myofibrils contraction, developing force (D, right axis), which is reflected by the ATP consumption of the ATPase activity associated with the formation of acto-myosin cross bridges, V_{AM} (E, left axis). In addition to V_{AM} and SERCA activity, the other processes that contribute to the demand of ATP are the activity of the Na^+, K^+ ATPase (V_{NaKA}) and the sarcolemmal Ca^{2+} pump (I_{pCa}) (E, right axis). ADP in both compartments the cytoplasm, ADP_i (F, left axis), and mitochondria, ADP_m (F left, axis) exhibit a transient increase which reflect the cytoplasmic increase in ATP consumption during systole and uptake by mitochondria, activating respiration and decreasing NADH levels (F, right axis). Mitochondrial Ca^{2+} (F, 2nd left axis) allows to recover from this transient mismatch between energy demand and supply, visualized as a restoration of NADH (F, right axis) through the TCA cycle activity. The shaded area in panels D-F corresponds to the same time range zoomed in panels A-C. Parameters and initial conditions of the simulation as described elsewhere (26).

(Reproduced from: Aon MA, Cortassa S, Lloyd D: Chaos in Biochemistry and Physiology. In: Advances in Molecular Biology and Medicine: Systems Biology p. (Ed. Meyers R.A.). Wiley-VCH: Weinheim, 2012)

Figure 2. Heterarchical control and regulation in the network of energetic and electro-mechanical processes of the heart cell

Heterarchical, as opposed to hierarchical, is the manner control and regulation is exerted in complex networks. In heterarchical control all edges (e.g. enzymes, channels) and nodes (e.g. metabolites, ions) control and are controlled by all other edges and nodes involved in the network of interest, e.g. energetic, mechanical, electric, and transport processes. The desired degree of detail in the description can be applied by zoom in on the network of integrated cardiomyocyte function (Excitation-Contraction coupling and Mitochondrial Energetic: ECME model) (84, 85) according to a generalized matrix method utilized for calculating its overall structure of control and regulation. More precisely, the top panel shows a scheme of the overall network of energetic and electro-mechanical processes from which it is possible to zoom in the mitochondria with its own reaction network (lower left panel) or yet deeper into the tricarboxylic acid (TCA) cycle (lower right panel) with its specific biochemical circuitry. Of note is that the TCA cycle was considered as an aggregated step in the two other schemes. The ability to zoom in and out the network also reflects that heterarchical control and regulation is bidirectional, i.e. bottom-up as well as top-down.

In the top panel, rectangular (ion or metabolites) or circular (myofibril conformations) boxes indicate state variables of the ECME network. Boxes depict a light blue background when the state variables participate in conservation relationships (ATP/ADP; creatine/creatine-P, Cr/CrP; NAD⁺/NADH; TRPN/TRPN-Ca, and the various conformations of myofibrils). Ionic species are indicated on a dark blue background. Hexagonal boxes denote inputs (ions or carbon substrate) that correspond to parameters in the model. Arrowheads point to the products of the numbered processes, whereas lines without arrowheads indicate inputs to those processes. In the mitochondrial energetics scheme, the TCA cycle was considered as a single step in the stoichiometric matrix. In the scheme of the TCA cycle (lower right panel) solid lines represent mass-energy transformation reactions, whereas regulatory interactions (with negative signs indicating inhibition, and positive signs activation) are denoted by dashed lines.

Processes accounted for by the ECME and ME models are numbered according to the following key:

Number	Abbreviation	Name
1	TCA	Tricarboxylic acid cycle
2	V_{RC}	Respiratory electron transport
3	HNe	Respiratory chain proton pumping
4	HFe	Succinate-driven proton pumping
5	ATPSy	Mitochondrial ATP synthase
6	Hu	Proton pumping through ATP synthase
7	Leak	Proton leak
8	ANT	Adenine nucleotide translocator
9	Ca_{uni}	Mitochondrial Ca^{2+} uniporter
10	V_{NCE}	Mitochondrial $Na^+ Ca^{2+}$ exchanger
11	AM-ATP	Myofibrillar ATPase
12	PMCA	Sarcolemmal Ca^{2+} ATPase
13	SERCA	Sarcoplasmic reticulum Ca^{2+} ATPase
14	I_{NaK}	Current through the $Na^+ K^+$ ATPase
15	I_{NCX}	Current through the sarcolemmal $Na^+ Ca^{2+}$ exchanger
16	I_{Cab}	Background Ca^{2+} current
17	$ATPase_c$	Constitutive cytosolic ATPase
18	I_{Na}	Na^+ inward currents
19	LCC	L type Ca^{2+} current
20	J_{Rel}	Ca^{2+} release from RyR
21	J_{xfer}	Ca^{2+} transport from subspace into cytoplasm
22	I_K	Outward potassium currents
23	-	Ca^{2+} association-dissociation to troponin
24	CK_i	Mitochondrial creatine kinase
25	-	Creatine species transport
26	CrKc	Cytosolic creatine kinase
27-35	-	Transitions between tropomyosin conformations

(*Reproduced from:* Aon MA, Cortassa S, Lloyd D: Chaos in Biochemistry and Physiology. In: Advances in Molecular Biology and Medicine: Systems Biology p. (Ed. Meyers R.A.). Wiley-VCH: Weinheim, 2012)

Figure 3. From direct to diffuse control loops in cardiomyocyte function

This figure describes several examples of diffuse control loops exhibiting different lengths as given by the number of intermediary steps involved. *Control by diffuse loops* was first defined as the control that a process A exerts over process C or D without an apparent direct mechanistic link among them. Unlike in a direct loop (I), in a diffuse control loop there is at least one intermediary process between A and C (II-IV). The positive and negative signs represent the type of control (positive or negative) that each edge (e.g. calcium uniporter, $\text{Ca}^{2+}_{\text{uni}}$, adenine nucleotide translocator, ANT) in the network has on other nodes or edges in the loop (see also text). In (I) $\text{Ca}^{2+}_{\text{uni}}$ exerts a negative control on cytoplasmic Ca^{2+} , $\text{Ca}^{2+}_{\text{i}}$, i.e. the higher the activity of the uniporter, the lower the concentration of $\text{Ca}^{2+}_{\text{i}}$. In (II) $\text{Ca}^{2+}_{\text{uni}}$ exerts an overall negative control on ATP synthesis which, mechanistically, is not direct as in (I), but indirectly mediated by the negative control of $\text{Ca}^{2+}_{\text{uni}}$ upon the mitochondrial membrane potential, $\Delta\Psi_{\text{m}}$. ATP synthesis will decrease following a decrease in $\Delta\Psi_{\text{m}}$ because the latter is the driving force of ADP phosphorylation by the ATP synthase. In (III) the ANT displays an overall positive control on the respiratory flux mediated by an increase in the rate of proton pumping by the ATP synthase, V_{hu} , which in turn control positively the rate of mitochondrial respiration, because the higher the ATP synthase activity the higher the consumption of its driving force, i.e. $\Delta\Psi_{\text{m}}$, stimulating the electron flow through the respiratory chain to reestablish the proton motive force. In (IV) the Na^+K^+ ATPase (NAK ATPase) overall control of respiration is negative, which is counter intuitive because one would expect the contrary from an ATP-consuming process. The explanation resides in the extensive diffuse loop that is mediating this control as follows: the increase in Na^+ pump activity lowers intracellular Na^+ , leading to an increased cellular extrusion of $\text{Ca}^{2+}_{\text{i}}$ via the sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX); this decrease in $\text{Ca}^{2+}_{\text{i}}$ lessens the extent of $\Delta\Psi_{\text{m}}$ dissipation associated with mitochondrial Ca^{2+} transport, which results in a higher $\Delta\Psi_{\text{m}}$ and a decrease of respiration.

In a complex network of reactions, the concept of control by diffuse loops is useful to interpret the changes that can be triggered by pharmacological agents among processes without direct mechanistic links between them.

Figure 4. Diffuse control loops in the *Digitalis* action on cardiac contractility

Inhibition of the NaK ATPase converts an overall negative diffuse control loop into a positive one, i.e. inhibiting a negative controller will increase overall downstream activity of the processes negatively controlled by the pump. The primary action of *Digitalis* and cardiotonic glycosides (e.g. ouabain) is to inhibit the NaK pump resulting in an overall positive effect on contractility, a well-known pharmacological action. The scheme shows the wide range of processes affected by NaK ATPase inhibition. The grey box represents the increase in cytoplasmic ATP and Ca^{2+} resulting from the activation of the diffuse control loop by NaK ATPase inhibition, both exerting opposing effects on mitochondrial respiration, and opening the question of which will prevail (i.e., the interrogation sign in the box). See text for a detailed explanation. Key to symbols: SERCA, sarcoplasmic reticulum Ca^{2+} ATPase; NCX (FM), $\text{Na}^+/\text{Ca}^{2+}$ exchanger forward mode; NCX (RM), $\text{Na}^+/\text{Ca}^{2+}$ exchanger reverse mode. See also the legend of Figure 3 and the text for the definition of other symbols.