

REVIEW

Mitochondrial mechanosensor in cardiovascular diseases

Cristina Caffarra Malvezzi¹, Aderville Cabassi¹ and Michele Miragoli^{1,2,3}

¹Department of Medicine and Surgery, University of Parma, Parma, Italy

²Center of Excellence for Toxicological Research, Department of Medicine and Surgery, University of Parma, Parma, Italy

³Department of Cardiovascular Medicine, Humanitas Clinical and Research Center - IRCCS, 20090 Rozzano, Milan, Italy

Correspondence should be addressed to M Miragoli: michele.miragoli@unipr.it

Abstract

The role of mitochondria in cardiac tissue is of utmost importance due to the dynamic nature of the heart and its energetic demands, necessary to assure its proper beating function. Recently, other important mitochondrial roles have been discovered, namely its contribution to intracellular calcium handling in normal and pathological myocardium. Novel investigations support the fact that during the progression toward heart failure, mitochondrial calcium machinery is compromised due to its morphological, structural and biochemical modifications resulting in facilitated arrhythmogenesis and heart failure development. The interaction between mitochondria and sarcomere directly affect cardiomyocyte excitation-contraction and is also involved in mechano-transduction through the cytoskeletal proteins that tether together the mitochondria and the sarcoplasmic reticulum. The focus of this review is to briefly elucidate the role of mitochondria as (mechano) sensors in the heart.

Key Words

- ▶ mechanoelectric feedback
- ▶ calcium handling
- ▶ arrhythmias
- ▶ microtubules
- ▶ oxidative stress

Introduction

Cardiovascular diseases (CVDs), with almost 18 million deaths per year, are the first cause of death globally (World Health Organization). The heart is one of the most energy-demanding organs in the body, due to its intrinsic nature to continuously pump blood into the systemic and pulmonary circulation. The heart modulates its function via a plethora of physiological and metabolic processes, necessary to supply oxygen, biochemical and biomolecular signals, and metabolites to the entire body (1). One of those physiological processes is the mechano-transduction that, via several actors like stretch-activated channels (SACS (2)), the sarcoplasmic reticulum (SR) (3), ryanodine receptor (RyR (4)), structural proteins (5) oxidative species (6), hormones (7, 8), and ion channels (9), modulate calcium signaling in the myocytes and the pumping function of the heart.

The cardiomyocytes, along with fibroblasts, endothelial cells, and other cells that form the heart, host the majority of most mitochondria when compared to all the other cells in the entire body (10). The role of mitochondria in cardiac diseases has been extensively studied both *in vitro* and *in vivo*, by mainly focusing on mitochondrial structural, functional, and metabolic remodeling (11). However, with respect to calcium-based contraction machinery, clear evidence showed that mitochondria 'sense' the mechanics of the heart and, by a complex mechanoelectric and mechanochemical feedback process, adjust their modulation of intracellular calcium homeostasis (12). Deciphering the mechanosensing features of mitochondria is challenging, as only a few instruments and approaches can localize or stimulate mitochondria in live cardiomyocytes (Fig. 1).

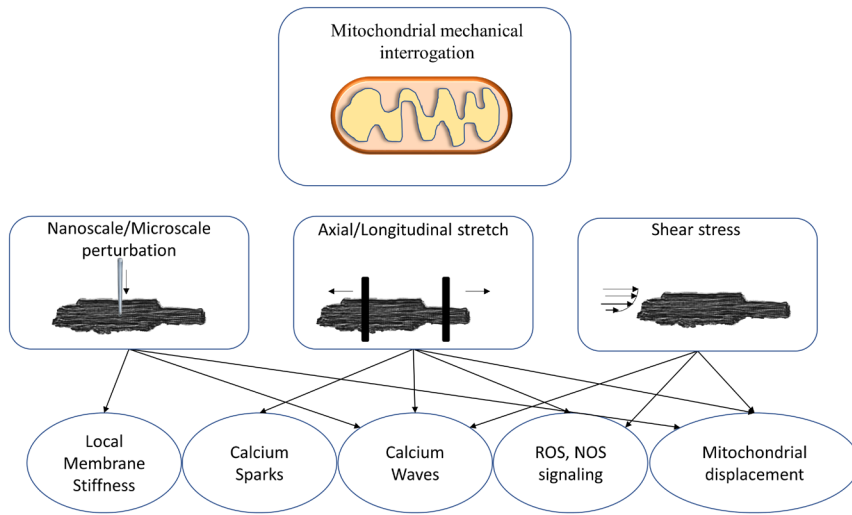


Figure 1

Schematic representation of the discussed technologies and approaches for mitochondrial interrogation in live cardiomyocytes. Left: Nanoscale perturbation, achieved by scanning probe microscopy. Middle: Axial stretch, achieved by piezoelectric translator of carbon fibers. Right: Shear stress, achieved by pressurized flow solution through microbarrels.

Mitochondria calcium handling regulation: mechanical-induced calcium propagation

Scanning ion conductance microscopy (SICM) is one of the few techniques capable of providing (13, 14) a detailed investigation into subsarcolemmal mitochondria which are located underneath the membrane crest (Fig. 1). SICM delivers a dedicated hydrojet of solution (normally Phosphate Buffered Saline, PBS, or Hanks' Balanced Salt Solution, HBSS) via a borosilicate pipette probe after having acquired a contactless high-resolution topographical scan with the same high-resistance nanopipette (100 MΩ). SICM can be combined with surface confocal microscopy (SSICM) (15) to increase scan details by showing fluorescent mitochondrial position within the conductance topographical images (10×10 μm, 512×512 pixels) (Fig. 2A). In our recent research (16), rat ventricular cardiomyocytes (either from healthy or failing heart) were first loaded with a calcium indicator. In particular, failing heart cardiomyocytes were isolated from ventricular zones remote from the scar, and failing heart was generated through myocardial infarction by coronary artery occlusion. In the present model, rats develop heart failure at 16 weeks with clear evidence of hypertrophy and left ventricular failure. After topography acquisition, 20 kPa of hydrojet for two seconds was delivered either on the crest (i.e. on top of the mitochondria) or in the unstriated part of the failing cardiomyocytes to perturb mitochondrial mechanical state. It was observed that, while in healthy adult cardiomyocytes the mechanical hydrojet activates a local mechanical-induced intracellular Ca²⁺ release (MiCai) (Fig. 2B and C), in failing cardiomyocytes the MiCai propagates throughout the cells, suggesting a possible mechano-sensing role of

mitochondria in arrhythmogenesis. The hydrojet released via SICM can also provide the membrane compliance on the perturbed area; it was also observed that the stiffer is the membrane the higher the probability of generating MiCai (Fig. 2D) (16). Mitochondria calcium serves as a trigger for activating calcium waves in failing cardiomyocytes, possibly involving the cytoskeleton or reactive species. Such remodeling, together with the 'loading' role of SR (17), has been recently suggested to play an active role in calcium propagation in failing cardiomyocytes. However, further investigations are needed.

Mitochondria calcium handling mechanisms: sarcoplasmic reticulum (SR), reactive oxygen species (ROS) and reactive nitrogen species (RNS)

The sarcoplasmic reticulum is the main source of calcium-induced-calcium release (CICR), and it is in intimate contact with mitochondria forming within the dyad a functional unit. Calcium sparks are the first elementary event of CICR, which are locally confined around the RyR cluster (mainly the same mechanism that we described previously in healthy nanomechanical stimulated cells). In CICR L-type, Ca channels that open RyR trigger a rise of intracellular Ca²⁺ moving from the SR, but such opening may occur spontaneously when RyR is sensitized to Ca²⁺ by phosphorylation, oxidation or nitrosylation. Of interest, shear stress and axial stretch applied to a cardiomyocyte immediately cause a release of bursts of Ca²⁺ sparks that terminate within a second with a time course that depends on the increment in ROS (6, 18, 19).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) in cardiomyocytes are normally generated

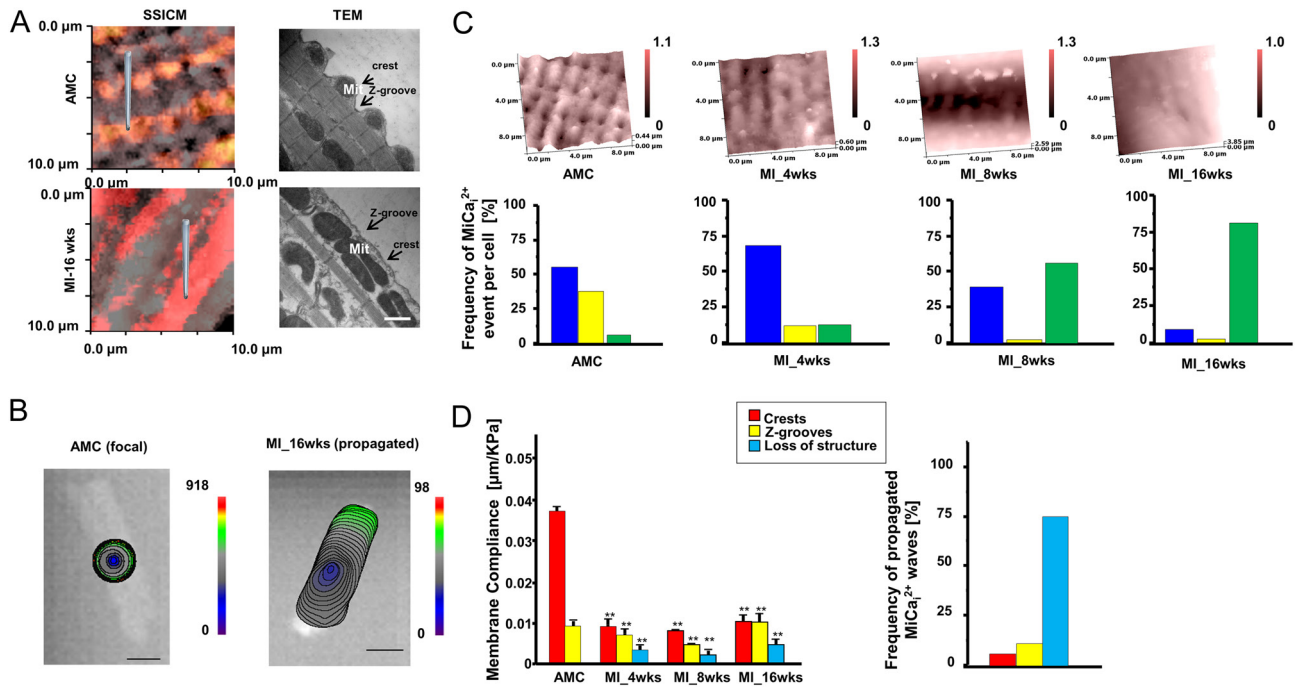


Figure 2

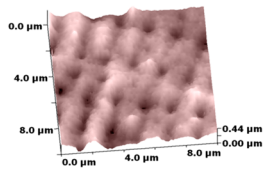
Mechanically-induced Ca²⁺ release in failing rat ventricular myocytes. (A) Surface topographical and confocal images (left) and transmission electronic microscopy images (right) of normal (top) and failing (bottom) portion of cardiomyocytes. In particular, as adult male Sprague-Dawley rats underwent proximal left anterior descending coronary ligation to induce chronic MI (heart failure was developed at 16 weeks), only cardiomyocytes from ventricular zones remote from the scar were utilized. (B) Representative optical images (color-coded isochrones) for AMC cell (local propagation) and infarcted-16-week-old cells (total-propagation). (C) Frequency of propagated mechanical induced Ca²⁺ transient (MiCaI) in aged match control (AMC) cells and in infarcted cells after 4, 8, and 16 weeks following anterior coronary ligation, respectively. Blue: not propagated; yellow: local propagation; green: total-cell propagation. (D) Membrane compliance obtained on crests, Z-grooves and unstriated parts of the cells in the aforementioned conditions (left) and frequency of propagated MiCaI. Modified from (14) and (16) with permission.

by mitochondria, NADPH oxidase (NOX), xanthine oxidase (OX), and nitric oxide synthase (NOS). In fact, mitochondria are the main source of ROS in cardiomyocytes, and superoxide generation is the result of oxygen leaking from the electron transport chain that, together with other enzymes, can participate in ROS generation, in particular, due to complexes I and III (20). ROS and RNS accumulation in the cell is finely controlled by several antioxidant components, including glutathione, catalase, superoxide dismutase, and others. While high concentrations of ROS and RNS are toxic to cells, by altering the activity of several antioxidant enzymes (21), low concentration can play an important role as signaling molecules. In particular, nitric oxide can regulate endothelium-dependent epicardial and microvascular vasodilatation, ROS provide cardiac protection during ischemic preconditioning, and RNS can modulate sympathetic cardiac activity (22, 23).

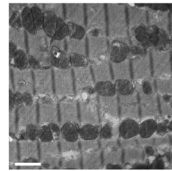
Furthermore, ROS and RNS are both protagonists in orchestrating calcium homeostasis within the cardiomyocytes (24); ROS are imperative in modulating

the amplitude of calcium signals, while RNS have a role in modulating the calcium uptake in the SR via the SERCA2a protein (25). Much evidence has shown the relationship between Ca²⁺ and ROS generation in mitochondria under physiological and pathological conditions (26). The combination of mitochondrial dealignment, microtubule disorganization, and uncontrolled level of oxidative stress, as happens in failing cardiomyocytes, may drive spontaneous calcium waves and arrhythmias (Fig. 3). Mitochondrial calcium concentration plays a critical role in maintaining the normal functionality of these organelles (27). Under physiological conditions, mitochondrial calcium uptake by calcium uniporter (MCU) complex is required to sustain energy demand and to keep the antioxidative capacity in a reduced state (28). In heart failure, the increment of diastolic calcium (29, 30) prevents mitochondrial calcium uptake when cytosolic Ca²⁺ > 400 nM (31), hampering regeneration of reduced forms of NAD and NADPH and leading to a status of energetic stress and ROS generation (32). In heart failure, Ca²⁺/Calmodulin 2 is upregulated and evidently increases

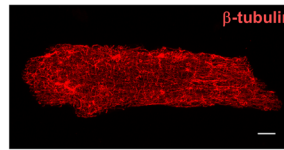
Preserved striation and membrane compliance



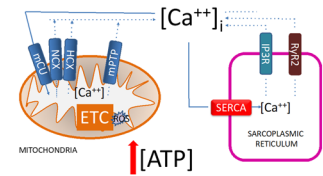
Healthy mitochondrial organization



Healthy microtubules organization

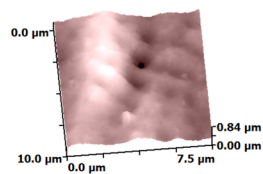


Physiological –OS levels

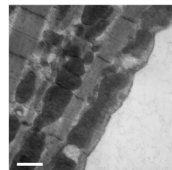


Physiological rhythm

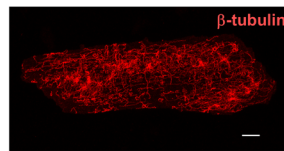
Unpreserved striation and higher stiffness



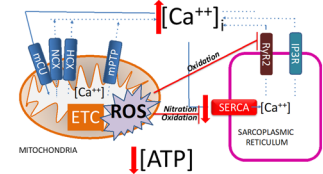
Mitochondrial dealignment and derangement



Microtubules derangement



Uncontrolled –OS levels



Increased mitochondrial mechanosensitivity: potentially driven arrhythmias

Figure 3

Schematic representation underpinning the known mechanism for potentially driven mitochondrial related mechanically induced arrhythmias. Top: healthy condition; Bottom: failing condition. Modified from (16) and (25) with permission.

the amount of cytosolic Na⁺ concentration and late inward I_{Na} (33). This increment can also lead spontaneous release of Ca²⁺ from SR, triggering arrhythmias.

Mitochondrial ROS generation induces more mitochondrial ROS production in a phenomenon called ROS-induced ROS release (34). Under physiological conditions, the proper handling of mitochondrial calcium concentrations is fundamental for efficient oxidative phosphorylation activation and maintenance of reduced NADPH, both contributing to cardiomyocyte pump function and energy. High NADPH generation is essential for ROS mitochondrial enzymes scavenging activities (35). A reduced ability to scavenge mitochondrial ROS generation occurs in experimental models of heart failure (36). In heart failure, the altered mitochondrial calcium uptake leads to higher concentration of ROS and to a reduced conversion of NADH to NADPH by the mitochondrial enzyme nicotinamide nucleotide transhydrogenase, lowering ROS enzymes scavenging activities, thereby weakening the cellular antioxidant defense (37). High ROS levels interfere with excitation–contraction coupling and induce cell death through mitochondrial permeability transition (6, 26). In a rat heart failure model, the reduction of mitochondrial ROS generation (in particular, hydrogen peroxide) and of calcium-induced mitochondrial permeability transition contributes to the beneficial effect of aerobic exercise on

mitochondria efficiency by reestablishing their number and size in cardiomyocytes (38). Prosser *et al.* (39) demonstrated that, under physiological conditions, stretching of heart cells activates NOX2 and leads to the generation of ROS in a process that depends on microtubules, called ‘X-ROS signaling’. Briefly, a mechano-chemical increase in ROS activates ryanodine receptors (RyRs) in the sarcoplasmic reticulum (SR) inducing a calcium burst, which leads to muscle contraction (4). After the contraction, calcium concentrations return to basal levels inducing muscle relaxation. On the contrary, in diseased cardiomyocytes the ‘X-ROS signaling’ generates arrhythmogenic Ca²⁺ waves, contributing to cardiomyopathy in a model of Duchenne muscular dystrophy (40). SERCA2a is an integral endo/sarcoplasmic reticulum (ER/SR) membrane protein that mediates active transport of calcium, helping to maintain normal calcium concentrations in the ER/SR lumen. In particular, in the heart the subtype SERCA2a is present and contribute to myocyte contraction and calcium reuptake in the SR. SERCA2a is located closely to mitochondria, exposing it to ROS and RNS species generated by oxidative phosphorylation. Pathological conditions implying high concentrations of peroxynitrite bring irreversible inactivation of SERCA2a through nitration. Lokuta *et al.* showed that, in human heart failure, the inactivation of SERCA2a by nitration might contribute to calcium pump failure (41).

Mitochondrial calcium handling mechanisms: microtubules-dependent mitochondrial trafficking, deformation and organization implicated in mechano-electrical transduction

The integrity of microtubules, as part of the cytoskeleton, is tightly controlled in the cytoplasm of cardiomyocytes. It has been demonstrated that, during the stretch-induced mitochondrial membrane ($\Delta\Psi_m$) potential hyperpolarization (42), microtubules integrity is necessary for the regulation of the respiratory function (16, 43, 44, 45). Tubulin is directly associated with mitochondrial voltage-dependent anion channels (VDACs), which are located in the mitochondrial outer membrane (MOM) (46). There is evidence that free tubulin dimers, in isolated cardiac mitochondria (*in vitro*), increase appKm for ADP, thus, decreasing ADP availability to ATP-ADP translocase (ANT). Moreover, VDAC and ANT are linked to mitochondrial creatine kinase (MitCK), and all three together have an important role in the control of metabolic energy and metabolic fluxes (43, 45, 47, 48). Recently, a link has been demonstrated between mitochondrial activity, cardiac preload modulation via Frank–Starling mechanisms, microtubules, and mitochondria X-ROS production (39, 42); however, further investigation is necessary to characterize the underlying mechanism. Subsarcolemmal mitochondria (and possibly interfibrillar mitochondria) deform during sarcomere contraction, and it is possible to appreciate a diastolic and a ‘systolic dimension’ of the organelles (49, 50). Importantly, mitochondria can move within the cytoplasm, trafficking among cells in neurons (51) and also fuse with adjacent mitochondria or split in two parts by fission mechanisms (Fig. 4A and B) (16, 49). In the failing heart, cells and microtubules encounter an upregulation of the constituent proteins, resulting in profound depolymerisation as well as derangement. This is one of the possible underlying mechanisms of MiCai, as induced by pharmacological depolymerisation of healthy cardiac cells with colchicine, followed by nanoperturbation via hydrojet (16) (Fig. 4C).

Considerations about mitochondrial pro-arrhythmogenicity via mechanosensing transduction

It is well known that single or minimal groups of cells are capable of evoking an abnormal bioelectrical impulse initiation and propagation. However, *in vivo*, the

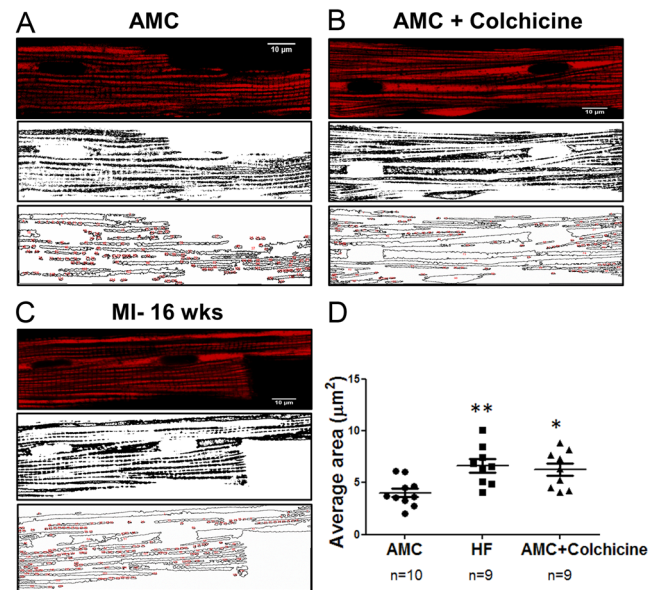


Figure 4

Effect of microtubules derangement and inhibited-polymerisation on the total mitochondrial area. (A) Aged-match control cells, whereby mitochondria were loaded with TMRM (top) and the same binarized image (middle) for mitochondrial area calculation (bottom). (B) Same as (A) for AMC-cells treated with 10 µmol/L of colchicine. (C) Same as (A) for MI-16 weeks cardiomyocyte. (D) Total mitochondrial area calculation in the three conditions. Data represented as Mean \pm s.e.m. Modified from (16) with permission.

mechanism has yet to be explored. At a single failing cell level, we observed that MiCai driven from mitochondria is able to trigger a secondary distant source of calcium waves, probably from stretch-activated channels (SACs) following the nanoindentation applied at the center of the failing cardiomyocyte (14). The local sarcomere contraction underneath the mechanical interrogation relaxes, while the more distant sarcomere contracts. This secondary phenomenon is abolished when SACs are blocked either with 30 µmol/L gadolinium or 100 µmol/L streptomycin. It has been speculated that the aforementioned conditions are able to mechanically perturb the neighboring cardiomyocytes, especially in the context of sarcomere desynchronization (52). Several mathematical and experimental models have shown that for triggering an impulse initiation, it is necessary to perturb a ‘liminal area’ in the cardiac tissue (53, 54, 55), as a single failing cardiomyocyte cannot jeopardize the entire electrical activity of the heart. All multicellular models consider cardiomyocytes to be almost identical among each other in terms of electrical, mechanical, and structural functions. However, in real, cardiomyocytes are not identical, thus with the intent to explain what is happening *in vivo*, it represents a notable limitation.

The 'domino' effect can represent this concept (Fig. 5). When all bricks (i.e. cardiomyocytes prone to be activated) have identical size and dimension, one has to 'activate' the first brick from the periphery of the failing row to cause the domino effect. Moreover, if the 'first brick' is in the center of the row or at other sites the domino effect does not happen or, if it does, it is limited only to the surrounding bricks (liminal area) (Fig. 5A). Considering a cell as a 'domino brick', if the bricks have different size, thickness, and 'propensity to fall' (i.e. different stiffness, energy, force, mitochondrial MiCai release, oxidative level or others unexplored trigger mechanisms), theoretically, they will be capable to partially activate a row, thus initiate an uncontrolled cumulative effect, such as re-entrant arrhythmias or propagated extrasystole (Fig. 5B).

Considerations about mitochondrial implication in heart failure via mechanosensing transduction

Failing myocardium is characterized by a severe impairment of energy metabolism and energy substrate utilization, resulting in an increment of glucose utilization that affects the metabolic state of the heart (12). Such remodeling is associated with a structural modification or loss of mitochondrial integrity, as observed by us and other colleagues (16, 52). Defective mitochondria, during heart failure, lack their morphology as well as cytoskeletal proteins (56) and mitofusines (57), and this alteration invariably affects mitochondrial biogenesis. Impairment of the structural interaction between mitochondria and

the surrounding domain leads to cardiomyopathies. The sarcomere structure accommodates the subsarcolemmal mitochondrion underneath the crest (25) that directly interacts with the calcium-induced calcium release machinery, SERCA2 pump (58), and myofilaments of the Z-disc (59). Therefore, in failing cardiomyocytes, the sarcolemma integrity is lost (Fig. 3) along with the intimate interaction between the sarcomere and the mitochondrion. As a consequence, the mitochondrial-sarcomere intermingle is a possible candidate for delaying the progression to heart failure, aimed to push, paradoxically, the metabolic demand toward 'controlled' glucose utilization together with a decrease in fatty acid oxidation (60) for reducing the oxidative stress-induced microdomain remodeling.

Conclusions

The mitochondrion is an ancient eukaryotic organelle that specialized its activity during the evolution, especially in the heart, where the energy demand is higher and consistent. Similar to prokaryotic bacteria (where mitochondrion originate from), it adapts its structure and activity by sensing the mechanical force in the environment and, within a dynamic organ such as the heart, it contributes to calcium homeostasis. This review suggests a possible pro-arrhythmic involvement of mitochondria in the heart mainly due to modification in its mechanosensation, thanks to a combination of pathophysiological structural and functional remodeling occurring during heart failure. Surely, a lot of basic research needs to be spent in the field of mitochondrial mechanobiology, as the background mechanisms are still largely unexplored.

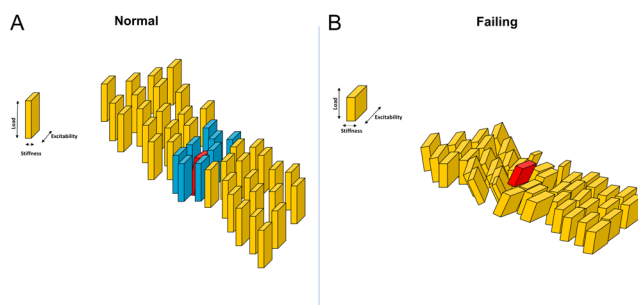


Figure 5

Domino-model cartoon showing liminal area concept revisited concerning the generation of abnormal impulse propagation. Left: not generated impulse propagation; Yellow bricks: remote resting cardiomyocytes; red brick: activated 'source' cardiomyocyte; blue bricks: surrounded 'sink' cardiomyocytes sensing the activated one. Right: generated abnormal impulse propagation. Yellow bricks: surrounding and remote 'sink' cardiomyocytes with different size; red brick: activated 'source' cardiomyocyte.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement

M M performed the experiments and the cartoon in Figs 1 and 5, while A C drew the cartoon in Fig. 3. C C M designed the review and wrote the manuscript with the supervision of M M and A C.

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