BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

Name of Journal: ***World Journal of Biological Chemistry***

ESPS Manuscript NO: 21290

Manuscript Type: REVIEW

Answering reviewers

Reviewed 00467115

The review by Santo-Domingo et al. gives a comprehensive overview on the molecules involved in mitochondrial calcium transport. The second part on redox signaling modulation is not well linked and should be revised.
To better link the first part (mitochondrial Ca^{2+} transport)with the second part (matrix redox signaling) we have added figure 3 which should clarify the causal relation between mitochondrial Ca^{2+} transport and mitochondrial redox signaling (recent evidence supporting this model are presented in this review).

In general, this review would gain from some critical assessment of the literature and more linking of the topics discussed to the diseases mentioned only in the outlook.
We have added a large section (page 15) describing links between redox signaling and specific disease areas. The relevant literature is cited (new references 140-149).

The abstract is somewhat self-contradictory: While first "the" protein involved in mitochondrial calcium transport is mentioned, later the (correct) referral is to several proteins.
We have corrected this in the abstract. We now refer to the "proteins" and the "transporters" involved in mitochondrial Ca^{2+} transport".

Bottom p. 3: NADPH is not – to my knowledge – generated by mitochondrial metabolism.
In proliferating cells, the largest contributor to cytosolic NADPH is the oxidative pentose phosphate pathway. Nevertheless mitochondrial NADPH production has been reported and for example it has been recently published that quantitative flux analysis reveals folate-dependent mitochondrial NADPH production (Fan et al., Nature 2014; 510(7504):298-302).

Mitochondrial sources of NADPH are the nicotinamide nucleotide transhydrogenase, isocitrate dehydrogenase-2, and malic enzyme (Yin et al., Antioxid Redox Signal 2012; 17(12):1714-1727; Rydstrom, Biocim. Biophys Acta 2006; 1757:721-726).
In our manuscript we have used the term "NAD(P)H" to include both NADH and NADPH, which can contribute to mitochondrial redox signaling.

p. 5: When discussing the composition of Unipex, all subunits should be referenced (not only MCU1).
We have amended as suggested by the referee.

p. 6: The OMM still is a membrane and ions will not "freely" diffuse across it. Present understanding would suggest calcium enters via VDAC which is regulated and not constitutively open.
We agree with the referee. Fig.2 already suggested that Ca^{2+} enters via VDAC. We have deleted the ambiguous sentence.

p. 6: When discussing the results on EMRE knock-outs, the authors should offer an (hypothetical) explanation for the conflicting results.
Despite an open discussion in the scientific community unfortunately there is no explanation for those conflicting results. The group that discovered the role of EMRE simply stated that incongruence (Sankac et al., Science 342 (2013) 1379-1382). The group supporting different results, recently commented that discrepancy, without a real explanation and admitted that: "additional experimental work is needed to solve the remaining issues". For discussion, see De Stefani et. Biochim and Biophys Acta, 1757 (2015) 2006-2011:
"…As discussed above, MCU is sufficient per se to form a Ca2+ channel in planar lipid bilayer, but what happens in vivo is unclear. EMRE is a broadly expressed 10kDa protein that spans the inner mitochondrial membrane and possesses a highly conserve C-terminus rich in aspartate residues, with a still unclear membrane topology (Sankac et al., Science 342 (2013) 1379-1382). Mootha and colleagues propose that this protein is required for Ca2+ channeling activity and keep the MICU1/MICU2 dimer attached to the MCU complex. In the contrast to its essential role, EMRE homologs are not present in plants, fungi or protozoa in with MCU and MICU1 are highly conserved. Downregulation or knockout of EMRE totally abolishes mitochondrial Ca2+ uptake, even when MCU is overexpressed. At protein level, EMRE stability is impaired in the absence of MCU, suggesting that other MCU complex components appear to be required in vivo for its correct function. It must be noted that the putative role of EMRE in mediating the binding between MCU and MICU1 is in contrast with the clear positive effect of MICU1 on MCU in planar lipid bilayer, where no other components are present. However, it is also clear that in the absence of EMRE the MCU complex became smaller, as revealed by blue-native PAGE (Sankac et al., Science 342 (2013) 1379-1382). This opens the possibility that

EMRE could be an essential protein for efficient assembly of the MCU complex. In line with this, it was recently shown that, in a heterologous system such as yeast, EMRE is required for the formation of a functional channel only with the mammalian MCU, but not with MCU derived from fungi (Kovacs-Bogdan et al, Proc. Natl. Acad. Sci. U.S.A. , 111(2014) 8985-8990). We think that additional experimental work is needed to solve the remaining issues".

p. 7: revise sentence: "Depolarization of the inner mitochondrial…"p. 8: "ryanodine receptors" should not be capitalized. p. 9, line 4: Na^+/Ca^{2+} exchanger. p. 9: "the inhibitor of mitochondrial Na/Ca" is slang. p. 12, line 16: membrane-permeant p. 13, line 8: our data establish p. 13, line 6 from bottom: ?-cell (not beta-cells) p. 14, line 4: italicise "S" (3 times) p. 14: TCA cycle enzymes should not be capitalized, similarly carnitine on p. 15.
We have revised, as suggested

The references have to be carefully checked; ie., second author ref. 17 should be Lillig HC.; second author ref. 18 is Schwarzl?nder. Formatting in references also must be checked (e.g., refs .44, 45, 65, 67 and 69).
The references were corrected.

Abbreviations should not be used without definition (e.g., MICU1) and a list of abbreviations should be included; once an abbreviation has been defined and introduced, it should be exclusively used (i.e., uncoupling protein 1, p. 14).
We have defined all the abbreviation and included a list of abbreviations.

All abbreviations used in a figure should be defined in the figure legend. Figure 2: histamine should not be capitalized in legend
We have corrected this.

Reviewed 00227420

In the present manuscript, Santo Domingo et al perform a nice review of what is known about the mechanisms that govern mitochondrial Ca2+ homeostasis and the role played by mitochondria in the regulation of cell redox equilibrium. The Authors also analyze the complex interplay between changes in mitochondrial Ca2+ levels and the regulation of mitochondrial matrix redox state. In my opinion, this is a well-written manuscript, characterized by a deep and thorough presentation of a rapidly moving field. The importance of the topic both in physiology and in pathology is

highlighted, and all its aspects are dissected in a comprehensive and updated manner.

Some minor typos: page 6, it is unclear the role of MICU1 oligomers (They should activate, not inhibit MCU1); page 7, depolarization of the inner mitochondrial (membrane) as a secondary consequence of LETM1.
We have corrected this.