Published in final edited form as:

Physiology (Bethesda). 2005 October ; 20: 303–315. doi:10.1152/physiol.00020.2005.

Mitochondrial Ion Channels: Gatekeepers of Life and Death

Brian O'Rourke, Sonia Cortassa, and Miguel A. Aon

Institute of Molecular Cardiobiology, Division of Cardiology, The Johns Hopkins University, Baltimore, Maryland

Brian O'Rourke: bor@jhmi.edu; Sonia Cortassa: ; Miguel A. Aon:

Abstract

Continuous generation of ATP by mitochondrial oxidative phosphorylation is essential to maintain function in mechanically active cells such as cardiomyocytes. Emerging evidence indicates that mitochondrial ion channels activated by reactive oxygen species can induce a mitochondrial "critical" state, which can scale to cause electrical and contractile dysfunction of the cardiac cell and, ultimately, the whole heart. Here we focus on how mitochondrial ion channels participate in life-and-death decisions of the cell and discuss the challenges ahead for translating recent findings into novel therapeutic applications.

We live in a dangerous environment, continuously immersed in a medium containing 21% oxygen, a highly reactive molecule. Fortunately for us, this impediment to life has been turned back on itself and has actually been harnessed by the cell to generate the stored chemical energy necessary to meet the major demands of cell physiology. Mitochondria are the organelles providing this protection, compartmentalizing and controlling the oxidation of metabolic fuels to produce ATP, the high-energy phosphate used to maintain ion gradients, synthesize new molecules, actively dispose of old molecules, and perform mechanical work. In the heart, the energetic demands are extreme: the heart is required to pump roughly 75 gallons of blood per hour, unceasingly, for up to 100 years. To meet this workload, the heart accounts for nearly 10% of the total oxygen consumption (V_{O_2}) of the body at rest, and this demand increases four- to fivefold during exertion. Thus it is vital for the cardiac cell to continuously adjust the fine balance between energy production and energy consumption and to limit the ill effects of the byproducts of metabolism, which include reactive oxygen species (ROS). Over the physiological range of activity, the multiple nonlinear control interactions governing mitochondrial oxidative phosphorylation elegantly and robustly adapt to large changes in demand while keeping the many positive and negative feedback loops in check to prevent instability in the metabolic network.

Unfortunately, age and/or disease can introduce a variety of stressors that can, under certain circumstances, result in the collapse of mitochondrial function, causing necrotic or apoptotic cell death. Failure at the level of the organelle can scale to the mitochondrial network and have a major impact on the basic excitation-contraction coupling processes of the cardiomyocyte. As a consequence of the syncytial nature of the myocardium, this cascade of failures can scale to level of the organ and, ultimately, determine the life or death of the organism. Intervention into this process requires a detailed understanding of the mechanisms underlying metabolic control, the factors responsible for loss of control, and the built-in mechanisms that might protect the cell from injury. Emerging evidence suggests that ion channels on the mitochondrial inner and outer membranes are key participants in the decision between cell life and death. Despite the central role of mitochondrial ion channels in cell injury, remarkably little effort has been directed toward developing specifically targeted therapeutic agents to modulate their activity. Furthermore, the molecular structures of key mitochondrial ion channels and/or transporters have not been determined, hampering progress toward a complete understanding

of their roles. Hopefully, recent discoveries and new techniques for identifying mitochondrial proteins and assaying mitochondrial ion channel activity will spur new initiatives to resolve long-standing questions in the field of bioenergetics and will inspire the development of tools for manipulating mitochondrial function in the future.

Mitochondrial Bioenergetics and ROS Production

Overview of cardiac energy metabolism

Investigating the effects of mitochondrial ion channels and transporters on bioenergetics requires a basic understanding of energy metabolism in the heart, as depicted schematically in FIGURE 1, which emphasizes the mitochondrial processes involved in oxidative phosphorylation. Glucose and fatty acids, the primary metabolic substrates of the heart, are sequentially oxidized to produce acetyl-CoA, the common intermediate driving the production of the reducing equivalents NADH and FADH₂ by the Krebs cycle (tricarboxylic acid cycle). NADH feeds electrons to the electron-transport chain (respiratory chain) through the NADH:ubiquinone oxidoreductase (complex I), which reduces ubiquinone (coenzyme Q). The reduced flavin moiety of succinate dehydrogenase (complex II), which is also a Krebs-cycle enzyme, passes electrons to ubiquinone directly. Ubiquinone is a lipid-soluble mobile carrier that transfers electrons to the cytochrome bc1 complex (complex III), which, in turn, reduces the peripherally associated carrier cytochrome c. In the terminal redox reaction, electrons are passed to cytochrome c oxidase (complex IV) and ultimately to molecular oxygen. The redox reactions occurring at complexes I, III, and IV are coupled to proton (H⁺) translocation across the mitochondrial inner membrane, establishing a proton-motive force (Δp), composed of the pH gradient (ΔpH) and the mitochondrial membrane potential ($\Delta \Psi_m$). This large driving force for proton influx is used by the mitochondrial ATP synthase (F₁F₀ ATPase) to produce ATP, which is exported to the cytosol via the adenine nucleotide translocase (ANT).

Maximum coupling between proton pumping by the respiratory chain and the phosphorylation of ADP is obtained when the leak of protons across the membrane is minimized. An increase in the ion permeability of the mitochondrial inner membrane can occur in the presence of protonophoric chemical uncouplers, in response to an increase in matrix ADP (through stimulation of proton flux through the ATP synthase), or as a consequence of opening mitochondrial ion channels. The energy dissipated by the increased ion permeability stimulates NADH oxidation, proton pumping, and respiration. Concomitant stimulation of NADH production is required to compensate for the higher rates of respiration, or else a mismatch in energy supply and demand will occur.

Sites of mitochondrial ROS production

Although the respiratory chain quite efficiently uses O₂ in the controlled oxidation of the redox carriers, the superoxide anion (O₂⁻) is an unavoidable byproduct of oxidative phosphorylation, making mitochondria a major site of ROS production. It has been estimated that ~1–5% of the electrons flowing through the electron-transport chain leak into the production of ROS (83). The dual effects of ROS on cell function have long been recognized. ROS can damage redox-sensitive target proteins (96), yet in small doses, they can paradoxically protect against cell injury and apoptosis (5,20). ROS also play an important physiological role as signal-transduction molecules within the cell (27,29,79). ROS production and/or mitochondrial permeability transition pore opening, with concomitant release of cytochrome c and apoptotic factors, may be the initial step in triggering the apoptotic cascade (32). Furthermore, recent studies from our laboratory (3) and others (86) have supported the notion that ROS generated in the mitochondrial matrix might activate inner-membrane anion channels (IMACs) to affect neighboring mitochondria or cytosolic targets (discussed below).

The relevant physiological and pathophysiological sites and conditions for mitochondrial ROS production are the subject of considerable current debate, in part depending on which model system and conditions are used. O_2^- can be generated at three main sites in the electron-transport chain, from complex I (NADH dehydrogenase), or at the outer or inner membrane faces of complex III (cytochrome bc1) (83) (FIGURE 2). The extent of electron leakage to O_2^- at complex I vs. complex III is regulated in very different and diametrically opposite ways. In the presence of NADH-linked substrates, O_2^- production by complex I is favored when the NADH/NAD⁺ redox couple is almost completely reduced. Indeed, in a recent study by Kushnareva et al. (52), the redox potential of the site of ROS production by complex I was estimated to be about -392 mV, which is 50 mV more negative than the NADH/NAD⁺ redox couple itself. Hence, ROS production from this thermodynamically unfavorable state is usually promoted by inhibiting respiration, for example, with rotenone, which blocks complex I at a site distal to the O_2^- -generating redox center. Blocking electron flow farther down the chain, e.g., by depleting cytochrome c, can also enhance O_2^- production at complex I (52) by reducing the electron carriers upstream from the inhibited site. Reduction of complex I and associated ROS production can also be induced by reverse electron flow from succinate to NAD⁺ (85). Thus O_2^- generation by complex I requires a highly reduced electron-transport chain and low electron flow (essentially state 4 conditions), as well as a ready supply of oxygen. This has led to the suggestion that a certain level of “mild uncoupling” of oxidative phosphorylation may be a mechanism for inhibiting this source of mitochondrial ROS production (78).

In contrast, the generation of O_2^- by the “Q-cycle” of complex III, which involves the formation of ubisemiquinone to donate electrons to molecular O_2 , requires both a supply of substrates to drive the reduction of coenzyme Q and the continuous availability of downstream electron acceptors (84). In other words, there is a positive correlation between electron flow through the respiratory chain and ROS production. In this case, blocking electron flow into the Q-cycle with rotenone or reducing the nonheme FeSIII electron acceptor of complex III by inhibiting sites downstream (e.g., by inhibiting cytochrome oxidase with CN^- or inducing state 4 by inhibition of the ATP synthase or the adenine nucleotide carrier) will inhibit the production of ROS by complex III. This was elegantly shown by Turrens et al. (84), who demonstrated the correlation between electron flow, respiration, and ROS production at complex III by titration of cytochrome c back into cytochrome c-depleted mitochondria.

Since these models of mitochondrial ROS production are almost exclusively based on data from isolated mitochondria, the question remains as to which site is important in intact myocytes or muscles. We have provided evidence that in intact myocytes, the majority of mitochondrial O_2^- production in cells subjected to local oxidative stress is from complex III, most likely from the matrix-oriented site (see Ref. 3 and below). A similar transition from low electron flow to high electron flow would be expected to occur early in reperfusion after ischemia and could contribute to the well-known burst of ROS produced during this period. It remains to be determined whether other sites of ROS production, both intrinsic and extrinsic to the mitochondria, are activated under specific conditions, such as during hypoxia.

Loss of Mitochondrial Function: Sequelae of Ischemia

There is widespread agreement that a reproducible series of events occurs in the myocardium during ischemia (FIGURE 3), although the cause-and-effect relationship of each with respect to ischemic injury is often difficult to ascertain. At the onset of ischemia, anaerobic metabolism is activated within 8 s of flow cessation (65), and contraction declines within 20 s. The myocardial electrical properties also change rapidly; the cellular action potential shortens, and the ST segment of the EKG is elevated within the first minute of ischemia. Intracellular pH declines and anaerobic glycolysis then becomes inhibited. Over the course of many minutes

of ischemia, intracellular Na^+ increases (2), in part through Na^+/H^+ and $\text{Na}^+/\text{Ca}^{2+}$ exchanger action, and the cytoplasmic and mitochondrial compartments load with Ca^{2+} (82). The $\Delta\Psi_m$ declines progressively during the ischemic period (30). Interestingly, when hearts are preconditioned (that is, subjected to one or more short periods of ischemia with reperfusion before a long ischemia), $\Delta\Psi_m$ may drop even more than with ischemia alone, yet the functional recovery is improved upon reperfusion (93).

Upon reperfusion, the Na^+ -loaded heart fosters additional cellular Ca^{2+} overload via reverse-mode $\text{Na}^+/\text{Ca}^{2+}$ exchange, and a major burst of ROS is evoked. During this important period, the course of cardiomyocyte survival or death is set, determined in large part by the ability of mitochondria to recover. The activation of destructive mitochondrial ion channels plays a role in this decision. Chemical or ischemic preconditioning of the heart appears to blunt most of the detrimental changes in cellular parameters, and mitochondrial function is better preserved. A number of intracellular signaling pathways and effects on oxidative phosphorylation, ROS production, and Ca^{2+} handling have been implicated in this native protective mechanism (84). A central role for the mitochondrial ATP-sensitive K^+ (mito K_{ATP}) channel is widely supported (63), but the precise mechanism of protection is still being investigated.

Mitochondrial Ion Channels

Function of mitochondrial ion channels

The physiological roles of many inner-membrane ion channels are largely unknown. For example, K^+ -selective and/or anion-selective pores are believed to be important for regulating mitochondrial volume (31), but in vivo confirmation of this function has been difficult to obtain (63). Mitochondrial swelling and contraction has been proposed to modulate the rate of substrate oxidation (36) under normoxic conditions and could be an important factor in determining the extent of ischemia-reperfusion injury (44,49,50). At a minimum, energy dissipation due to channel opening will increase the flux through the electron-transport chain, and if this effect is uncompensated by increased substrate production, then net oxidation of the matrix, as well as a change in ROS production, will occur. Thus one potential physiological role of mitochondrial channels could be redox regulation, which is known to be an important intracellular signaling mechanism influencing transcription, translation, phosphorylation/dephosphorylation cascades, and programmed cell death. Paradoxically, net reduction of the cellular redox state can also activate transcription factors; for example, oxygen deprivation activates the transcription factor hypoxia inducible factor-1 (HIF-1), leading to an increased expression of adaptive enzymes (72). Recent evidence suggests that hypoxia might also increase ROS production (86), but the precise site of ROS production and the downstream effects on cell function are still under investigation.

Mitochondrial swelling assays and later direct patch-clamp studies of mitoplasts (which are mitochondria stripped of their outer membranes) have identified a number of ion-conductive pores on the inner membrane (94). In general, these mitochondrial ion channels have been associated with either protective or destructive effects on cells during metabolic stress, as illustrated schematically in FIGURE 4.

Ca^{2+} uniport

The Ca^{2+} uniporter of the mitochondrial inner membrane has perhaps the most well-understood role in physiology. This ruthenium red-sensitive Ca^{2+} uptake pathway is the primary route for Ca^{2+} entry into the mitochondrial matrix, and there is strong evidence that an increase in matrix Ca^{2+} is important for stimulating oxidative phosphorylation at several sites, including the Ca^{2+} -sensitive dehydrogenases of the Krebs cycle (26) and one or more sites in the electron-transport chain (7,21). Although recent evidence supports the notion that the Ca^{2+} uniporter is

a highly Ca^{2+} -selective, low-conductance ion channel (46), the molecular structure of this channel has not yet been determined. Intriguing recent studies have proposed that type I (skeletal type) ryanodine receptors (RyR1) are present on the cardiac mitochondrial inner membrane and may participate in either Ca^{2+} influx or efflux (12). Several major discrepancies between the behavior of the Ca^{2+} uniporter and RyR1 will need to be resolved before the ryanodine receptor can be considered as a viable candidate for the protein responsible for Ca^{2+} uniprot activity in heart cells.

The Ca^{2+} uniporter has also been implicated in the pathophysiological consequences of ischemia and reperfusion. Mitochondrial Ca^{2+} loading has been associated with cell injury (58), although the relative contributions of cytoplasmic vs. mitochondrial Ca^{2+} overload are often difficult to assess individually due to their interdependence, and there is not always a clear correlation between mitochondrial Ca^{2+} and survival (34). Although mitochondria are normally capable of tolerating large amounts of Ca^{2+} uptake with minimal effects on oxidative phosphorylation, there is ample evidence demonstrating that mitochondrial Ca^{2+} loading under simulated or true ischemia-reperfusion conditions can trigger a mitochondrial permeability transition (37). The latter may depend on the simultaneous contribution of multiple factors such as Ca^{2+} , ROS, ADP, NAD^+ , etc. (67).

Cytoprotective potassium channels

There are at least two types of potassium channels in the mitochondrial inner membrane that have been shown to be protective against ischemia-reperfusion injury. An ATP-sensitive channel (mitoK_{ATP}) with properties somewhat resembling those described for surface membrane K_{ATP} channels has been found to be present on intact mitoplasts (42) and in bilayers reconstituted with purified mitochondrial proteins (66). The mitoK_{ATP} observed in these electrophysiological studies is thought to be the ion channel corresponding to ATP-sensitive and K⁺ channel opener/blocker-sensitive K⁺ fluxes studied in isolated mitochondria, proteoliposomes (10,43), and mitochondrial swelling studies (24). MitoK_{ATP} activation has been implicated in the mechanism of protection against ischemic injury induced by ischemic or chemical preconditioning and in protecting cells against apoptosis and PTP activation (1, 38,44). Several questions and controversies related to the specificity of certain K⁺ channel openers and the mechanism of protection by mitoK_{ATP} activation are the subject of ongoing investigation, as discussed in recent reviews of the topic (35,60,63).

A second mitochondrial inner membrane K⁺ channel (mitoK_{Ca}), having properties resembling the Ca^{2+} -activated K⁺ channel (K_{Ca}) of the surface membrane, has also been identified in cardiac (92) and glioma cell (73) mitochondria. This channel is blocked by the scorpion-venom toxins charybdotoxin and iberiotoxin and by paxilline, known blockers of K_{Ca} channels in other cell types. MitoK_{Ca} channel openers have been shown to confer protection against ischemia-reperfusion injury in a manner analogous to that of mitoK_{ATP} (56), but through distinct pathways (71). A role for the mitoK_{Ca} channel in delayed preconditioning, which is manifested 24–72 h after a preconditioning treatment, has also been suggested by a recent study (89). The molecular structure of this mitochondrial channel is currently under investigation, but a 55- to 80-kDa protein cross-reacting with anti-BKCa channel antibodies was identified in purified mitochondrial membranes using 2D-PAGE analysis (92). Future studies will be required to establish the role of mitoK_{Ca} in the normal physiology of the cell, but we propose that this channel might be activated at high mitochondrial Ca^{2+} loads, acting as a relief valve to prevent further Ca^{2+} accumulation or to fine-tune mitochondrial volume to optimize oxidative phosphorylation.

In addition to the two K⁺ channels mentioned above, a voltage-activated K⁺ channel of the Kv1.3 type has recently been identified in lymphocyte mitochondria (80). This channel, however, has been suggested to participate in apoptotic cell death, since knockout of the

channel prevented cytochrome c release and $\Delta\Psi_m$ depolarization induced by actinomycin D (15).

Channels contributing to dysfunction under stress

PERMEABILITY TRANSITION PORE—The inner-membrane ion channel receiving the most attention is the permeability transition pore (PTP), a large-conductance (~ 1 nS) pore that permits the passage of solutes up to 1,500 Da nonselectively (67). It is thought to be activated by oxidative stress, Ca^{2+} , and depolarization and is strongly inhibited by cyclosporin A (CsA). The PTP described in mitochondrial suspension studies is believed to be identical to the mitochondrial mega- (or multiconductance) channel (MMC) observed in electrophysiological studies (81). From a structural point of view, it has been argued that the PTP is a multiprotein complex comprised of (at least) the mitochondrial ANT, the voltage-dependent anion channel of the outer membrane (VDAC), the F_1F_0 ATPase, and cyclophilin D, the receptor for CsA. The pore structure has not been conclusively identified, but it has been observed that, in high Ca^{2+} , the ANT can be converted into a large channel (~ 600 pS) that is nonselective with respect to cations and partially selective for cations over anions ($P_K/P_{\text{Cl}} \sim 4:1$) (17). This channel is inhibited by bongrekic acid and ADP but not carboxyatractyloside, resembling the profile of the PTP. Furthermore, a recombinant bacterial ANT type protein can form similar channels when expressed in *Escherichia coli*, excluding the argument that contaminating proteins in the mitochondrial preparation could have been responsible for the channel activity (18). On the other hand, knockout of ANT1 in mice does not eliminate the mitochondrial permeability transition in response to metabolic stress but alters its sensitivity to Ca^{2+} and ANT modulators (48). In addition, the MMC can still be observed in mammalian cells lacking mitochondrial DNA (ρ^0) and in yeast in which the VDAC or ANT have been knocked out (53,54).

There is considerable evidence that the PTP opens during ischemia-reperfusion, and it has been linked to cytochrome c release and the triggering of apoptosis (23). Griffiths et al. (33) reported that the PTP does not open during ischemia but is activated upon reperfusion. Since the PTP opens when there is significant cellular Ca^{2+} overload and severe energy depletion, and it mediates loss of matrix constituents, PTP activation likely represents an irreversible terminal event for the mitochondria. There are several reports that PTP opening can be visualized in intact cells (62,67,68) or in isolated mitochondria (40) by monitoring the fluorescence of voltage-sensitive dyes (indicating membrane polarization) or calcein (indicating permeability transition) loaded into the mitochondrial matrix compartments. PTP opening can also mediate fast Ca^{2+} release from Ca^{2+} -loaded mitochondria, perhaps through a Ca^{2+} -induced release mechanism; this has led to the concept of mitochondria as excitable organelles with Ca^{2+} representing the signaling molecule (41). A model of ROS-induced ROS release involving the PTP has also been described by Zorov et al. (95). We have also recently described mitochondrial ROS-induced ROS release (discussed below) in cardiac cells subjected to local oxidative stress, but the mechanistic details appear to be quite different from the previously described studies implicating the PTP.

ANION CHANNELS—The mitochondrial inner membrane is also home to several anion-selective ion channels with undetermined physiological roles. Anionic conductances of 15 pS, 26 pS (SMAC), 57 pS (INMAC), and 107 pS (centum pS channel) have been reported. The 107-pS channel was the first channel identified in patch-clamp studies (76). This channel, also referred to as the centum picosiemen channel or mCS (19,61), is only slightly anion selective ($P_{\text{Cl}}/P_K = 4.5$) and is strongly voltage sensitive, being driven to the closed state by hyperpolarization. Thus it could be a prime candidate for positive-feedback activation of ion flux under conditions of $\Delta\Psi_m$ depolarization. Although the 108-pS channel has been tentatively associated with the IMAC (16) characterized in swelling assays of isolated mitochondria (9), this match is not definitive, and other candidates (e.g., the 15-pS channel) have been proposed.

IMAC is permeable to a variety of anions and small anionic metabolites (e.g., Cl^- malate, oxaloacetate, malonate, citrate, etc.), including ATP. It is blocked by a wide variety of amphipathic compounds that can accumulate in the mitochondria, including many clinically effective classes of drugs: antiarrhythmics (amiodarone), anesthetics (dibucaine), β -adrenergic antagonists (propranolol), antidepressants (amitriptyline), and benzodiazepines (clonazepam) (9). Reactive thiols are also present on IMAC, and it is regulated by pH, Mg, and P_i . Importantly, it is not blocked by CsA, which permits one to discriminate between IMAC vs. PTP opening (3). Moreover, IMAC does not allow passage of fluorescent markers with sizes of ~ 500 mol wt (e.g., calcein), and its opening is independent of Ca^{2+} (for other evidence that distinguishes IMAC from the PTP see Ref. 3).

OUTER-MEMBRANE CHANNELS—Patch-clamp and bilayer-reconstitution studies have also detected ion channels in the mitochondrial outer membrane, which was originally shown to be highly permeable to large solutes and protein. In recent years, the outer membrane has been the target of intense interest, with the discovery that cytochrome c (as well as other factors) can be released from the intermembrane space to induce apoptosis (88). Although there is a paucity of molecular information on mitochondrial inner-membrane ion channels, the sequence and isoform distribution of the major outer membrane protein, VDAC (or porin), is known (55). Changes in VDAC permeability have been implicated in the cell death process and activation of the PTP, and they can modulate the rate of metabolite transport in and out of the mitochondria (70,87). Current investigations have focused on determining if the apoptogenic proteins Bad, Bax, or tBid independently form channels that allow passage of cytochrome c to the cytoplasm, or if they modulate VDAC activity. Recent patch-clamp studies suggest that either Bax or Bad can directly form cytochrome c-permeable channels, referred to as mitochondrial apoptosis-inducing channels, in the outer membrane (25). Several studies have also recently reported that an increase in outer membrane permeability does not always require a concomitant permeability transition on the inner membrane (88).

Mitochondrial ion channels can also be formed by the proteins involved in the translocation of mitochondrial protein precursors. The translocase of the outer membrane and its partner on the inner membrane can form ion-conductive pores that are blocked by mitochondrial polypeptide-targeting fragments (59). It remains to be determined if these pores are permeable to ions in the intact cell and when they may be active. A variety of other ion-channel conductances have also been observed in the outer membrane (57), but they have not been well characterized or attributed to a specific function.

Mitochondrial Criticality and Emergent Network Behavior

The mitochondrial network of cardiac cells consists of thousands of mitochondria packed between the myofilaments in an ordered, three-dimensional array (FIGURE 5A, LEFT). This arrangement is required to place the sites of ATP production close to the sites of ATP consumption (e.g., the myosin ATPase of the thick filament and the ion pumps required for Ca^{2+} sequestration and ion homeostasis). This spatial organization begs the question of whether mitochondria behave independently or are synchronized and what factors might be responsible for intermitochondrial communication. These questions apply to both the normal range of function, when the bioenergetic status must rapidly adapt to changes in workload to maintain a constant supply of ATP (that is, mitochondria must be both flexible and robust), and to pathological situations, when metabolism becomes severely compromised by ischemia, reperfusion, or other forms of metabolic stress (e.g., oxidative stress, substrate deprivation). In these situations, the inherent vulnerability of the mitochondrial network is revealed, as previously reported (39, 41, 74) (for review see Refs. 23, 28).

The idea that biological systems can operate at the edge of dynamic instability is a new way to view physiological control (6,45) (for review see Ref. 77). It is thought that this adaptation may have evolved to favor a quick and flexible response to environmental change. However, the downside of this type of nonlinear response can be the appearance of critical behavior when cells are exposed to pathological conditions, leading to deadly outcomes such as apoptosis or necrosis (51,69). It is under these circumstances that the mitochondrial network can display a remarkably rich set of dynamic spatial and temporal patterns that are, in many ways, reminiscent of other physical or manmade connected systems. For example, networks of non-linearly coupled elements that are subjected to excessive loads or to failure of parts of the system can approach a critical state, which is characterized by an extremely large susceptibility to external factors and strong correlation between individual components. Consequently, new emergent macroscopic behavior can appear, including spatiotemporal patterns of self-organization, visualized as oscillations and/or waves in activity or the level of an intermediate. Our recent experimental and theoretical work shows that such conditions are met when mitochondrial ROS accumulates to a critical threshold in a certain fraction of the mitochondrial network (FIGURE 5, B AND C). At this point of mitochondrial criticality (4), oxidative metabolism becomes unstable, undergoing a rapid transition involving $\Delta\Psi_m$ depolarization or self-sustained oscillation (FIGURE 5, A–C) reflected by a coordinated response of almost the entire population of mitochondria in the network.

Uniquely, the ability to trigger reproducible cell-wide synchronized autonomous oscillations of $\Delta\Psi_m$, NADH, ROS, and glutathione in cardiac myocytes using a highly localized oxidative trigger has enabled us to explore the mechanism and the functional implications of this spatiotemporal dynamic response in unprecedented detail (3,22). The oscillatory behavior could also be reproduced in a mathematical model of a single mitochondrion, which incorporates ROS production by the electron-transport chain, a ROS-activated mitochondrial channel (IMAC), and extramitochondrial ROS scavenging (FIGURE 5D). In this model, ROS produced by the electron-transport chain accumulates to a threshold level, triggering the opening of IMAC in a positive feedback loop. IMAC activation is terminated by a reduction in ROS at the activator site of the channel as a result of membrane depolarization (decreasing ROS production and efflux from the mitochondrial matrix) and ROS scavenging by the antioxidant enzymes. Thus the system acts as a relaxation oscillator, in which a controlling factor builds up to a critical point (akin to the buildup of force in the earth's crust prior to an earthquake), and then a rapid change is triggered, with the process then repeating in a stereotypical pattern. These events, which the model suggests can occur in single mitochondria, are transmitted to the whole mitochondrial network through neighbor-neighbor interactions among mitochondria, synchronized and spatially organized by a process that can be described as a percolation lattice (4). The emergent macroscopic patterns therefore depend on the spatial arrangement and the proximity of the mitochondria to each other; hence, the tight and ordered packing of mitochondria in the cardiac cell provides a unique three-dimensional lattice to explore this type of interaction. Similar investigations in other cell types, with various mitochondrial distributions, should in the future yield more information on the importance of organization in the propagation of mitochondrial signals.

Linkage to integrated cell function

The biological complexity of the control mechanisms described above does not end at the level of the mitochondria, for the integrated functions of the cardiac cell are intimately coupled to the energetic status. Both the electrical and Ca^{2+} -handling subsystems of the myocyte are strongly influenced by the energy state of the mitochondria. In the oscillatory phenomenon described above, the repeating cycles of mitochondrial $\Delta\Psi_m$ depolarization and repolarization allow us to examine the phase relationships between $\Delta\Psi_m$, ROS bursts, and their effects on cellular action potentials and Ca^{2+} transients. The rapid phase of mitochondrial uncoupling

was closely correlated with the activation of energy-sensing K_{ATP} channels of the sarcolemmal membrane (3,64), and also with the suppression of intracellular Ca^{2+} release (64), demonstrating that mitochondrial criticality scales to produce critical behavior in the primary function of the cardiomyocyte, excitation-contraction coupling. These effects are presumed to be the result of the mitochondria switching from energy-producing to energy-consuming organelles. The drop in the ATP/ADP ratio will affect multiple intracellular targets, including the K_{ATP} channel, the sarcoplasmic reticulum Ca^{2+} pump, the ryanodine receptor, the L-type Ca^{2+} channel, and energy-dependent ion pumps. The repetitive switching of the cell between electrically excitable and inexcitable states would be expected to raise havoc with whole-heart electrical propagation. Thus we had previously proposed that if these transitions in the energy state are present in hearts subjected to metabolic stress, they could set the stage for global arrhythmias (64). Recent experiments from our laboratory have provided support for this hypothesis and have suggested a potentially important new therapeutic target in the mitochondrial membrane.

Scaling to the whole heart: ischemia-related arrhythmias

MECHANISM OF ISCHEMIA-RELATED ARRHYTHMIAS—Many investigators have explored the mechanisms underlying ischemia- or reperfusion-induced arrhythmias, and a number of hypotheses have been promulgated (reviewed in Refs. 47 and 90). Both reentrant and nonreentrant mechanisms have been implicated, and the mechanism may differ for arrhythmias occurring during ischemia vs. after reperfusion. Triggered automaticity is thought to be related to cellular electrical disturbances, including early or delayed depolarization, whereas reentry has been attributed to heterogeneous conduction block in the myocardial syncytium (47). The latter can occur as a result of an anatomic obstruction (47) or dynamic instabilities related to the restitution properties of the cellular action potential (91). It has been well documented that an increase in regional action potential (AP) dispersion predisposes the heart to ventricular tachycardias and fibrillation. Although ample descriptive evidence is available about the initiation and propagation of reentrant arrhythmias, there is much less consensus about the cellular mechanisms that make the electrical substrate more vulnerable to abnormal conduction. Certainly, altered ion homeostasis is important, including extracellular K^+ accumulation, acidification of the cytosol, and Na^+ and Ca^{2+} loading during ischemia. Especially for reperfusion-induced arrhythmias, sarcolemmal K_{ATP} channels have been implicated, and K^+ channel blockers have been reported to decrease the incidence of postischemic arrhythmias (13,14). Mitochondrial $\Delta\Psi_m$ depolarization could be involved in the activation of K_{ATP} channels during ischemia and reperfusion, but few studies have investigated how or when this occurs. In a recent study, whole-heart $\Delta\Psi_m$ measurements suggested that mitochondrial depolarization and a lack of $\Delta\Psi_m$ recovery upon reperfusion may contribute to fibrillation through a mechanism that is largely insensitive to CsA (11). Thus it is possible that the mechanism of mitochondrial criticality that we have described above might contribute to these postischemic arrhythmias.

EFFECTS OF THE MITOCHONDRIAL BENZODIAZEPINE RECEPTOR

INHIBITORS ON POSTISCHEMIC ARRHYTHMIAS—Ongoing studies in our laboratory are now testing the hypothesis that failure of the mitochondrial network could be the basis of postischemic arrhythmias. With respect to the ROS-induced ROS-release mechanism, the early reperfusion phase would be particularly conducive to the mitochondrial critical state, since a burst of ROS production and antioxidant depletion is known to occur. Preliminary data from our lab employing two-photon fluorescence imaging in intact perfused hearts suggests that spatial and temporal heterogeneities of $\Delta\Psi_m$ are, in fact, present in the postischemic heart (75). The complete collapse or oscillation of $\Delta\Psi_m$ leading to modification of the cardiac action potential could potentially introduce enormous gradients of electrical excitability and repolarization across individual cells or groups of cells that have reached mitochondrial

criticality. These “islands” of inexcitability could constitute regions of metabolic block that could result in reentry and ventricular fibrillation. Recently, we tested whether inhibitors of the mitochondrial benzodiazepine receptor (mBzR), which are known to inhibit IMAC (8) and can block $\Delta\Psi_m$ oscillations by stabilizing $\Delta\Psi_m$ (3), could alter the electrophysiology of isolated perfused hearts exposed to 30 min of ischemia followed by reperfusion. Epicardial action potentials were mapped using the fluorescent voltage-sensitive indicator di-4-ANEPPS and a high-resolution photodiode array system. Although almost all (~86%) of the control hearts displayed ventricular tachycardia and/or fibrillation upon reperfusion, stable postischemic action potentials were observed in hearts treated with 4-Cl-diazepam throughout the ischemia-reperfusion protocol or given as a bolus just prior to reperfusion. Moreover, arrhythmias were observed in <10% of the treated hearts (1a).

Mitochondria as Therapeutic Targets

These data support the central hypothesis that mitochondrial ion channels play a central role in the decision between life and death. The activation of energy-dissipating mitochondrial ion channels can result in the uncoupling of oxidative phosphorylation at the level of the individual mitochondrion, which can scale to cause the failure of the mitochondrial network, loss of cardiomyocyte function, and whole-heart dysfunction (FIGURE 6). In contrast, the opening of K^+ -selective mitochondrial ion channels could protect against cell injury by activating intracellular pathways that resist the approach to the critical state. One must always keep in mind that the mitochondrial channels are probably there for physiological reasons we do not yet understand. For example, IMAC may modulate mitochondrial volume to counteract excessive swelling (9), or it may play a role in ROS-mediated intracellular signaling, so one must proceed with caution before recommending chronic inhibition to protect against ischemia-reperfusion injury.

Continued investigative effort will be required to devise ways of exploiting this newfound knowledge to develop novel therapeutic strategies. There are several challenges ahead that stand as impediments to progress in this area. First and foremost is the lack of identification of the molecular structures responsible for protection (e.g., the mitochondrial K^+ channels), mitochondrial ion homeostasis (e.g., the Ca^{2+} uniporter and Na^+/Ca^{2+} exchanger), or injury (e.g., the PTP and IMAC). With regard to the widely reproduced model of the PTP as a multiprotein complex of the ATP synthase, ANT, the inorganic phosphate carrier, VDAC, mBzR, and cyclophilin, contradictory results from knockout mice (48) and gene deletions in yeast (53,54) need to be resolved. New methods for genomic and proteomic analysis of mitochondrial targets, as well as gene-deletion techniques, will facilitate progress in this area.

A second consideration is that our recent work indicates that the collapse of $\Delta\Psi_m$ can occur through the opening of mitochondrial ion channels distinct from the classical PTP, highlighting the importance of finding out which specific conditions or factors are responsible for the opening of reversible and irreversible pores in the inner membrane. More potent and specific tools will be required to characterize existing and novel ion-conductive pores in mitochondrial membranes. A difficult, but not impossible, job lies ahead in designing compounds that can cross the surface membrane to target the mitochondria without affecting other cellular sites.

From the point of view of both basic science and therapeutic development, it will also be imperative to identify the important control sites of metabolism and ROS production and how they are remodeled by cardiovascular disease. Thus a parallel course of discovery lies ahead to answer longstanding questions in bioenergetics while focusing on the essential questions of life.

References

1. Akao M, Ohler A, O'Rourke B, Marban E. Mitochondrial ATP-sensitive potassium channels inhibit apoptosis induced by oxidative stress in cardiac cells. *Circ Res* 2001;88:1267–1275. [PubMed: 11420303]
- 1a. Akar FG, Aon MA, Tomaselli GF, O'Rourke B. The mitochondrial origin of postischemic arrhythmias. *J Clin Invest*. In press
2. Anderson SE, Murphy E, Steenbergen C, London RE, Cala PM. Na-H exchange in myocardium: effects of hypoxia and acidification on Na and Ca. *Am J Physiol Cell Physiol* 1990;259:C940–C948.
3. 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. *J Biol Chem* 2003;278:44735–44744. [PubMed: 12930841]
4. Aon MA, Cortassa S, O'Rourke B. Percolation and criticality in a mitochondrial network. *Proc Natl Acad Sci USA* 2004;101:4447–4452. [PubMed: 15070738]
5. Baines CP, Goto M, Downey JM. Oxygen radicals released during ischemic preconditioning contribute to cardioprotection in the rabbit myocardium. *J Mol Cell Cardiol* 1997;29:207–216. [PubMed: 9040035]
6. Bak, P. *How Nature Works: The Science of Self-Organized Criticality*. New York: Copernicus; 1996.
7. Balaban RS, Bose S, French SA, Territo PR. Role of calcium in metabolic signaling between cardiac sarcoplasmic reticulum and mitochondria in vitro. *Am J Physiol Cell Physiol* 2003;284:C285–C293. [PubMed: 12529248]
8. Beavis AD. On the inhibition of the mitochondrial inner membrane anion uniporter by cationic amphiphiles and other drugs. *J Biol Chem* 1989;264:1508–1515. [PubMed: 2492277]
9. Beavis AD. Properties of the inner membrane anion channel in intact mitochondria. *J Bioenerg Biomembr* 1992;24:77–90. [PubMed: 1380509]
10. Beavis AD, Lu Y, Garlid KD. On the regulation of K⁺ uniport in intact mitochondria by adenine nucleotides and nucleotide analogs. *J Biol Chem* 1993;268:997–1004. [PubMed: 8380420]
11. Berkich DA, Salama G, LaNoue KF. Mitochondrial membrane potentials in ischemic hearts. *Arch Biochem Biophys* 2003;420:279–286. [PubMed: 14654067]
12. Beutner G, Sharma VK, Giovannucci DR, Yule DI, Sheu SS. Identification of a ryanodine receptor in rat heart mitochondria. *J Biol Chem* 2001;276:21482–21488. [PubMed: 11297554]
13. Billman GE, Avendano CE, Halliwill JR, Burroughs JM. The effects of the ATP-dependent potassium channel antagonist, glyburide, on coronary blood flow and susceptibility to ventricular fibrillation in unanesthetized dogs. *J Cardiovasc Pharmacol* 1993;21:197–204. [PubMed: 7679152]
14. Billman GE, Englert HC, Scholkens BA. HMR 1883, a novel cardioselective inhibitor of the ATP-sensitive potassium channel. Part II: effects on susceptibility to ventricular fibrillation induced by myocardial ischemia in conscious dogs. *J Pharmacol Exp Ther* 1998;286:1465–1473. [PubMed: 9732412]
15. Bock J, Szabo I, Jekle A, Gulbins E. Actinomycin D-induced apoptosis involves the potassium channel Kv1.3. *Biochem Biophys Res Commun* 2002;295:526–531. [PubMed: 12150982]
16. Borecky J, Jezek P, Siemen D. 108-pS channel in brown fat mitochondria might be identical to the inner membrane anion channel. *J Biol Chem* 1997;272:19282–19289. [PubMed: 9235923]
17. Brustovetsky N, Klingenberg M. Mitochondrial ADP/ATP carrier can be reversibly converted into a large channel by Ca²⁺. *Biochemistry* 1996;35:8483–8488. [PubMed: 8679608]
18. Brustovetsky N, Tropschug M, Heimpel S, Heidkamper D, Klingenberg M. A large Ca²⁺-dependent channel formed by recombinant ADP/ATP carrier from *Neurospora crassa* resembles the mitochondrial permeability transition pore. *Biochemistry* 2002;41:11804–11811. [PubMed: 12269823]
19. Campo ML, Tedeschi H, Muro C, Kinnally KW. Effects of carbonyl cyanide phenylhydrazones on two mitochondrial ion channel activities. *J Bioenerg Biomembr* 1997;29:223–231. [PubMed: 9298707]
20. Chen W, Gabel S, Steenbergen C, Murphy E. A redox-based mechanism for cardioprotection induced by ischemic preconditioning in perfused rat heart. *Circ Res* 1995;77:424–429. [PubMed: 7614726]

21. Cortassa S, Aon MA, Marban E, Winslow RL, O'Rourke B. An integrated model of cardiac mitochondrial energy metabolism and calcium dynamics. *Biophys J* 2003;84:2734–2755. [PubMed: 12668482]
22. Cortassa S, Aon MA, Winslow RL, O'Rourke B. A mitochondrial oscillator dependent on reactive oxygen species. *Biophys J* 2004;87:2060–2073. [PubMed: 15345581]
23. Crompton M. The mitochondrial permeability transition pore and its role in cell death. *Biochem J* 1999;341:233–249. [PubMed: 10393078]
24. Debska G, Kicinska A, Skalska J, Szewczyk A, May R, Elger CE, Kunz WS. Opening of potassium channels modulates mitochondrial function in rat skeletal muscle. *Biochim Biophys Acta* 2002;1556:97–105. [PubMed: 12460666]
25. Dejean LM, Martinez-Caballero S, Guo L, Hughes C, Tejjido O, Ducret T, Ichas F, Korsmeyer SJ, Antonsson B, Jonas EA, Kinnally KW. Oligomeric Bax is a component of the putative cytochrome c release channel MAC, mitochondrial apoptosis-induced channel. *Mol Biol Cell* 2005;16:2424–2432. [PubMed: 15772159]
26. Denton RM, McCormack JG. Ca^{2+} as a second messenger within mitochondria of the heart and other tissues. *Annu Rev Physiol* 1990;52:451–466. [PubMed: 2184763]
27. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* 2002;82:47–95. [PubMed: 11773609]
28. Duchen MR. Contributions of mitochondria to animal physiology: from homeostatic sensor to calcium signalling and cell death. *J Physiol* 1999;516:1–17. [PubMed: 10066918]
29. Duranteau J, Chandel NS, Kulisz A, Shao Z, Schumacker PT. Intracellular signaling by reactive oxygen species during hypoxia in cardiomyocytes. *J Biol Chem* 1998;273:11619–11624. [PubMed: 9565580]
30. Fuchs J, Zimmer G, Thurich T, Bereiter-Hahn J, Packer L. Noninvasive fluorometric measurement of mitochondrial membrane potential in isolated working rat hearts during ischemia and reperfusion. *Methods Enzymol* 1990;186:723–729. [PubMed: 2233329]
31. Garlid KD. Cation transport in mitochondria—the potassium cycle. *Biochim Biophys Acta* 1996;1275:123–126. [PubMed: 8688444]
32. Green DR, Reed JC. Mitochondria and apoptosis. *Science* 1998;281:1309–1312. [PubMed: 9721092]
33. Griffiths EJ, Halestrap AP. Mitochondrial nonspecific pores remain closed during cardiac ischaemia, but open upon reperfusion. *Biochem J* 1995;307:93–98. [PubMed: 7717999]
34. Griffiths EJ, Ocampo CJ, Savage JS, Stern MD, Silverman HS. Protective effects of low and high doses of cyclosporin A against reoxygenation injury in isolated rat cardiomyocytes are associated with differential effects on mitochondrial calcium levels. *Cell Calcium* 2000;27:87–95. [PubMed: 10756975]
35. Grover GJ, Garlid KD. ATP-Sensitive potassium channels: a review of their cardioprotective pharmacology. *J Mol Cell Cardiol* 2000;32:677–695. [PubMed: 10756123]
36. Halestrap AP. The regulation of the matrix volume of mammalian mitochondria in vivo and in vitro and its role in the control of mitochondrial metabolism. *Biochim Biophys Acta* 1989;973:355–382. [PubMed: 2647140]
37. Halestrap AP, Connern CP, Griffiths EJ, Kerr PM. Cyclosporin A binding to mitochondrial cyclophilin inhibits the permeability transition pore and protects hearts from ischaemia/reperfusion injury. *Mol Cell Biochem* 1997;174:167–172. [PubMed: 9309682]
38. Hausenloy DJ, Yellon DM, Mani-Babu S, Duchen MR. Preconditioning protects by inhibiting the mitochondrial permeability transition. *Am J Physiol Heart Circ Physiol* 2004;287:H841–H849. [PubMed: 15072953]
39. Huser J, Blatter LA. Fluctuations in mitochondrial membrane potential caused by repetitive gating of the permeability transition pore. *Biochem J* 1999;343:311–317. [PubMed: 10510294]
40. Huser J, Rechenmacher CE, Blatter LA. Imaging the permeability pore transition in single mitochondria. *Biophys J* 1998;74:2129–2137. [PubMed: 9545072]
41. Ichas F, Jouaville LS, Mazat JP. Mitochondria are excitable organelles capable of generating and conveying electrical and calcium signals. *Cell* 1997;89:1145–1153. [PubMed: 9215636]
42. Inoue I, Nagase H, Kishi K, Higuti T. ATP-sensitive K^{+} channel in the mitochondrial inner membrane. *Nature* 1991;352:244–247. [PubMed: 1857420]

43. Jaburek M, Yarov-Yarovoy V, Paucek P, Garlid KD. State-dependent inhibition of the mitochondrial KATP channel by glyburide and 5-hydroxy-decanoate. *J Biol Chem* 1998;273:13578–13582. [PubMed: 9593694]
44. Juhaszova M, Zorov DB, Kim SH, Pepe S, Fu Q, Fishbein KW, Ziman BD, Wang S, Ytrehus K, Antos CL, Olson EN, Sollott SJ. Glycogen synthase kinase-3 β mediates convergence of protection signaling to inhibit the mitochondrial permeability transition pore. *J Clin Invest* 2004;113:1535–1549. [PubMed: 15173880]
45. Kauffman, SA. *Origins of Order: Self-Organization and Selection in Evolution*. New York: Oxford University Press; 1993.
46. Kirichok Y, Krapivinsky G, Clapham DE. The mitochondrial calcium uniporter is a highly selective ion channel. *Nature* 2004;427:360–364. [PubMed: 14737170]
47. Kleber AG, Rudy Y. Basic mechanisms of cardiac impulse propagation and associated arrhythmias. *Physiol Rev* 2004;84:431–488. [PubMed: 15044680]
48. Kokoszka JE, Waymire KG, Levy SE, Sligh JE, Cai J, Jones DP, MacGregor GR, Wallace DC. The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature* 2004;427:461–465. [PubMed: 14749836]
49. Korge P, Honda HM, Weiss JN. Protection of cardiac mitochondria by diazoxide and protein kinase C: implications for ischemic preconditioning. *Proc Natl Acad Sci USA* 2002;99:3312–3317. [PubMed: 11867760]
50. Kowaltowski AJ, Seetharaman S, Paucek P, Garlid KD. Bioenergetic consequences of opening the ATP-sensitive K⁺ channel of heart mitochondria. *Am J Physiol Heart Circ Physiol* 2001;280:H649–H657. [PubMed: 11158963]
51. Kroemer G, Dallaporta B, Resche-Rigon M. The mitochondrial death/life regulator in apoptosis and necrosis. *Annu Rev Physiol* 1998;60:619–642. [PubMed: 9558479]
52. Kushnareva Y, Murphy AN, Andreyev A. Complex I-mediated reactive oxygen species generation: modulation by cytochrome c and NAD(P)⁺ oxidation-reduction state. *Biochem J* 2002;368:545–553. [PubMed: 12180906]
53. Lohret TA, Kinnally KW. Multiple conductance channel activity of wild-type and voltage-dependent anion-selective channel (VDAC)-less yeast mitochondria. *Biophys J* 1995;68:2299–2309. [PubMed: 7544166]
54. Lohret TA, Murphy RC, Drgon T, Kinnally KW. Activity of the mitochondrial multiple conductance channel is independent of the adenine nucleotide translocator. *J Biol Chem* 1996;271:4846–4849. [PubMed: 8617754]
55. Mannella CA. Conformational changes in the mitochondrial channel protein, VDAC, and their functional implications. *J Struct Biol* 1998;121:207–218. [PubMed: 9615439]
56. Matsushita K, Morrell CN, Cambien B, Yang SX, Yamakuchi M, Bao C, Hara MR, Quick RA, Cao W, O'Rourke B, Lowenstein JM, Pevsner J, Wagner DD, Lowenstein CJ. Nitric oxide regulates exocytosis by S-nitrosylation of N-ethylmaleimide-sensitive factor. *Cell* 2003;115:139–150. [PubMed: 14567912]
57. Moran O, Sciancalepore M, Sandri G, Panfili E, Bassi R, Ballarin C, Sorgato MC. Ionic permeability of the mitochondrial outer membrane. *Eur Biophys J* 1992;20:311–319. [PubMed: 1373115]
58. Murata M, Akao M, O'Rourke B, Marban E. Mitochondrial ATP-sensitive potassium channels attenuate matrix Ca²⁺ overload during simulated ischemia and reperfusion: possible mechanism of cardioprotection. *Circ Res* 2001;89:891–898. [PubMed: 11701616]
59. Muro C, Grigoriev SM, Pietkiewicz D, Kinnally KW, Campo ML. Comparison of the TIM and TOM channel activities of the mitochondrial protein import complexes. *Biophys J* 2003;84:2981–2989. [PubMed: 12719229]
60. Murphy E. Primary and secondary signaling pathways in early preconditioning that converge on the mitochondria to produce cardioprotection. *Circ Res* 2004;94:7–16. [PubMed: 14715531]
61. Murphy RC, Diwan JJ, King M, Kinnally KW. Two high conductance channels of the mitochondrial inner membrane are independent of the human mitochondrial genome. *FEBS Lett* 1998;425:259–262. [PubMed: 9559661]

62. Nieminen AL, Byrne AM, Herman B, Lemasters JJ. Mitochondrial permeability transition in hepatocytes induced by t-BuOOH: NAD(P)H and reactive oxygen species. *Am J Physiol Cell Physiol* 1997;272:C1286–C1294.
63. O'Rourke B. Evidence for mitochondrial K^+ channels and their role in cardioprotection. *Circ Res* 2004;94:420–432. [PubMed: 15001541]
64. O'Rourke B, Ramza BM, Marban E. Oscillations of membrane current and excitability driven by metabolic oscillations in heart cells. *Science* 1994;265:962–966. [PubMed: 8052856]
65. Owen P, Dennis S, Opie LH. Glucose flux rate regulates onset of ischemic contracture in globally underperfused rat hearts. *Circ Res* 1990;66:344–354. [PubMed: 2297807]
66. Paucek P, Mironova G, Mahdi F, Beavis AD, Woldegiorgis G, Garlid KD. Reconstitution and partial purification of the glibenclamide-sensitive, ATP-dependent K^+ channel from rat liver and beef heart mitochondria. *J Biol Chem* 1992;267:26062–26069. [PubMed: 1464617]
67. Petronilli V, Miotto G, Canton M, Brini M, Colonna R, Bernardi P, Di Lisa F. Transient and long-lasting openings of the mitochondrial permeability transition pore can be monitored directly in intact cells by changes in mitochondrial calcein fluorescence. *Biophys J* 1999;76:725–734. [PubMed: 9929477]
68. Petronilli V, Miotto G, Canton M, Colonna R, Bernardi P, Di Lisa F. Imaging the mitochondrial permeability transition pore in intact cells. *Biofactors* 1998;8:263–272. [PubMed: 9914828]
69. Plas DR, Thompson CB. Cell metabolism in the regulation of programmed cell death. *Trends Endocrinol Metab* 2002;13:75–78. [PubMed: 11854022]
70. Rostovtseva T, Colombini M. VDAC channels mediate and gate the flow of ATP: implications for the regulation of mitochondrial function. *Biophys J* 1997;72:1954–1962. [PubMed: 9129800]
71. Sato T, Saito T, Saegusa N, Nakaya H. Mitochondrial Ca^{2+} -activated K^+ channels in cardiac myocytes: a mechanism of the cardioprotective effect and modulation by protein kinase A. *Circulation* 2005;111:198–203. [PubMed: 15623543]
72. Semenza GL. Regulation of mammalian O_2 homeostasis by hypoxia-inducible factor 1. *Annu Rev Cell Dev Biol* 1999;15:551–578. [PubMed: 10611972]
73. Siemen D, Loupatatzis C, Borecky J, Gulbins E, Lang F. Ca^{2+} -activated K channel of the BK-type in the inner mitochondrial membrane of a human glioma cell line. *Biochem Biophys Res Commun* 1999;257:549–554. [PubMed: 10198249]
74. Siemens A, Walter R, Liaw LH, Berns MW. Laser-stimulated fluorescence of submicrometer regions within single mitochondria of rhodamine-treated myocardial cells in culture. *Proc Natl Acad Sci USA* 1982;79:466–470. [PubMed: 6952198]
75. Slodzinski MK, Aon MA, O'Rourke B. Intracellular and intercellular mitochondrial membrane potential oscillations in the Langendorff perfused heart (Abstract). *Biophys J* 2004;86:461a. [PubMed: 14695289]
76. Sorgato MC, Keller BU, Stuhmer W. Patch-clamping of the inner mitochondrial membrane reveals a voltage-dependent ion channel. *Nature* 1987;330:498–500. [PubMed: 2446143]
77. Sornette, D. Chaos, Fractals, Selforganization and Disorder: Concepts and Tools. Berlin: Springer; 2000. Critical Phenomena in Natural Sciences.
78. Starkov AA, Fiskum G. Regulation of brain mitochondrial H_2O_2 production by membrane potential and NAD(P)H redox state. *J Neurochem* 2003;86:1101–1107. [PubMed: 12911618]
79. Suzuki YJ, Ford GD. Redox regulation of signal transduction in cardiac and smooth muscle. *J Mol Cell Cardiol* 1999;31:345–353. [PubMed: 10093047]
80. Szabo I, Bock J, Jekle A, Soddemann M, Adams C, Lang F, Zoratti M, Gulbins E. A novel potassium channel in lymphocyte mitochondria. *J Biol Chem* 2005;280:12790–12798. [PubMed: 15632141]
81. Szabo I, Zoratti M. The mitochondrial megachannel is the permeability transition pore. *J Bioenerg Biomembr* 1992;24:111–117. [PubMed: 1380498]
82. Tani M, Neely JR. Na^+ accumulation increases Ca^{2+} overload and impairs function in anoxic rat heart. *J Mol Cell Cardiol* 1990;22:57–72. [PubMed: 2157854]
83. Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol* 2003;552:335–344. [PubMed: 14561818]

84. Turrens JF, Alexandre A, Lehninger AL. Ubisemiquinone is the electron donor for superoxide formation by complex III of heart mitochondria. *Arch Biochem Biophys* 1985;237:408–414. [PubMed: 2983613]
85. Turrens JF, Boveris A. Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. *Biochem J* 1980;191:421–427. [PubMed: 6263247]
86. Vanden Hoek TL, Becker LB, Shao Z, Li C, Schumacker PT. Reactive oxygen species released from mitochondria during brief hypoxia induce preconditioning in cardiomyocytes. *J Biol Chem* 1998;273:18092–18098. [PubMed: 9660766]
87. Vander Heiden MG, Chandel NS, Li XX, Schumacker PT, Colombini M, Thompson CB. Outer mitochondrial membrane permeability can regulate coupled respiration and cell survival. *Proc Natl Acad Sci USA* 2000;97:4666–4671. [PubMed: 10781072]
88. Von Ahsen O, Waterhouse NJ, Kuwana T, Newmeyer DD, Green DR. The 'harmless' release of cytochrome c. *Cell Death Differ* 2000;7:1192–1199. [PubMed: 11175256]
89. Wang X, Yin C, Xi L, Kukreja RC. Opening of Ca^{2+} -activated K^{+} channels triggers early and delayed preconditioning against I/R injury independent of NOS in mice. *Am J Physiol Heart Circ Physiol* 2004;287:H2070–H2077. [PubMed: 15217801]
90. Wit AL, Janse MJ. Experimental models of ventricular tachycardia and fibrillation caused by ischemia and infarction. *Circulation* 1992;85:132–142.
91. Xie F, Qu Z, Yang J, Baher A, Weiss JN, Garfinkel A. A simulation study of the effects of cardiac anatomy in ventricular fibrillation. *J Clin Invest* 2004;113:686–693. [PubMed: 14991066]
92. Xu W, Liu Y, Wang S, McDonald T, Van Eyk JE, Sidor A, O'Rourke B. Cytoprotective role of Ca^{2+} -activated K^{+} channels in the cardiac inner mitochondrial membrane. *Science* 2002;298:1029–1033. [PubMed: 12411707]
93. Ylitalo KV, Ala-Rami A, Liimatta EV, Peuhkurinen KJ, Hassinen IE. Intracellular free calcium and mitochondrial membrane potential in ischemia/reperfusion and preconditioning. *J Mol Cell Cardiol* 2000;32:1223–1238. [PubMed: 10860765]
94. Zoratti M, Szabo I. Electrophysiology of the inner mitochondrial membrane. *J Bioenerg Biomembr* 1994;26:543–553. [PubMed: 7534761]
95. 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;192:1001–1014. [PubMed: 11015441]
96. Zucchi R, Yu G, Galbani P, Mariani M, Ronca G, Ronca-Testoni S. Sulfhydryl redox state affects susceptibility to ischemia and sarcoplasmic reticulum Ca^{2+} release in rat heart Implications for ischemic preconditioning. *Circ Res* 1998;83:908–915. [PubMed: 9797339]

Figure 1. Overview of oxidative phosphorylation in the cardiac cell

The sequential oxidation of fuels (e.g., fatty acids and glucose) leads to the common substrate for the Krebs cycle, acetyl-CoA, which drives the production of the reducing equivalents NADH and FADH₂. Electrons are passed to the electron-transport chain, where coupled redox reactions mediate proton translocation across the inner membrane to establish an electrical potential and pH gradient (proton-motive force) that drives ATP synthesis by the mitochondrial ATP synthase. Ion-selective or nonselective mitochondrial ion channels dissipate energy and alter the ionic balance and volume of the mitochondrial matrix, which is partly compensated by antiporters coupled to H⁺ movement. See text for further details. ANT, adenine nucleotide translocase; G-6-P, glucose-6-phosphate; IMAC, inner-membrane anion channel; MCU, mitochondrial Ca²⁺ uniporter; mitoK_{Ca}, mitochondrial Ca²⁺-activated K⁺ channel; mitoK_{ATP}, mitochondrial ATP-sensitive K⁺ channel; PIC, phosphate carrier; PTP, permeability transition pore; PYR, pyruvate; KHE, K⁺/H⁺ exchanger; NHE, Na⁺/H⁺ exchanger; NCE, Na⁺/Ca²⁺ exchanger; IDH, isocitrate dehydrogenase; KDH, α -ketoglutarate dehydrogenase; MDH, malate dehydrogenase; PDH, pyruvate dehydrogenase; SDH, succinate dehydrogenase.

Figure 2. Possible sites of mitochondrial superoxide production

Superoxide ($O_2^{\bullet-}$) can be produced as a byproduct of oxidative phosphorylation at several sites in the electron transport chain, including complex I (NADH dehydrogenase) and the Q-cycle of complex III (cytochrome bc₁). Specific metabolic poisons can help to discriminate which sites are physiologically relevant. UQ, ubiquinone; UQ[•], ubisemiquinone; UQH₂, ubiquinol.

Figure 3. Sequelae of ischemia and reperfusion

Loss of electrical and contractile function of the heart occurs rapidly after the onset of ischemia. Changes in intracellular ions and high-energy phosphates occur throughout the ischemic period, contributing to injury upon reperfusion. The time course of mitochondrial membrane potential ($\Delta\psi_m$) depolarization is not well defined, but failure to recover $\Delta\psi_m$ upon reperfusion is likely to be the main determinant of cellular life or death. A burst of reactive oxygen species (ROS) and cellular and mitochondrial Ca^{2+} overload is known to occur within the first 5 min of reperfusion. Sarck_{ATP} , sarcolemmal K_{ATP} channel; pCr, phosphocreatine.

Figure 4. Protective and destructive mitochondrial ion channels participating in the life-and-death decisions of the cell

Activation of either of two protective mitochondrial K^+ channels ($\text{mitoK}_{\text{ATP}}$ and mitoK_{Ca}) has been shown to protect the heart against ischemia and reperfusion injury, whereas the opening of the PTP or the IMAC can result in the collapse (or oscillation in the case of the latter) of $\Delta\psi_{\text{m}}$. The protective channels are known to inhibit PTP opening. This could occur either as a result of inhibition of the factors that cause PTP opening (e.g., ROS, Ca^{2+}) or perhaps by direct physical interaction with the PTP protein complex.

Figure 5. Critical behavior of the mitochondrial network induced by oxidative stress

A: images of $\Delta\Psi_m$ in an isolated guinea pig cardiomyocyte before local mitochondrial oxidative stress (*left*), after local $\Delta\Psi_m$ depolarization, but before whole-cell depolarization of the mitochondrial network (*middle*; note square region of low $\Delta\Psi_m$), and during global depolarization (*right*). B: approach to mitochondrial criticality depends on the history of oxidative stress in the mitochondrial matrix, reported by the oxidation of the mitochondrially located fluorophore, chloro-methyl dichlorofluorescein (CM-DCF). When a significant fraction ($\sim 60\%$) of the network shows evidence of elevated ROS production, a small further perturbation can lead to the catastrophic cell-wide depolarization of $\Delta\Psi_m$ (image sequence corresponds to the same time points for the images shown in A). C: time course of whole-cell $\Delta\Psi_m$ oscillation triggered by the local oxidative stress (red trace) and ROS production (indicated by the rate of change of the CM-DCF signal shown in green). Experiment was carried

out as described in Aon et al. (3) using two-photon laser scanning fluorescence imaging. *D*: the mitochondrial oscillator, as previously described in the computational modeling study of Cortassa et al. (22). SOD, superoxide dismutase; GPX, glutathione peroxidase; CAT, catalase.

Figure 6. Scaling from the level of the organelle to the organ