

To the
The Austrian Science Fund (FWF)
Sensengasse 1
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TITLE OF PROPOSAL:

Mitochondrial Ca²⁺ homeostasis in endothelial cells:
regulation and function

Short title: *Mitochondrial calcium homeostasis in endothelial cells*

RESEARCH LOCATION: *Molecular & Cellular Physiology Research Unit, MCPRU*
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ABSTRACT

For a long time, mitochondria were thought to serve as passive Ca²⁺ sinks that accumulate Ca²⁺ along the organelle's negative membrane potential. This paradigm has changed radically. Nowadays, mitochondria are known to specifically respond to environmental Ca²⁺ and to contribute actively to the sophisticated regulation of the spatial and temporal patterns of intracellular Ca²⁺ signaling. Accordingly, our recent work describes mitochondria to be essential for the maintenance of capacitative Ca²⁺ entry, the accomplishment of Ca²⁺ refilling of the endoplasmic reticulum and Ca²⁺-dependent protein folding. Although these findings foster our understanding of the physiological role of mitochondria in Ca²⁺ signaling, the actual proteins involved in mitochondrial Ca²⁺ homeostasis, the molecular mechanisms of its regulation and the interdependency with other ions are largely unknown.

In our previous grant, we demonstrated that two isoforms of the uncoupling protein family, UCP2 and UCP3, are essential for mitochondrial Ca²⁺ uniport (Trenker et al. *Nat. Cell Biol.* in press). However, each protein alone failed to accomplish Ca²⁺ fluxes in a heterologous environment, thus, pointing to additional proteins that might be essential to assemble the mitochondrial Ca²⁺ uniporter. Accordingly, in **work package A**, we will explore *UCP2 and UCP3 as fundamental components of the mitochondrial Ca²⁺ uniporter*. In particular, utilizing UCP2/UCP3 as bait the protein composition of the assumed signalplex that forms the mitochondrial Ca²⁺ uniporter, the functional *interaction of UCP2- and UCP3-containing signalplexes with intracellular sites of Ca²⁺ release/uptake and Ca²⁺ entry* and the importance of existing *phosphorylation sites for UCP2/UCP3 function and activity* will be explored.

Our recent findings on the contribution of UCP2/UCP3 on mitochondrial Ca²⁺ uptake apparently stands, against the common hypothesis on the physiological uncoupling function of these proteins. Consequently, **work package C** is designed to provide *a critical in-depth evaluation whether the fundamental contribution of UCP2/UCP3 to MCU can represent the molecular mechanism behind already reported phenomena* by correlating their impact on mitochondrial Ca²⁺ uptake with mitochondrial oxidative phosphorylation and ROS generation.

Another important aspect of mitochondrial Ca²⁺ signaling is its interrelation with the cellular Na⁺ homeostasis. Mitochondrial permeability for these ions is strongly associated with mitochondrial Ca²⁺ homeostasis and the initiation of endothelial Ca²⁺ signaling is accompanied with activation of Na⁺-permeable ion channels or antiporters in the plasma membrane. Moreover, Na⁺ dependent Ca²⁺ efflux from mitochondria strictly depends on SERCA activity. Consequently, **work package C** is designated to explore *the importance of Na⁺-permeable plasma membrane ion channels and NCX_{pm} and SERCA activity for mitochondrial Ca²⁺ homeostasis in endothelial cells*.

The project outcome will reveal molecular insights of mitochondrial Ca²⁺ homeostasis and principles of its regulation. Furthermore the recently discovered "Ca²⁺ function" of UCP2 and UCP3 will be critically evaluated in comparison to already existing data regarding these UCP isoforms. Despite this grant focuses on mitochondrial Ca²⁺ homeostasis in endothelial cells as well defined test models, the research outcome will have important implications for Ca²⁺ handling in other cell types as well. This aspect deserves to be explored subsequently to the molecular assessment implemented in this project.

Key words: Endothelial cells, Calcium signaling, Mitochondrial calcium, Organelle calcium signaling