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Review

Mitochondrial calcium transport in trypanosomes

Roberto Docampo[a,b,*], Anibal E. Vercesi[b], Guozhong Huang[a]

[a] Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA 30602, USA
[b] Departamento de Patologia Clínica, State University of Campinas, Campinas 13083, SP, Brazil

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ABSTRACT

The biochemical peculiarities of trypanosomes were fundamental for the recent molecular identification of the long-sought channel involved in mitochondrial Ca^{2+} uptake, the mitochondrial Ca^{2+} uniporter or MCU. This discovery led to the finding of numerous regulators of the channel, which form a high molecular weight complex with MCU. Some of these regulators have been bioinformatically identified in trypanosomes, which are the first eukaryotic organisms described for which MCU is essential. In trypanosomes MCU is important for buffering cytosolic Ca^{2+} changes and for activation of the bioenergetics of the cells. Future work on this pathway in trypanosomes promises further insight into the biology of these fascinating eukaryotes, as well as the potential for novel target discovery.

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1. Introduction

Trypanosomatids belong to one of the oldest branches of eukaryotic cells that possess mitochondria [1]. These organelles have very unusual properties in these cells. A special structure, known as the kinetoplast, was the first extranuclear DNA ever described [2] and consists of thousands of concatenated DNA minicircles and a few DNA maxicircles encoding a few gene products and having a very complex mechanism of replication (reviewed in [3]). Most mitochondrial mRNAs are subjected to editing by a process first discovered in these cells [4] (reviewed in [5]). The mitochondrial genome of trypanosomes does not contain tRNAs and the whole set of these tRNAs needs to be imported by a mechanism that share components with the protein import machinery [6,7]. Some respiratory complexes are incomplete or absent in some trypanosomatids, such as in *Phytomonas* spp. [8], and in the bloodstream stages of the *Trypanosoma brucei* group [9]. These species are also characterized by the presence of an alternative oxidase [10,11] similar to those present in plants and fungi, by the presence of an ATP synthase functioning in reverse, as an ATPase, to maintain the mitochondrial membrane potential [8,12–15], and by a partially functional, in *Phytomonas* spp. [8,16], or absent, in the case of bloodstream forms of *T. brucei* [16], tricarboxylic acid cycle.

Despite these peculiarities, trypanosomatids are one of the eukaryotic groups that have conserved a mitochondrial Ca^{2+} transport mechanism (mitochondrial calcium uniporter or MCU) with similarities to those of animal cells, as first demonstrated in

* Corresponding author at: Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, 500 D. W. Brooks Drive, Athens, GA 30602, USA. Tel.: +1 706 542 8104; fax: +1 706 542 9493.
E-mail address: rdocampo@uga.edu (R. Docampo).

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Trypanosoma cruzi in 1989 [17,18]. Interestingly, this finding together with the reported absence of the uniporter described in *Saccharomyces cerevisiae* [19], and the availability of sequenced genomes of many species, led to the discovery of the molecular identity of the *MCU* [20,21] and one modulator of the uniporter, the mitochondrial calcium uptake 1 or *MICU1* [22]. The early history of mitochondrial Ca^{2+} transport [23], and of the studies that led to the discovery of the uniporter [24–26] have been reviewed elsewhere.

2. The components of the mitochondrial Ca^{2+} uniporter

The ability of mitochondria to take up Ca^{2+} was discovered more than 50 years ago when it was found that rat kidney mitochondria were able to take up large amounts of Ca^{2+} [27,28] and that this process was energized by coupled respiration [28]. The properties of this process were soon identified: Ca^{2+} uptake is inhibited by respiratory chain blockers and oxidative phosphorylation uncouplers [28] and does not require ATP hydrolysis, except when the respiratory chain is blocked, and in this case it is inhibited by oligomycin [29]; other divalent cations, such as Mn^{2+} [30,31] and Sr^{2+} [32], can be taken up by this mechanism, while Mg^{2+} is a competitive inhibitor [33]; Ca^{2+} uptake is saturable and accompanied by H^+ extrusion [34] and could be accompanied by phosphate that can precipitate in the matrix [35]; the uniporter is inhibited by the dye ruthenium red [36] and its derivative, Ru360 [37], and is a gated, Ca^{2+}-selective, ion channel [38].

Since the discovery of the molecular nature of the uniporter [20,21] there has been a flurry of activity to identify all the components of the mitochondrial calcium uniporter complex (MCUC or uniplex).

HeLa cells MCU is a 40 kDa protein that loses its cleavable target sequence during mitochondrial import resulting in a 35 kDa mature form [20]. The protein has two transmembrane domains and topology studies have convincingly demonstrated that both its N- and C-terminal domains span into the mitochondrial matrix [39] while these two domains are connected in the intermembrane space by a short loop containing the DIME motif, which is highly conserved [20,21] (Fig. 1). It has been suggested that the protein forms oligomers, probably tetramers, as part of a larger complex of about ~480 kDa [20], with eight helices lining the putative pore region where the DIME motif is, and charged residues in proximity of the pore favoring Ca^{2+} flux [40]. The reconstitution of MCU into planar lipid bilayers [21] and patch-clamp studies of mitoplasts (mitochondria devoid of outer membranes) [41] have demonstrated that this protein is the pore-forming subunit of the uniporter complex.

Homologs of MCU are found together with homologs to MICU1 in nearly all metazoa, including plants, as well as some fungi (i.e., *Cryptococcus neoformans*, *Neurospora crassa*) that do not have a MICU1 homolog, and other protists (i.e., trypanosomatids, ciliates, *Naegleria gruberi*, *Dictyostelium discoideum*, *Chlamydomonas reinhardtii*) and in some bacteria of the Bacteroides/Chlorobi group, but are absent in Apicomplexan (i.e., *Plasmodium* spp., *Toxoplasma gondii*, *Cryptosporidium* spp., *Eimeria* spp.) and in organisms lacking classical mitochondria (i.e., *Giardia intestinalis*, *Trichomonas vaginalis*, *Entamoeba hystolitica*) [42]. Downregulation of *MCU* expression leads to autophagy [43] while overexpression leads to mitochondrial Ca^{2+} overload [21], which in turn leads to mitochondrial membrane permeabilization, and apoptosis [44]. Interestingly, expression of *D. discoideum MCU* alone in *S. cerevisiae* is sufficient to reconstitute MCU activity, while expression of the human *MCU* requires the co-expression of another component (essential MCU regulator or EMRE, see below) [45]. Surprisingly, *MCU* knockout mice are viable, although smaller in size, and with marked reduced ability to perform strenuous work, potentially linked to alterations in the phosphorylation of pyruvate dehydrogenase (PDH) [46].

MCU has a paralog, named MCUb, which in HEK-293 cells is a 35-kDa protein whose primary sequence is 50% similar to that of MCU and, as MCU, possesses two transmembrane domains (Fig. 1). MCUb has key mutations in the predicted pore-forming region and does not transport Ca^{2+} when inserted in planar lipid bilayers [40]. *MCUb* has lower expression level and a different expression profile from *MCU*, being more abundantly expressed in heart and lung and appears to be a subunit of the complex with inhibitory properties. MCUb is inserted into the MCU oligomer and exerts a dominant-negative effect [40] (Fig. 2A). Direct patch-clamp recordings from the inner mitochondrial membrane of different tissues of mice have indicated that the activity of the MCU varies greatly between them

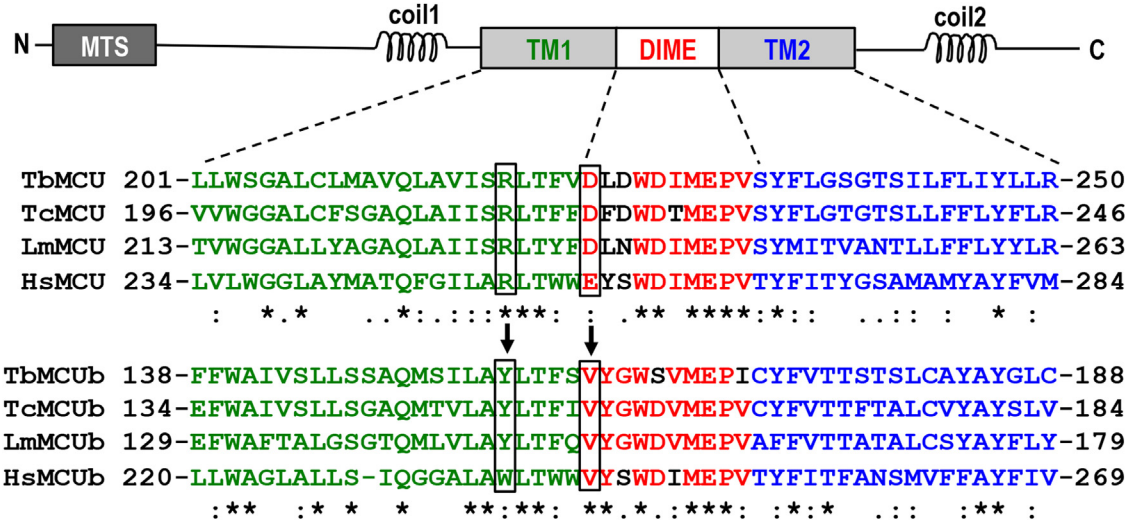

Fig. 1. Domain organization of MCU and MCUb proteins highlighting two highly conserved transmembrane domains and one putative pore region, from *Trypanosoma brucei* (TbMCU, Tb427tmp.47.0014; TbMCUb, Tb427.10.300), *Trypanosoma cruzi* (TcMCU, TcCLB.503893.120; TcMCUb, TcCLB.504069.4), *Leishmania major* (LmMCU, LmjF.27.0780; LmMCUb, LmjF.21.1690), and *Homo sapiens* (HsMCU, NP_612366.1; HsMCUb, NP_060388.2). Two critical conserved substitutions from MCU to MCUb are boxed and indicated with arrows. MTS, mitochondrial targeting sequence; coil, coiled-coil domain; TM, transmembrane domain; DIME, functional "DIME" motif, the putative Ca^{2+} selectivity filter.