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Mitofusin 2 Joins the Sarcoplasmic Reticulum and Mitochondria at the Hip to Sustain Cardiac Energetics

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The divalent cation calcium (Ca^{2+}) is best known in cardiomyocytes for its role in excitation-contraction coupling, where it triggers myosin-actin cross-bridge formation. However, Ca^{2+} regulates myriad fundamental processes in all cells. Among these are the activities of enzymes, channels, and pumps, transcription, translation, motility, exocytosis, metabolism, growth, differentiation, and death[1]. Mitochondria are the primary venue for some of these processes, such as metabolism and cell death. This raises a question that appears seemingly straightforward, but has proven to be complex and contentious: How does Ca^{2+} get from its major storage depot, the sarcoplasmic/endoplasmic reticulum (SR/ER), to mitochondria? In this issue of *Circulation Research*, Chen et al[2] address this issue in cardiomyocytes.

Uptake of Ca^{2+} by mitochondria is critical for coupling the energy demands of cardiac work with metabolism[3]. These demands can change on a beat-to-beat basis necessitating a messenger, such as Ca^{2+}, with a rapid response time. The most intensive catabolism of substrates occurs in the mitochondrial matrix via the Krebs cycle, several rate-limiting enzymes of which are Ca^{2+}-dependent. The phasic increases in cytosolic Ca^{2+} with each heart beat, which are more frequent or larger under conditions of increased energy demand (e.g. increased heart rate or contractility), are sensed and averaged by the complex buffering system in mitochondria to accelerate cardiac metabolism through uptake of Ca^{2+} into the matrix[4].

The story of mitochondrial Ca^{2+} handling is long and convoluted, but a bolus of clarity was recently infused with the molecular identification of the mitochondrial Ca^{2+} uniporter (MCU) in the inner mitochondrial membrane (IMM)[5, 6] (Figure 1). Ca^{2+} traverses the outer mitochondrial membrane (OMM) through non-specific high-conductance voltage dependent anion channels (VDAC) to reach the intermembrane space. The MCU, which is highly selective for Ca^{2+}, then moves Ca^{2+} from the intermembrane space to the matrix.

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ATP production by aerobic metabolism requires a large negative voltage gradient across the IMM, which is generated by the electron transport chain. However, these conditions also favor Ca^{2+} entry into the matrix via the MCU. Perhaps to protect cardiomyocytes from the potentially lethal consequences of mitochondrial Ca^{2+} overload, the MCU is relatively insensitive to the average ambient cytosolic Ca^{2+} concentrations in cardiomyocytes (~100 nM diastole and 0.5 μM systole). Yet, there needs to be some conditions under which the MCU can accomplish its job of bringing Ca^{2+} into the matrix. This conundrum gave rise to the concept of Ca^{2+} microdomains, localized areas of high Ca^{2+} concentration[7, 8]. Microdomains with Ca^{2+} concentrations ~10 μM flank the ends of intermyofibrillar mitochondria in cardiomyocytes[9] (Figure 1), and are adequate to drive efficient uptake of Ca^{2+} into the matrix via the MCU. These microdomains, which persist 10–20 ms per heart beat, originate in the "subspace" between the transverse tubules and the junctional sarcoplasmic reticulum (jSR). The influx of Ca^{2+} through L-type Ca^{2+} channels in the transverse tubules triggers the massive release of Ca^{2+} from the jSR to the subspace via ryanodine receptors, most of which face the transverse tubules[10]. The resulting very high concentrations of Ca^{2+} in the subspace (100–300 μM)[11] then diffuses ~100 nanometers to produce the Ca^{2+} microdomains in juxtaposition to the mitochondria.

For Ca^{2+} microdomains to exist in proximity to mitochondria, the SR/ER and mitochondria must be held in close apposition. While contact points between SR/ER and mitochondria have been recognized and membrane fractions containing SR/ER and mitochondrial proteins ("mitochondrial associated membranes") have been isolated, the molecular basis of SR/ER-mitochondrial tethering remained obscure until recently. A major breakthrough was provided by experiments in non-excitable cells demonstrating that the dynamin-related GTPase mitofusin 2 (MFN2) is critical in linking the two organelles[12]. MFN2 in the ER membrane interacts with MFN2 in the outer mitochondrial membrane as well as with MFN1, a homologous protein located solely on the outer mitochondrial membrane, to bring ER and mitochondria together. Reconstitution experiments in knockout cells have shown, however, that only MFN2 specifically at the ER is indispensable in this tethering process.

In the present study, Chen et al conduct experiments that move from *Drosophila* to mice to interrogate the consequences of mitofusin-mediated mitochondrial-SR tethering across cardiac evolution. The starting point of these investigations is that Ca^{2+} microdomains are important in overcoming the low efficiency of the MCU in moving Ca^{2+} into the mitochondrial matrix. The authors ask how microdomains are created in cardiomyocytes, and what are their downstream consequences? Since microdomains reflect the close apposition of SR and mitochondria, the investigators sought to differentiate between two hypotheses: Was the nearness of these organelles in cardiomyocytes mediated by contact points dependent on MFN2, as in non-excitable cells? Alternatively, was the physical sandwiching of the junctional SR (jSR) between T-tubules and mitochondria, by itself, sufficient to account for this close proximity? To sort out these possibilities, Chen et al first determined the effect of RNAi-mediated suppression of the sole Drosophila mitofusin, MARF, in fruit fly heart tubes; phasic SR Ca^{2+} release increased, possibly reflecting decreased SR-to-mitochondrial Ca^{2+} transfer. Then, they created mice in which each of the two vertebrate mitofusins, MFN1 or MFN2, was individually deleted specifically in cardiomyocytes beginning in postnatal life. Comparing ablation of MFN1 and MFN2 controlled for the potential confounding factor of MFN2 as a mediator (along with MFN1) of fusion between mitochondria. Cardiomyocyte-specific inactivation of MFN1 would, therefore, have the same effects on mitochondrial fusion as MFN2, but would not affect SR-mitochondrial coupling.

The most important finding of this study is that the contact area between SR and mitochondria in cardiomyocytes is reduced ~30% in MFN2 knockout mice as compared

with wild type controls, an abnormality not observed in MFN1 knockout mice. This structural uncoupling correlated with decreased SR to mitochondrial Ca^{2+} transfer in isolated cardiomyocyte experiments. Moreover, the conclusion that MFN2 deletion decreases SR to mitochondrial Ca^{2+} transfer was strengthened by the observation that loss of MFN2 actually increases SR to cytosol release, although the precise mechanism of the latter is not known. The study went on to show that the depressed transfer of Ca^{2+} to mitochondria in cells lacking MFN2 is of physiological significance as it impairs the Krebs cycle. These abnormalities are most dramatic under conditions of increased workload, as modeled by isoproterenol administration combined with accelerated rates of cellular pacing. While wild type cardiomyocytes transiently augment the rate of SR to mitochondria Ca^{2+} transfer to drive the Krebs cycle, MFN2 null cardiomyocytes can do neither. Taken together, these data indicate that MFN2 is critical in coupling cardiac work with metabolism, most likely by maintaining the intimacy between SR and mitochondria required for optimal Ca^{2+} transfer.

The main lesson we learn from this study is that a commonality exists in the mechanisms that maintain SR/ER-mitochondrial apposition in non-excitable cells and cardiomyocytes. While the rigid architecture of the latter may contribute to bringing these organelles together, it does not suffice, and MFN2 is critical. Given the conservation of MFNs, why consider that cell type-specific differences might exist? One compelling reason is that cardiomyocytes have already proven to differ drastically with respect to their glacial rates of mitochondrial fusion[13], another key function of these proteins.

The investigators excluded many of the alternative explanations for their data, the most obvious being MFN2-related alterations in the electrical potential difference across the IMM, a key driving force for Ca^{2+} import via the MCU. One possibility that was not investigated, however, is whether MFN2 plays a direct role in mitochondrial Ca^{2+} uptake, independent of in its tethering of SR/ER to mitochondria. This notion could be tested using isolated mitochondria. While there is currently no evidence for this hypothesis, it would not be surprising for MFN2 to have yet additional functions. In this vein, MFN2 has been implicated in the Ca^{2+} sensitivity of the mitochondrial permeability transition pore[14, 15].

The results of the current study appear to differ from those of Papanicolaou et al, who concluded that MFN2 deletion does not alter the relationship of SR to mitochondria in cardiomyocytes[14]. This discrepancy, however, appears to be largely the result of variation in the parameters that were assessed. The conclusions of the present study are based on decreases in the contact area between SR and mitochondria, similar to the major parameter evaluated in the initial study in non-excitable cells[12]. A trend toward increased distance between jSR and mitochondria was observed in the present study, but did not reach statistical significance. In contrast, Papanicolaou et al found that MFN2 absence does not affect the distance between the transverse tubule and mitochondria. The magnitude of this distance, however, is much larger than the gap between jSR and mitochondria and may, therefore, have obscured small changes in the latter. Rates of Ca^{2+} uptake were not assessed in Papanicolaou et al.

Mice with combined deletion of MFN1 and MFN2 manifest a spontaneous lethal dilated cardiomyopathy[13]. In contrast, the present study shows that mice with inactivation of MFN2 (or MFN1) alone have normal cardiac dimensions and function. This may not be surprising given the low metabolic demands of the basal state. It will be interesting to test the effects of various energy-demanding stresses on cardiac function in these mice. If the pathway described in this report exists to match bioenergetics with increased work load, one would predict that increases in preload, afterload, chronotropy, or inotropy would be poorly tolerated in MFN2 knockout mice.

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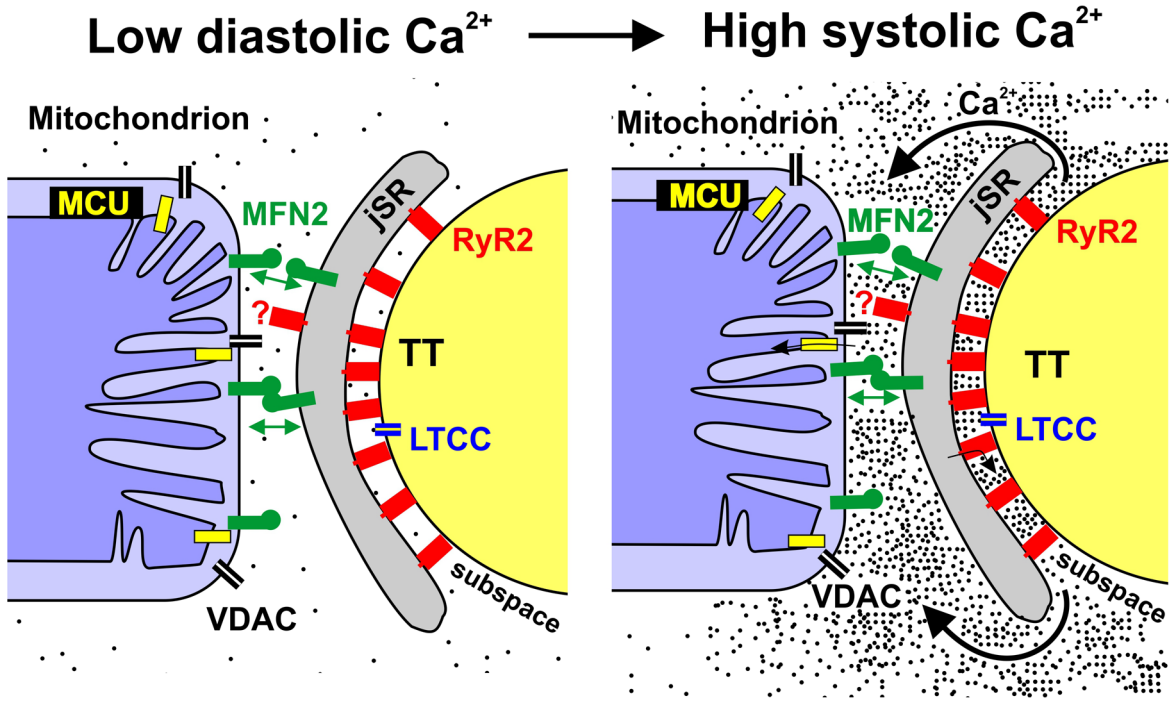

Figure 1. Ca^{2+} microdomains in heart

Mitochondria span Z-disk to Z-disk and in close proximity to the junctional SR (jSR). The inner mitochondrial membrane contains the mitochondrial Ca^{2+} uniporter (MCU), and the outer mitochondrial membrane contains non-specific Ca^{2+}-permeable voltage dependent anion channels (VDAC). The dynamin-related GTPase mitofusin-2 (MFN2) in the jSR membrane engages in homotypic interactions with MFN2 in the outer mitochondrial membrane (indicated by double arrows). During systole, extracellular Ca^{2+} enters the subspace between the transverse tubule (TT) and the jSR through L-type Ca^{2+} channels (LTCC) in the membrane of the TT and the sarcolemma (SL), and this Ca^{2+} triggers the release of large amounts of additional Ca^{2+} from the jSR into the subspace via ryanodine receptors (RyR2). The very high local concentrations of Ca^{2+} in the subspace subsequently diffuse to create microdomains of high Ca^{2+} around the ends of the mitochondria. Ca^{2+} can cross the outer mitochondrial membrane through VDACs and be transported into the matrix via the MCU. Rogue RyR2[16] (indicated by the question mark) may be found in the microdomain.