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Cardiac mitochondrial network excitability: insights from computational analysis

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Zhou L, O'Rourke B. Cardiac mitochondrial network excitability: insights from computational analysis. *Am J Physiol Heart Circ Physiol* 302: H2178–H2189, 2012. First published March 16, 2012; doi:10.1152/ajpheart.01073.2011.—In the heart, mitochondria form a regular lattice and function as a coordinated, nonlinear network to continuously produce ATP to meet the high-energy demand of the cardiomyocytes. Cardiac mitochondria also exhibit properties of an excitable system: electrical or chemical signals can spread within or among cells in the syncytium. The detailed mechanisms by which signals pass among individual elements (mitochondria) across the network are still not completely understood, although emerging studies suggest that network excitability might be mediated by the local diffusion and autocatalytic release of messenger molecules such as reactive oxygen species and/or Ca^{2+}. In this short review, we have attempted to described recent advances in the field of cardiac mitochondrial network excitability. Specifically, we have focused on how mitochondria communicate with each other through the diffusion and regeneration of messenger molecules to initiate and propagate waves or oscillations, as revealed by computational models of mitochondrial network.

mitochondrial energetics; reactive oxygen species-induced reactive oxygen species release; oxidative stress; cardiovascular disease; network modeling

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Introduction

Many biological systems have properties resembling an excitable medium, in which a wave of regenerative signal or activity propagates at a constant velocity without damping. In such a system, individual elements are connected to each other either physically, electrically, or chemically, so that signals can pass among neighboring regions in an ordered pattern (47). One characteristic of an excitable medium is that an impulse or stimulus beyond a certain threshold can trigger a response in a state variable much larger than the stimulus itself (i.e., regeneration). Another one is that the propagation of a wave is dependent on a diffusion-like local transport instead of simple diffusion over the whole network length. Cardiac muscle is a typical example of an excitable medium, in which cells are coupled electrotonically and the regenerative signals (currents) are supported by voltage gradients induced by the active movements of ions through sarcolemmal ion channels. Loss of excitability in an individual cardiac cell, or group of cells, is a harbinger of arrhythmias and sudden cardiac death.

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Mitochondria are the hubs of cellular metabolic and signaling pathways, which form dynamic networks with morphologies that vary by tissue (52), for example, a highly ordered lattice in cardiac cells (8, 11, 88), versus a more irregular tubular arrangement in neurons. Functionally, the mitochondrial network displays highly nonlinear, multiplicative, and collective dynamics that span wide temporal scales (i.e., from milliseconds to hours) (10). Interestingly, the mitochondrial network also exhibits excitability, mediated by intracellular secondary messengers such as Ca^{2+} and reactive oxygen species (ROS). Ichas et al. (45) reported that Ca^{2+} can induce transitory opening of the mitochondrial permeability transition pore (mPTP), evoking Ca^{2+}-induced Ca^{2+} release. Opening of the mPTP can cause release of cytochrome *c*, leading to initiation and propagation of apoptotic signals throughout the cell (71).

Work from our group showed that oscillations of mitochondrial inner membrane potential ($\Delta\Psi_m$) can be observed in either small clusters of mitochondria, synchronized throughout the whole cell (7), or as propagating waves between cells during metabolic stress (79). These events were shown to involve a mechanism that could be inhibited by ligands of the mitochondrial benzodiazepine receptor, but not by cyclosporin A (CsA), an inhibitor of mPTP (65), suggesting the involvement of mitochondrial ROS-induced ROS release (RIRR) and the activation of the inner membrane anion channel (IMAC) (5, 7, 9). Alternatively, others have found that direct laser-induced mitochondrial $\Delta\Psi_m$ fluctuations (104), as well as traveling $\Delta\Psi_m$ depolarization waves (23), are delayed or partially sup-

pressed by CsA treatment, suggesting that the mPTP may also underlie RIRR-triggered responses.

Interestingly, a detailed frequency domain analysis of $\Delta\Psi_m$ provided evidence for correlated oscillations of $\Delta\Psi_m$ spanning a wide frequency range even under physiological conditions, indicating that the mitochondrial network can display oscillatory dynamics with a varying degree of ROS-dependent coupling under many different conditions (5). The strength of coupling is likely to determine both the spatial organization of the response (i.e., how locally restricted it is) as well its amplitude and frequency. Recent studies employing multiple wavelet analysis demonstrated that the frequency of $\Delta\Psi_m$ oscillation depends inversely on cluster size (49), supporting the notion that the mechanism scales from high-frequency single mitochondrion fluctuations to whole cell periodic oscillations (64).

Taken together, these findings provide strong evidence that mitochondria form an excitable network that is able to convey signals across the cell. Various mechanistic hypotheses have been proposed to explain mitochondrial network excitability and depolarization wave propagation (11, 24, 91); however, it remains unclear how discrete subcellular loci could initiate the excitation waves and how subsets of mitochondria communicate to achieve wave propagation throughout the cell.

As an alternative tool, mathematical modeling has been increasingly used over the past few decades, alone, or in combination with experimental measurements to explore the complex behavior of biological systems. However, the application of this powerful tool to the study of mitochondrial network excitability has only recently begun. In this short review, we describe several recently published models of mitochondrial network excitability and oscillations that are based on observations of ROS- or Ca^{2+}-induced $\Delta\Psi_m$ depolarization (70, 72, 96, 98). These computational studies provide additional evidence to support the concept of mitochondrial network excitability.

Mitochondria: Important Intracellular Signaling Organelles

Mitochondria, since their discovery in the late 19th century (3, 80), have been recognized as the cell's powerhouse, producing energy to maintain various cellular functions. In addition, mitochondria function as critical signaling organelles in the delivery of signals crucial for cell survival or death (12). Mitochondria are not only a major source of ROS production but also a main target of oxidative damage. Elevated ROS accumulation and resulting mitochondrial dysfunction have been implicated in various human diseases, such as cancer, cardiovascular disease, diabetes, neurodegenerative diseases, and age-associated disease (41, 86, 89, 92, 97).

ROS source and target. Under physiological conditions, cardiac mitochondria produce over 95% of the cell's ATP requirement, which is hydrolyzed in the cytosol to support cellular mechanical work, molecular synthesis, and ion homeostasis (62). In addition to functioning as the cellular powerhouse, mitochondria are also a major site of ROS production. Even under physiological conditions, up to 0.5–2% of electrons flowing through the electron transport chain are partially reduced to form superoxide anion ($O_2^{\cdot -}$) (28). The possible electron leakage sites include complex I and complex III (60), but the precise mechanisms are still unclear. Superoxide is an unstable radical and has a short half-life; it is rapidly dismutated by superoxide dismutase (e.g., MnSOD in the mitochondrial matrix and Cu/ZnSOD in the intermembrane space and cytoplasm) to form the more stable molecule, hydrogen peroxide (H_2O_2). H_2O_2 can be further reduced to H_2O by various antioxidant enzymes such as catalase, glutathione peroxidase, and peroxiredoxin (38, 68, 69). As natural by-products of oxygen metabolism, ROS are important signaling molecules that regulate gene expression and other oxygen-sensing machineries (15, 85, 87). However, excessive ROS, caused by uncontrolled production or impaired scavenging, are toxic components due to their high reactivity with large molecules such as proteins, DNA, and lipids, leading to oxidative damage (60, 69, 89). Elevated ROS can increase the permeability of mitochondrial outer membrane, facilitating the release of cytochrome *c* that activates the cellular apoptotic process (33, 48). Moreover, oxidative stress promotes the opening of the mPTP, causing mitochondrial swelling, loss of molecules from the matrix, and membrane rupture. It has been shown that mitochondrial ROS accumulation in cells suffering from ischemia-reperfusion can cause rapid and spatiotemporally heterogeneous discharge of $\Delta\Psi_m$ (35), resulting in cell death, arrhythmias, and ischemia-perfusion injury (2). ROS overload could also cause a depletion of the intracellular redox pool (6) and decrease of ATP production (90). This would affect cellular Ca^{2+} handling, ion channel activity, and ROS-mediated signaling, which are very sensitive to intracellular redox and phosphorylation states (27, 34, 93).

Ca^{2+} handling and signaling. As a ubiquitous intracellular second messenger, Ca^{2+} is involved in the modulation of diverse cellular functions including energy metabolism, electrical signaling, fertilization, gene transcription, and programmed cell death (21). The role of Ca^{2+} as a signaling molecular is highly dependent on its "local" concentration; therefore, a precise regulation of Ca^{2+} handling is vital. Abnormal Ca^{2+} handling can contribute to the pathogenesis of various diseases such as diabetes, neurodegeneration, and cardiac disease (74, 78, 81).

In cardiomyocytes, Ca^{2+} dynamics are controlled by a complex system composed of ion channels and exchangers located at the sarcolemma and subcellular membranes, including L-type Ca^{2+} channel, Na^+/Ca^{2+} exchanger, ryanodine receptor, and sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA). Emerging evidence indicate that intracellular Ca^{2+} dynamics are also shaped by mitochondria (13, 14, 43). Mitochondria can influence Ca^{2+} cycling either directly, via inner membrane Ca^{2+} transport, or indirectly, via ROS-mediated signaling pathways. The ruthenium red-sensitive Ca^{2+} uniporter is the primary route for Ca^{2+} entry into the mitochondrial matrix (61), 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 (32) and one or more sites in the electron transport chain (16). Physiological mitochondrial Ca^{2+} uptake may cause a measurable transient mitochondrial depolarization (53), whereas mitochondrial Ca^{2+} overload can trigger mPTP opening and persistent mitochondrial depolarization. The extrusion of Ca^{2+} from the mitochondria is mediated by a mitochondrial Na^+/Ca^{2+} exchanger (61). A H^+/Ca^{2+} exchanger may also be present in noncardiac tissues, and it has also been postulated that transient mPTP opening may be a

third Ca^{2+} release mechanism (40). Under pathological conditions, mitochondria can affect Ca^{2+} handling indirectly by producing and releasing ROS close to the ion transporters involved in cytoplasmic Ca^{2+} handling. Both the L-type Ca^{2+} channel (44) and the ryanodine receptor (93) are sensitive to oxidative stress, as is the SERCA (51). We have recently found that in quiescent cardiomyocytes, mitochondrially derived ROS, released during self-sustained oscillations in $\Delta\Psi_m$, can dynamically modulate the spontaneous Ca^{2+} release from the ryanodine receptors (Ca^{2+} sparks) (99). Finally, mitochondria can function as a Ca^{2+} sink by taking up a large amount of Ca^{2+}, allowing matrix free Ca^{2+} to be kept in the micromolar per liter range, which minimizes the osmotic effects of Ca^{2+} uptake (66).

Mitochondrial Network Excitability Induced by IMAC-Mediated RIRR

Mitochondrial oscillations and RIRR: experimental observations. Under physiological conditions, intracellular ROS are maintained at very low levels by the efficient antioxidant system. However, under certain circumstances such as ischemia, hypoxia, or substrate starvation, $O_2^{\cdot-}$ and H_2O_2 concentrations can increase dramatically as the ROS scavenging capacity is overwhelmed and/or excessive oxygen intermediates are produced. Mitochondrial ROS generation is a self-amplifying process (RIRR) that can be triggered by intracellular or exogenous ROS sources (103). One mechanism of RIRR involves the activation of IMAC (a benzodiazepine-sensitive energy-dissipating channel in the inner membrane) and depolarization of $\Delta\Psi_m$. Aon et al. (7, 29) showed that a local laser flash which triggers a burst of ROS production in a small number of mitochondria is followed by synchronized, cell-wide oscillations of ROS, $\Delta\Psi$m, NADH, and GSH/GSSG. An interesting question to ask is how the perturbation of a few mitochondria triggers the global self-organized and synchronized spatiotemporal behavior. Early studies suggested that mitochondria are organized as clusters and may be electrically coupled through cable properties (4). On the contrary, other investigators have provided ample evidence that individual mitochondria can depolarize independent of their neighbors (36, 46, 79), arguing against electrical continuity. The precise mechanisms under which individual mitochondria communicate with each other to spread the ROS signals over the cellular distance remain unclear.

Based on previous studies (7, 11), a hypothesis based on RIRR and ROS diffusion was proposed. According to this hypothesis, a few initially depolarized mitochondria (e.g., triggered by local photooxidation) recruit their neighbors within a cluster through ROS diffusion and RIRR, amplifying the initial mitochondrial event. After a critical mass of mitochondria reach a threshold of oxidative stress, termed "mitochondrial criticality" (11), synchronized cell-wide oscillations occur. The basis of coupling in this model is ROS-mediated mitochondrial excitability, i.e., the local diffusion and regeneration of ROS within the mitochondrial network, along with a ROS-activated mitochondrial ion channel.

Mitochondrial oscillations: model simulation. To test the hypothesis, we recently developed a computational model of the cardiac mitochondrial network (98). The model was based on the reaction-diffusion properties of $O_2^{\cdot-}$ and the RIRR mechanism (29), and it incorporated detailed mitochondrial energetics and ROS metabolism pathways, including $O_2^{\cdot-}$ production and dismutation to H_2O_2, $O_2^{\cdot-}$ release through IMAC, activation of IMAC, and scavenging and diffusion of $O_2^{\cdot-}$ in the cytoplasm. In the model, the mitochondrial network was divided up into individual nodes (mitochondria), with each mitochondrion chemically coupled with its four nearest neighbors (Fig. 1*A*) through local ROS diffusion. The dynamics of state variables of each mitochondrion are derived from the principle of mass/ion balance. At each node (*i,j*) in the network, the dynamics are given by:

$$V(k)_{i,j}\frac{dC(k)_{i,j}}{dt} = R(k)_{i,j} + J(k)_{i,j} \quad (1)$$

where *V(k)* is the effective volume of species *k* in the mitochondria, *C(k)* is the concentration, *R(k)* is the net reaction rate and *J(k)* is the net transport rate. The extramitochondrial $O_2^{\cdot-}$ dynamics were taken to be a combination of reaction and diffusion according to the partial differential equation:

$$\frac{\partial C_{O_2^{-}i}(x,y,t)}{\partial t} = D_{O_2^{-}i}\left(\frac{\partial^2 C_{O_2^{-}i}(x,t)}{\partial x^2} + \frac{\partial^2 C_{O_2^{-}i}(y,t)}{\partial y^2}\right) + f(C_{O_2^{-}i}, t) \quad (2)$$

with the following boundary and initial conditions: $\partial C_{O_2^{-}i}(0,t)/\partial x = 0$, $\partial C_{O_2^{-}i}(X,t)/\partial x = 0$, $\partial C_{O_2^{-}i}(0,t)/\partial y = 0$, $\partial C_{O_2^{-}i}(Y,t)/\partial y = 0$, and $C_{O_2^{-}i}(x,y,0) = g(x,y)$. In *Eq. 2*, $D_{O_2^{-}i}$ is the diffusion coefficient of $O_2^{\cdot-}$ in the cytosol, and $f(C_{O_2^{-}i}, t)$ is the net extramitochondrial $O_2^{\cdot-}$ production rate (release from mitochondria minus scavenging by SOD).

This network model successfully simulated $\Delta\Psi_m$ depolarization wave propagation observed in experiments in response to oxidative stress. Specifically, the results showed that a few oscillating mitochondria (~1% of the total volume) could entrain the whole network into an oscillatory mode (Fig. 1*B*). The observations that *1*) local diffusion (1 to 2 μm distances between neighboring mitochondria) of ROS can give long range (~100 μm cellular length) global behavior and *2*) the velocity of the wave and the rate of depolarization do not decline with distance from the site of wave origin demonstrate that the cardiac mitochondrial network is an excitable medium. Another insight is that the observed dynamics of the mitochondrial network do not necessarily need to invoke physical connections between mitochondria (although some may exist), as chemical communication among mitochondrial neighbors can convey information across long distances.

In addition to reproducing the experimentally observed mitochondrial oscillations and propagation of $O_2^{\cdot-}$ waves, the model also revealed several interesting findings that may help to elucidate the mechanisms underlying the initiation and maintenance of $\Delta\Psi_m$ depolarization and $O_2^{\cdot-}$ waves.

RIRR, ROS diffusion, and wave propagation. As shown in Fig. 1*B*, the network model successfully simulated the initiation and propagation of waves of $O_2^{\cdot-}$ and $\Delta\Psi_m$ depolarization, the latter reproducing data obtained in cardiomyocytes exposed to oxidative stress. The key question that the model answers is how the local events scale to the network level. In the single mitochondrion model (29), RIRR occurs when $O_2^{\cdot-}$ produced from the electron transport chain builds up in the

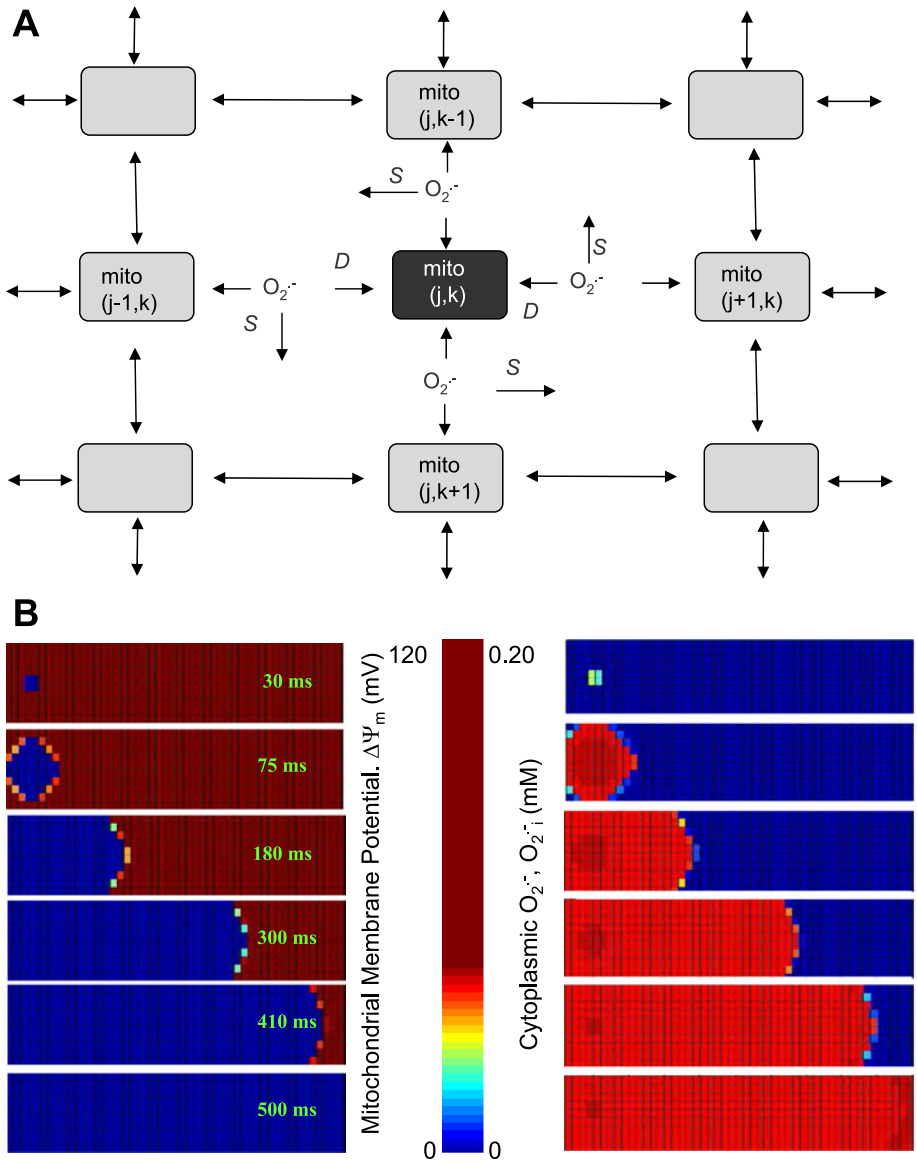

Fig. 1. *A*: scheme of the two-dimensional (2-D) reaction-diffusion reactive oxygen species (ROS)-induced ROS release (RD-RIRR) mitochondrial network model. In the RD-RIRR model, neighboring mitochondria are chemically coupled with each other through superoxide anion ($O_2^{\cdot-}$) diffusion. Light and dark gray indicate polarized and depolarized mitochondria, respectively. Arrows indicate release of superoxide anion ($O_2^{\cdot-}$) and its effect on mitochondrial neighbors. D stands for extramitochondrial $O_2^{\cdot-}$ diffusion, and S for $O_2^{\cdot-}$ scavenging by Cu/ZnSOD and catalase. *B*: simulation of spatial propagations of mitochondrial inner membrane potential ($\Delta\Psi$m) depolarization wave (*left*) and ROS wave (*right*) by the 2-D RD-RIRR model. This network consisted of 500 mitochondria (10 × 50), in which 6 were initially depolarized, whereas the others were polarized. Figure was obtained from (98) with permission.

matrix and subsequently leaks out to a level that triggers IMAC opening via cytoplasmic activation site. The increasing ion flow across the inner membrane results in the rapid collapse of mitochondrial $\Delta\Psi_m$ and a transient increase ROS generation, followed by a termination of the release due to both ROS scavenging at the activator site and depolarization of $\Delta\Psi_m$ (which decreases the $O_2^{\cdot-}$ efflux rate). This RIRR mechanism explains very well the behavior of a single mitochondrion in response to stress. However, to elicit the whole network response, this local ROS burst must reach the neighboring mitochondria at concentration above the threshold to active their IMACs. In other words, a suprathreshold level of cytoplasmic $O_2^{\cdot-}$ must be delivered from the originally perturbed mitochondrion to its neighbors for the response to be regenerative.

In the model, cytoplasmic $O_2^{\cdot-}$ surrounding a mitochondrion is governed by the following terms: $O_2^{\cdot-}$ release from mitochondrial matrix, the diffusion rate to its immediate neighbors, and the rate of scavenging by superoxide dismutase (*Eq. 2*). Increasing the diffusion rate or reducing the scavenging capacity, therefore, increases the surrounding $O_2^{\cdot-}$ concentration (up to suprathreshold levels), resulting in the activation of IMAC and the initiation of RIRR. The dependence on scavenger rate explains why spontaneous cell-wide oscillations can be triggered in saponin-permeabilized cardiomyocytes by decreasing the medium GSH-to-GSSG ratio (6). The role of ROS diffusion in supporting the propagation of ROS and $\Delta\Psi_m$ depolarization waves was suggested by simulations showing that an oscillating mitochondrion (mito_4) can entrain the oscillation of other mitochondria in the network (total 30) only when the ROS diffusion coefficient is large enough (Fig. 2). ROS diffusion-mediated mitochondrial network excitability can be further demonstrated by simulations shown in Fig. 3, which show the effect of $O_2^{\cdot-}$ produced by the perturbed mitochondrion (i.e., high ROS production) on the entrainment of $\Delta\Psi_m$ depolarization in other mitochondria that were not initially oxidatively stressed (i.e., they were initialized with low rates of ROS production). When $O_2^{\cdot-}$ produced by the perturbed mitochondrion is relatively low (0.0018 mM), the $O_2^{\cdot-}$ surrounding its neighbors is low as well and does not

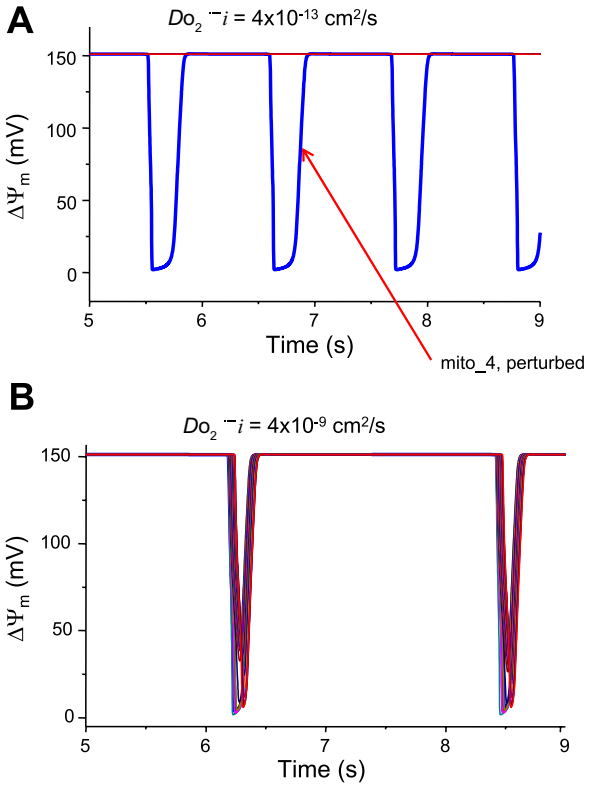

Fig. 2. Effect of ROS coupling on the entrainment of mitochondrial network (30 mitochondria in a row) oscillations. Model simulations show that the diffusion (or coupling) of $O_2^{\cdot-}$ among neighboring mitochondria must be big enough to ensure a fast diffusion rate that can deliver enough amount of $O_2^{\cdot-}$ from the perturbed mitochondrion (mito_4) to its immediate neighbors to activate their inner membrane anion channels (IMACs). *A*: when the $O_2^{\cdot-}$ diffusion coefficient is low (4×10^{-13} cm^2/s), the network depolarization and oscillations cannot be entrained. $D_{O_2^{\cdot-}}i$ is the diffusion coefficient of $O_2^{\cdot-}$ in the cytosol. *B*: when the diffusion coefficient is 10^4 times higher (4×10^{-9} cm^2/s), mito_4 can induce whole network depolarization and oscillations. In these 2 simulations, all model parameters are the same except the $O_2^{\cdot-}$ diffusion coefficient.

reach the threshold for activation of IMAC; therefore, propagation and entrainment of the network (30 mitochondria in total) does not occur. When the perturbed mitochondrion produces more $O_2^{\cdot-}$ (0.02 mM), the $O_2^{\cdot-}$ reaching its neighbor is sufficiently high (0.008 mM) to activate the IMAC, consequently eliciting whole network oscillations. Finally, as cytoplasmic $O_2^{\cdot-}$ is also governed by scavenging capability, network oscillation and propagation only occur when etSOD (concentration of cellular superoxide dismutase) is in the appropriate range. Therefore, it is the interplay between the production, scavenging, and diffusion of $O_2^{\cdot-}$ that determines whether a local perturbation can trigger a network response, i.e., the network excitability. When all parameters are in the right ranges such that the network is close to its criticality, perturbing even a single mitochondrion can induce cell-wide mitochondrial depolarization (98).

Mitochondrial excitability: network versus single mitochondrion. In the single mitochondrion model of RIRR developed by Cortassa et al. (29), oscillation occurs when there is a bifurcation in the dynamics of the model, creating upper (polarized) and lower (depolarized) branches of the steady state. Stability analysis revealed that the mitochondrion could exhibit oscillatory behavior with stable limit cycles only when key parameters are within the oscillatory domain (e.g., small ROS scavenging, etSOD, or large ROS production, shunt); outside these domains, the mitochondrion displays either a stable depolarized or polarized state. For the network model, however, entrainment of mitochondria that would not normally oscillate is possible (98). This situation more closely resembles evidence from intact cardiomyocytes where we observe that the network behaves as a system of oscillators with a variable degree of ROS-dependent coupling as one moves from the physiological to the pathophysiological state (8). In other words, individual mitochondria might be oscillating at high frequencies and low amplitudes at all times but become entrained under stress to produce network-wide large amplitude, slow oscillations. The transition to synchronized oscillations has significant consequences because it induces large changes in the cellular ATP-to-ADP ratio and much larger bursts of ROS, which could impact multiple targets in the cell. As shown in Fig. 3, a high ROS-producing mitochondrion located at the center of a row of 30 mitochondria can entrain the oscillations of other low ROS-producing mitochondria, as long as ROS diffusion is rapid enough between neighboring mitochondria (determined by the diffusion coefficient). The summation of the responses of the source mitochondrion and the target mitochondria result in the $O_2^{\cdot-}$ concentration surrounding the low ROS-producing mitochondria to increase dramatically compared with those without ROS-dependent coupling. The elevated ROS, upon reaching the critical threshold, activates RIRR in the follower mitochondria and entrains $\Delta\Psi_m$ oscillations. Therefore, a mitochondrion does not necessarily need to be in an "oscillatory parametric domain" to oscillate; the determining factor is the surrounding ROS concentration. The source of this ROS could be either endogenous (produced from the electron transport chain and exported through the IMAC) or exogenous (e.g., diffusing from neighboring mitochondria or extramitochondrial sources of ROS). For example, an addition of 20 μM KO_2 (98) or depletion of cytoplasmic-reduced glutathione (6) can cause mitochondrial depolarization and/or $\Delta\Psi_m$ oscillation in partially permeabilized cardiomyocytes.

Taking the question of how $\Delta\Psi_m$ loss in individual mitochondria (flickering) scales to produce synchronization at the whole cell level one step further, Nivala et al. (64) recently developed a Markov gating model of IMAC incorporated into individual mitochondria, which were then organized into a network of mitochondria. Stochastic gating of the channel resulted in flickering of $\Delta\Psi_m$, and when the mitochondria were initialized with high $O_2^{\cdot-}$ production rates, coupling occurred in the network, resulting in propagating waves and slower periodic oscillations at the whole cell level. This study illustrates how a seemingly random high-frequency process at the nanoscale level can lead to self-organized criticality through coupling in the network.

Other potential mechanisms participating in IMAC-mediated mitochondrial oscillations. The model simulations provide supporting evidence that local $O_2^{\cdot-}$ regeneration and diffusion are central to the propagation of ROS and $\Delta\Psi_m$ depolarization waves in the mitochondrial network. Our model of RIRR is centered on the ROS-dependent activation of IMAC, based on our experimental observations that RIRR induced by substrate deprivation (65), local laser excitation (7), or GSH depletion (6) is inhibited by benzodiazepine receptor

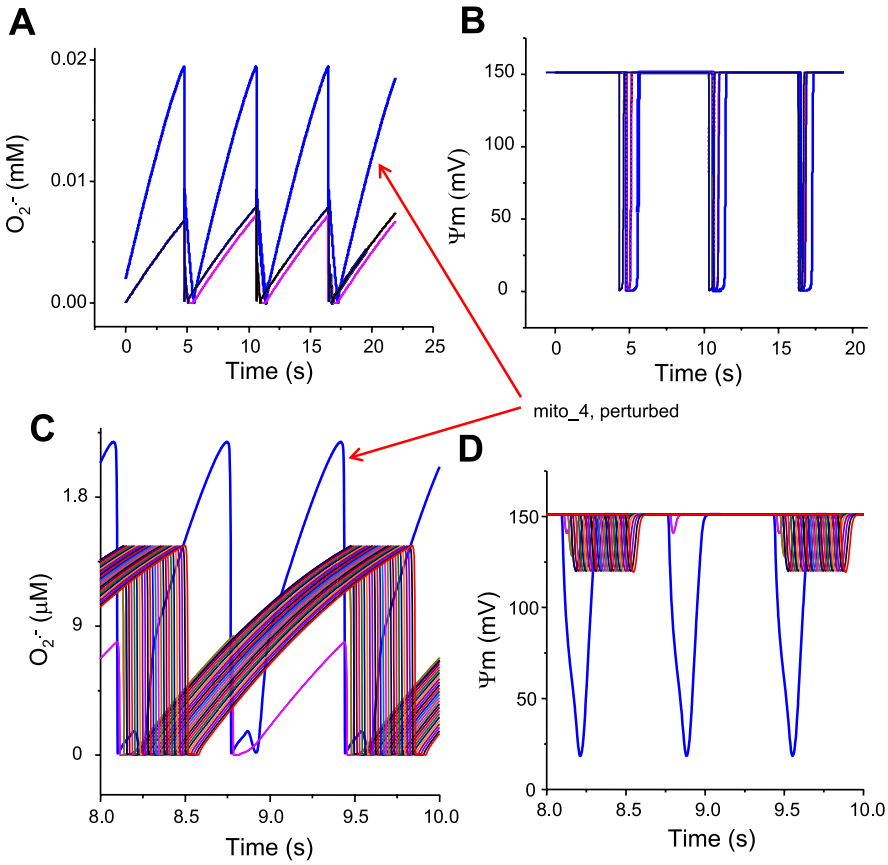

Fig. 3. Effect of $O_2^{\cdot-}$ produced by the mito_4 on the network (30 mitochondria in total) entrainment. *A*: cytoplasmic $O_2^{\cdot-}$ level surrounding (and produced by) the perturbed mitochondrion is high (0.02 mM); as a result, the $O_2^{\cdot-}$ arriving the adjacent mitochondria is above the threshold (0.008 mM) to activate their IMACs and entrain the whole network oscillations (*B*). *C*: cytoplasmic $O_2^{\cdot-}$ level surrounding (and produced by) the mito_4 is relatively low (0.0022 mM), and the $O_2^{\cdot-}$ reaching its neighbors is below the threshold level (0.0015 mM); thus it cannot entrain the full depolarization of the network (*D*).

ligands, which block IMAC in isolated mitochondria (17), but does not involve mPTP activation (7). IMAC is permeable to a variety of inorganic (e.g., Cl^- and P_i) and organic (e.g., $citrate^{3-}$, $malate^{2-}$ and ATP^{4-}) anions (17) and has properties similar to the 108pS conductance anion channel found in mitoplast patch-clamp studies (22). Other potential IMAC modulatory factors might also be suggested by these early studies. For example, Beavis and Powers (20) showed that in isolated mitochondria, IMAC is inhibited by Mg^{2+} from both the inside and the outside of the membrane, and this regulation is dependent on the matrix pH. However, the assay procedure they used to activate IMAC-mediated mitochondrial swelling was to totally deplete free Mg^{2+} from the mitochondrial matrix (18), which is unlikely to occur in the living cells. In addition, the inhibitory effect of protons and Mg^{2+} is highly sensitive to the temperature, suggesting that IMAC is poised to respond to small changes in pH or Mg^{2+} under more physiological conditions (19).

With respect to the observed RIRR-mediated mitochondrial oscillations, there is no direct experimental evidence to implicate free Mg^{2+} depletion and pH changes as a trigger of the RIRR, at least under our experimental conditions. Although they are not essential players in the fundamental oscillator mechanism, changes in both ions and energetics do occur during $\Delta\Psi_m$ oscillations and they are likely to modulate either the amplitude or frequency of oscillations, as suggested by a recent study (98). It is essential to incorporate the modulation of IMAC by these factors in future modeling studies, pending additional experimental support.

Mitochondrial Network Excitability Induced by mPTP-Mediated RIRR

In addition to the IMAC-mediated pathway, RIRR might also be triggered by the ROS-activated opening of the mPTP, as originally described by Zorov et al. (103) for direct laser excitation of individual mitochondria. A slow depolarization wave (<0.1 μm/s) associated with mPTP activation was also described by Brady et al. (23), that is, slow compared with the propagation rate of RIRR measured in studies from our group (~25 μm/s) (7, 11). The mPTP-mediated RIRR has been implicated in both the preconditioning and the apoptotic pathways in cardiac cells (46, 83). mPTP activation has been invoked to explain these phenomena, as well as the more recent observations of $O_2^{\cdot-}$ transients in single mitochondria (94). However, the effects of CsA on these responses are to delay (103) or decrease the frequency of the $\Delta\Psi_m$ depolarizations (94), and other investigators have observed no CsA sensitivity (76). Moreover, these groups have found that Ca^{2+} is not required to activate the mPTP via RIRR, leaving the nature of the $\Delta\Psi_m$ depolarization somewhat unclear.

These differences in mechanistic interpretation need to be resolved in future studies, but it is likely that more than one energy-dissipating ion channel can mediate transient $\Delta\Psi_m$ depolarization. In addition, there may be synergistic interactions among different channels. For instance, IMAC and mPTP can be activated in a sequential manner by oxidation of the GSH pool in cardiac cells (6), so it is important to consider the cooperative effects of both channels in the context of RIRR

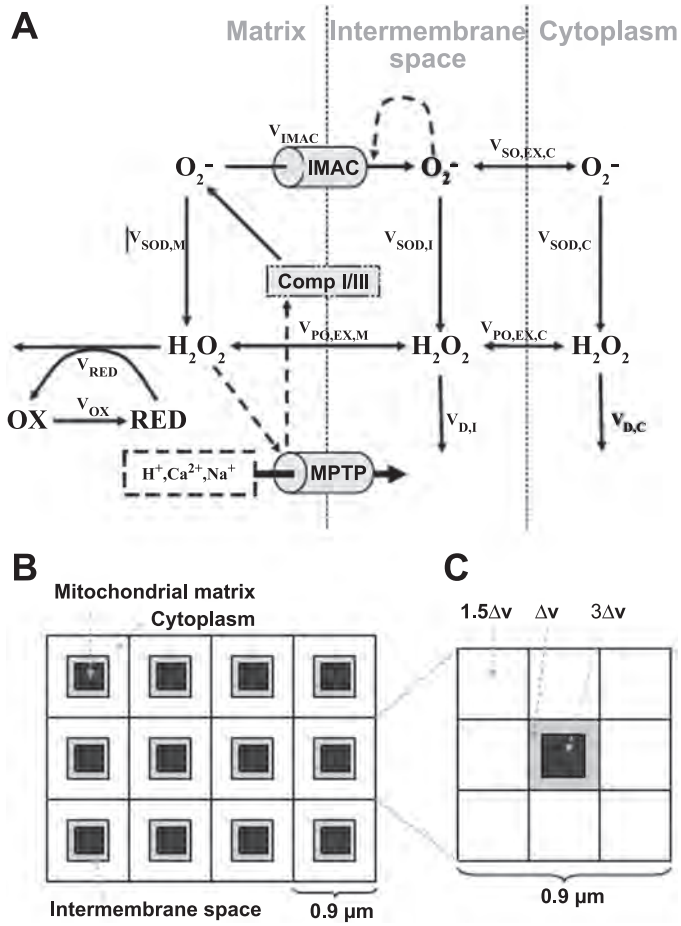

Fig. 4. Scheme of mitochondrial model that includes both IMAC-mediated RIRR and mitochondrial permeability transition pore (mPTP)-mediated RIRR. In this model, mitochondria are coupled via ROS diffusion in a spatially extended 2-D array of voxels, each containing the single mitochondrion model, the intermembrane space, and cytoplasmic volumes. Reproduced from (96).

mitochondrial network excitability. Yang et al. (96) developed a mitochondrial model that included both IMAC-mediated RIRR and mPTP-mediated RIRR. In their model, mitochondria are coupled via ROS diffusion in a spatially extended two-dimensional array of voxels, each containing the single mitochondrion model, the intermembrane space, and cytoplasmic volumes (Fig. 4). The IMAC-mediated RIRR was modeled using the same strategy as that developed by Cortassa et al. (29). To simulate the mPTP-mediated RIRR, the authors assumed that the accumulation of downstream product of antioxidant pathways, such as hydroxyl radicals (modeled as the linear function of H_2O_2), activates the mPTP opening, leading to $\Delta\Psi_m$ depolarization and RIRR. In this model, both $O_2^{\cdot-}$ and H_2O_2 were allowed to diffuse freely between the intermembrane space and the adjacent cytoplasmic space.

The authors showed that their model was able to simulate both IMAC-mediated and mPTP-mediated mitochondrial oscillations and depolarization wave propagation, depending on how much $O_2^{\cdot-}$ production (K_{shunt}) was induced by external factors. With relatively low K_{shunt} (e.g., <0.105 mM/s), mitochondria exhibited limit cycle oscillations that were regulated by IMAC-mediated RIRR, whereas when K_{shunt} was higher (e.g., 0.25 mM/s), the accumulated H_2O_2 triggered the complete opening of mPTP, forming a positive feedback loop that leads to bistability. The lower excitability of mPTP-mediated RIRR was perhaps due to the higher H_2O_2 scavenging rate (compared with $O_2^{\cdot-}$), as increasing H_2O_2 degradation rate significantly reduced the velocity of wave propagation. When scaled up to the mitochondrial network, these two dynamics resulted in different spatiotemporal behaviors in the cell. Specifically, the IMAC-mediated RIRR, induced by increasing ROS production in a small area in the center of the network, triggered synchronized cell-wide $\Delta\Psi_m$ oscillations. The resulting depolarization wave propagated at a speed of ~25 μm/s that was comparable with that observed by Aon et al. (11). The mPTP-mediated RIRR, induced by increasing ROS production (K_{shunt}) in all mitochondria in the network, led to a $\Delta\Psi_m$ depolarization wave that propagated much more slowly (~2.1 μm/s). Because of the self-amplifying nature of the RIRR process, opening of mPTP eventually led to irreversible $\Delta\Psi_m$ depolarization. The authors also simulated the effect of increasing K_{shunt} (as a linear function of time) on the network dynamics to mimic a pathological condition of progressive depletion of antioxidant capability or damage to the distal electron transport chain. They showed that under this condition, a series of mitochondrial oscillations evoked by IMAC-mediated RIRR were observed at the first, which then developed into a slow depolarization wave as a result of mPTP-mediated RIRR (Fig. 5). These results indicate that the mitochondrial network can exhibit two different types of excitabilities, fast waves induced by the IMAC-mediated RIRR (triggered by $O_2^{\cdot-}$) and slower waves caused by mPTP-mediated RIRR (triggered by H_2O_2) processes, respectively. These two processes could occur alone or together, depending on the

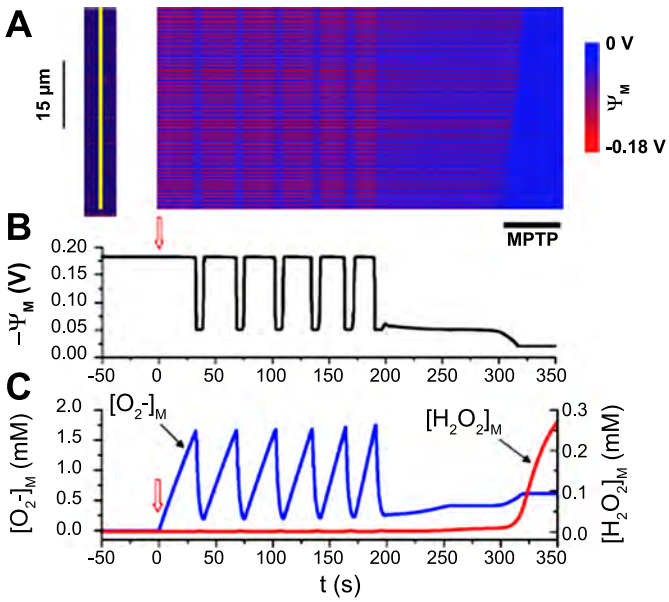

Fig. 5. IMAC-mediated ΔΨm oscillations triggering an mPTP-mediated final depolarization wave. *A*: space-time plot of ΔΨm recorded along the yellow line (*left*). $O_2^{\cdot-}$ production rate increased progressively over time, most rapidly in the lower 4 rows of mitochondria {where k_{shunt} = 0.35[0.3 + 0.7 min $(1,e^{0.02t}/150)$]} and more slowly elsewhere {k_{shunt} = 0.2[0.3 + 0.7 min $(1,e^{0.02t}/150)$]}. *B*: average ΔΨm vs. time. *C*: mitochondrial $O_2^{\cdot-}$ (blue) and H_2O_2 (red) concentrations vs. time. k_{shunt} was initially zero and started to increase at the upward arrows. Black bar below the snapshot indicates the final mPTP-mediated slow wave. Reproduced from (96).

oxidative status of the cell. This model of differential sensitivity of IMAC and mPTP to different ROS remains hypothetical at present, and other factors may contribute to coupling between IMAC and mPTP. In this regard, it is very likely that disturbances of the Ca^{2+} handling subsystem associated with mitochondrial $\Delta\Psi_m$ depolarization and the concomitant oxidative stress (99) would predispose the mPTP to open during sustained RIRR, as suggested by experimental observations (6). It should be emphasized that the details of the mechanism of activation of energy-dissipating ion channels in the mitochondrial inner membrane are not well understood and the molecular structures of IMAC and PTP have not been determined. For example, PTP activation in isolated mitochondria is strictly dependent on Ca^{2+} as the trigger, although its sensitivity is altered by the redox state. Limitations of available ROS probes also make it impossible to quantitatively measure local ROS concentrations near the mitochondrial inner membrane owing to the robust scavenger systems present in the matrix and the intermembrane space. Hence, certain aspects of the proposed models will require additional investigation to refine them in the future.

Agent-Based Model of Mitochondrial Network Excitability

More recently, to determine the role of ROS (i.e., $O_2^{\cdot -}$ and H_2O_2) in the mediation of mitochondrial network excitability, Park et al. (72) developed a mathematical model of the mitochondrial network using an agent-based modeling (ABM) approach. The ABM technique has a rule-based modeling format that determines the probabilities of a transition to the next state at every step; therefore, it can be used to simulate interactions between agents in a complex system. Their simulations showed that mitochondrial network organization (i.e., the mitochondrial spatial distribution in the cytosol or the number of mitochondria in the cell) could significantly affect intracellular ROS propagation profiles and cellular sensitivity to oxidative stress. This was achieved by incorporating differential effects of specific free radicals (i.e., $O_2^{\cdot -}$ and H_2O_2) that have an impact on inter-mitochondrial communication. Remarkably, they showed that increasing the superoxide dismutase activity, either in the mitochondria or in the cytosol, significantly reduced the reactivity of mitochondria in a regular lattice and blocked ROS propagation, but this protective effect was largely abolished when the mitochondrial network was irregularly distributed. In contrast, raising the rate of cytosolic H_2O_2 decomposition capability had a protective effect on the irregular mitochondrial network but did not protect the lattice-like network. These results indicate that there is a correlation between mitochondrial organization and ROS signaling and suggest that in a regular lattice-like mitochondrial network, such as in cardiomyocytes, $O_2^{\cdot -}$ is the major species the regulates mitochondrial excitability, whereas in irregularly distributed mitochondria, such as in neurons, H_2O_2 may play a larger role in mediating RIRR and mitochondrial signal propagation. The switch between $O_2^{\cdot -}$-triggered RIRR and H_2O_2-triggered RIRR, depending on mitochondrial network organization, is probably determined by differences in the kinetics, diffusability, and lifetime of these two messenger molecules. H_2O_2 is more stable compared with $O_2^{\cdot -}$ and can also diffuse through membranes; therefore, it can travel much farther in the cytosol and between cells.

Mitochondrial Excitability Induced by mPTP-Mediated Mitochondrial Ca^{2+}-Induced Ca^{2+} Release

Mitochondrial excitability through Ca^{2+}-induced Ca^{2+} release (mCICR), propagated by mPTP opening, has been proposed by Ichas et al. (45). In their scheme, transitory opening of mPTP in a reversible low-conductance mode can dissipate the transmembrane potential, thus releasing any stored Ca^{2+} in the matrix. This Ca^{2+} would then be taken up by neighboring mitochondria, triggering mPTP and propagating the signal (31, 45). To simulate the experimentally observed mCICR, Selivanov et al. (82) developed a kinetic model of mitochondria that composes of Ca^{2+} uniporter, pH-gated mPTP, and respiratory chain. This simplified model successfully simulates all mCICR profiles observed experimentally, as well as the notable triggering characteristics of mCICR (82). To better understand the role of mPTP in the propagation of waves of depolarization and Ca^{2+} release, Oster et al. (70) modified the Magnus-Keizer model (55–57) by incorporating the PTP (75, 82) and mitochondrial pH buffering systems. In their model, the low conductance mPTP opening (PTP_l) is assumed to be initialized by pH changes (H_m).

$$\frac{dPTP_l}{dt} = \frac{[PTP_l^{\infty}(H_m) - PTP_l]}{\tau_l(H_m)} \quad (3)$$

where τ_l is the time constant and PTP_l^{∞} is the opening rate. To simulate the traveling of depolarization and Ca^{2+} waved observed by Ichas et al. (45), mitochondria were assumed to be spatially coupled through the cytosolic Ca^{2+} diffusion described by Fick's law. The cytoplasmic (extramitochondrial) Ca^{2+} dynamics (Ca_c) were modeled as

$$\frac{\partial}{\partial t}Ca_c(x,t) = f_c\left\{ D_{Ca}\frac{\partial^2}{\partial x^2}Ca_c(x,t) + \frac{1}{v_c\tau_{\min}} \left[M\left(J_{na,ex}^{Ca} - J_{uni}^{Ca} - J_{PTP}^{Ca}\right) + J_{stim}^{Ca}\right]\right\} \quad (4)$$

where D_{Ca} is the cytosolic Ca^{2+} diffusion coefficient and J represents individual Ca^{2+} fluxes (via Na^+/Ca^{2+} exchanger, uniporter, PTP, and stimulus, respectively). Their model, with the inclusion of mPTP, successfully simulated the excitable behavior of mitochondria demonstrated by Ichas et al. (45), i.e., the traveling of Ca^{2+} and depolarization waves across the length of the domain. Interestingly, they showed that mCICR-mediated mitochondrial excitability does not require Ca^{2+} efflux through the permeability transition pore, since Ca^{2+} can flow out of mitochondria via the uniporter (operating in reverse) (39, 58, 61) and the slower mitochondrial Na^+/Ca^{2+} exchanger. In addition, model simulations demonstrated that the pH buffering capability in the mitochondrial matrix is important in shaping the resulting dynamics, and the cyclical variations in the mitochondrial pH can lead to PTP oscillations. This is not surprising since in their model PTP is explicitly gated by proton gradient. Moreover, they proposed a heuristic model for how prolonged Ca^{2+} overload can induce nonreversible mPTP openings.

It is worth pointing out that the experiments of Ichas et al. (45) were done on isolated mitochondria suspended in gels that lack extramitochondrial Ca^{2+} buffering or nearby rapid Ca^{2+} sequestering pathways such as sarco(endo)plasmic reticulum uptake or plasmalemmal Ca^{2+} transporters and involve Ca^{2+}

pulses well above the physiological range (>60 μM). Thus there is still little experimental evidence to indicate that mCICR-mediated mitochondrial excitability occurs in intact cells or in vivo. In fact, most studies of RIRR-mediated propagation have shown that Ca^{2+} is not required for $\Delta\Psi_m$ depolarization to be propagated (7, 103), and there is evidence that enhanced Ca^{2+} release from neighboring SR stores (Ca^{2+} sparks) is a consequence, rather than a cause, of $\Delta\Psi_m$ depolarization in the resting, nonbeating cardiomyocytes. Nevertheless, since oxidative stress sensitizes the mPTP to Ca^{2+} activation as mentioned above, it is possible that there is an interaction between RIRR and mCICR such that the propagation of $\Delta\Psi_m$ depolarization has a mixed mechanism when intracellular Ca^{2+} overload occurs. Although cross talk between Ca^{2+} and ROS signaling pathways has been discussed intensively (30, 37, 95), more work is required to gain a complete understanding of the interactions between intracellular Ca^{2+} handling and mitochondria.

Mitochondrial Network Excitability and Cardiac Pathology

These network model simulations, in combination with experimental data, suggest that the mitochondrial network can serve as an intracellular signal amplifier by scaling up local processes to produce emergent macroscopic behaviors such as sustained oscillations, damped oscillations, bistability, or waves. Signaling molecules such as ROS, nitric oxide, Ca^{2+}, and ADP play a prominent role in modulating the network properties, and these factors are known to change significantly in pathological situations. Ischemia-reperfusion injury, for example, is known to involve increased ROS accumulation, especially during the restoration of coronary flow and the concomitant increase in mitochondrial respiration. The increase in oxidative stress during and after ischemia can lead to rapid loss of ΔΨm in the whole heart (54, 84), along with changes in the cytoplasmic phosphorylation potential (100). Changes in the intracellular redox environment will trigger redox cascades that alter the signal transduction pathways, ion transport processes, and Ca^{2+} handling systems of the cell (1, 93). A well-known effect of a decrease in ATP-to-ADP ratio is the activation of sarcolemmal ATP-sensitive K^+ (K_{ATP}) channels, causing shortening of action potential duration (Fig. 6) (2, 63, 67, 100), and this will profoundly affect the electrical properties of the myocardial syncytium. Oxidative stress also influences cytosolic Ca^{2+} transients, as many enzymes involved in Ca^{2+} handling pathways are sensitive to intracellular redox state and energy state, such as the ryanodine receptors and the SERCA pump (102). During whole cell metabolic oscillations, our group has previously shown that Ca^{2+} transients are suppressed during the phase of NADH oxidation (67). On the other hand, inhibition of sarcoplasmic reticulum Ca^{2+} uptake by oxidative stress has been shown to induce larger cytoplasmic Ca^{2+} transients in neurons (73). The implication is that the collapse of $\Delta\Psi_m$ in a small number of depolarized mitochondria can scale to the level of the mitochondrial network and then to the whole heart because of the multiplicative interdependency of the bioenergetic, electrophysiological, and Ca^{2+} handling subsystems. This has been demonstrated in several experimental studies in which ventricular arrhythmias were induced by ischemia-reperfusion (2, 25), ROS exposure (42, 50, 59), or depletion of intracellular reduced glutathione (26). Treatment with ROS scavengers or mitochondrially targeted antioxidant peptides, or blocking IMAC during the metabolic stress (2, 25, 26), can significantly decrease the incidence of cardiac arrhythmias.

Understanding the role of the cardiac mitochondrial network in intracellular signaling under both physiological and pathological conditions requires a multiscale (77) and ultimately a multicellular modeling approach. This will be extremely important in developing a detailed mechanistic knowledge of how the failure of the energy generation, combined with redox-dependent modulation of ion transporters and channels, contributes to arrhythmias. To that end, expanding on our integrated cardiac cell models of RIRR (100), we have recently developed two-dimensional models of the cardiac syncytium to study the impact of $\Delta\Psi_m$ loss on electrical propagation (101). Preliminary results show that mitochondrial depolarization in a region of a cardiac cell monolayer through the activation of sarcolemmal K_{ATP} currents, results in shortening of the action potential, dispersion of repolarization, and increased vulnerability to reentry, providing further motivation to explore novel therapeutic strategies based on manipulation of mitochondrial inner membrane ion fluxes.

Summary

Mitochondria form dynamic networks, both morphologically and functionally, to generate energy, as well as to trans-

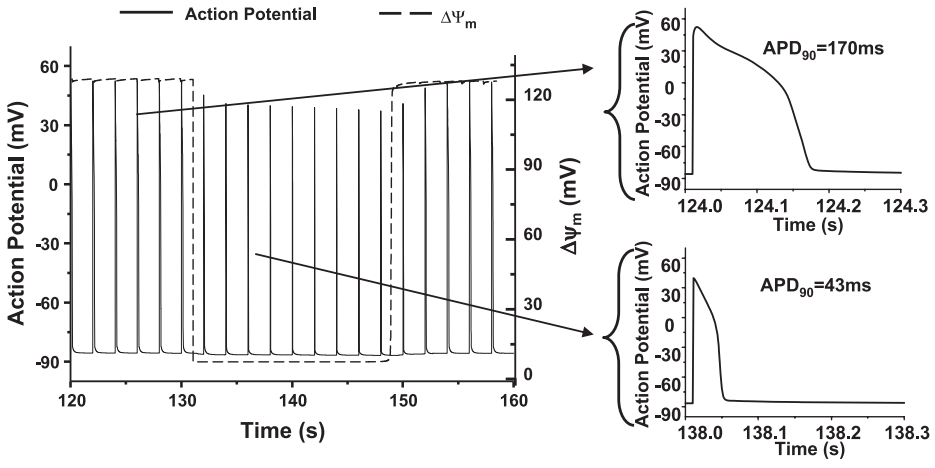

Fig. 6. Model simulated shortening of action potential during mitochondrial depolarization. When mitochondria are polarized, the action potential duration (APD) is 170 ms, whereas when mitochondria depolarize, the APD decreased to 43 ms, representing about 75% shortening. Reproduced from (100).

mit critical intracellular signals. To investigate how mitochondria communicate with each other to initiate and propagate these signals, an increasing number of experimental and computational studies have been carried out over the past decades. Whereas more studies need to be done to understand the precise mechanism(s) behind propagated mitochondrial $\Delta\Psi_m$ and redox waves, these studies clearly demonstrate that mitochondrial network excitability can be mediated by various messenger molecules. Our studies have focused on a mechanism of RIRR mediated by IMAC, which, in the regular lattice-like arrangement of mitochondria in the cardiomyocyte can support wave propagation, K_{ATP} channel current activation and oscillation through local $O_2^{\cdot-}$-mediated coupling. Alternatively, H_2O_2 may transmit mitochondrial ROS effects over longer distances. In addition, mitochondrial excitability can also be evoked by mCICR and the mPTP under certain circumstances. Despite differences in the type of messenger molecule and targets implicated in different studies, the common finding is that the local diffusion of messenger molecules, in the range of few micrometers (mitochondrion size) can elicit a much longer range (~100 μm, cellular size) synchronized behavior. In addition, the velocity of the wave and the rate of depolarization do not decline with distance from the site of wave origin, supporting a regenerative model that involves diffusive signaling between neighboring mitochondria. The results of model simulations also reinforce the concept that intermitochondrial communication represents an effective means to ensure progressive spreading of signals throughout the network.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: L.Z. and B.O. conception and design of research; L.Z. prepared figures; L.Z. drafted manuscript; L.Z. and B.O. edited and revised manuscript; L.Z. and B.O. approved final version of manuscript.

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