Review of: "PERM1 interacts with the MICOS-MIB complex to connect the mitochondria and sarcolemma via ankyrin B"

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In this timely and interesting study, the authors analyze the localization and function of PGC-1- and ERR-induced regulator in muscle 1 (PERM1), a protein mostly present in heart and skeletal muscles. PERM1 has been previously described to localize to nucleus and cytosol, as well as to associate with an outer mitochondrial membrane. PERM1 has been connected to mitochondrial biogenesis in response to exercise and adaptive oxidative metabolism in skeletal muscles. In this study, however, the authors show predominantly mitochondrial localization of PERM1 and, interestingly, identify the MICOS-MIB complex as one of the PERM1 interaction partners, along with ankyrin B (ANKB). This observation extends the spectrum of functions of MICOS-MIB complex beyond its role in the maintenance of mitochondrial cristae morphology and protein assembly. Previously, it has been shown that Miro GTPases connect MICOS-MIB complex with TRAK motor adaptor proteins, whereas at the same time influencing the number of contact sites between endoplasmic reticulum and mitochondria (Modi et al, 2019). In this study, we see that in specialized cells such as those belonging to the muscle tissue, MICOS-MIB complex mediates interactions of mitochondria with sarcolemma through PERM1 and ANKB, influencing mitochondrial positioning, as well as functionality, in these cells. These findings point to the possible existence of a range of tissue-specific adaptor proteins, which interact with MICOS-MIB complex to influence mitochondrial adaptations to specific requirements.

Considering that mitochondrial function is extremely important for muscle tissues, the authors expectedly show that even though PERM1 knockout in mice has no effect on mitochondrial cristae structure, it reduces the number of subsarcolemmal mitochondria (SSM), as well as muscle force of affected animals. The authors next show that in comparison to interfibrillar mitochondria (IFM), SSM contain more PERM1, and also that the MICOS-MIB complex in SSM is affected by PERM1 knockout. Very detailed proteomic analysis in this study offer interesting insights into the turnover speed of different mitochondrial proteins and show that PERM1-/- cells have a higher protein turnover than WT cells, in addition to the increased membrane depolarization and decreased oxidative phosphorylation. Next, the authors analyzed the interaction partners of PERM1 using 293T cells stably or C2C12 cells transiently overexpressing PERM1 fused to the FLAG tag. Here, they identified several MICOS-MIB components, as well as SLC25A5 transporter, ANKB, vimentin and nestin as proteins which interact with PERM1. Analysis of the MICOS-MIB complex from

muscle cells showed that PERM1 associates with the core MICOS complex, and that for this the C-terminal anchor domain of the protein is important. ANKB interaction, however, occurs through a different part of the protein and loss of PERM1 negatively influences the ANKB signal intensity at subsarcolemmal sites. Taken together, the results of the authors indicate that ANKB interaction with PERM1, which in turn interacts with the MICOS-MIB complex is important for the positioning of mitochondria in the vicinity of the sarcolemma.
The following comments could have been additionally addressed by the authors.

1.) Isolation of the SSM from PERM1 -/- mice could require the adaptation of the isolation protocol due to the changes in the interaction between these mitochondria and surrounding structures. How did the authors control for this effect?
2.) Due to the reduction in MICOS-MIB complex in SSM from PERM1 -/- mice, it is possible that there is a reduction in TOM complex and consequently Tom20, which would not make it an ideal mitochondrial marker for analyzing mitochondrial distribution in PERM1-/- cells. Although proteomic analyses probably indicate this is not the case, it should have been addressed in the text.
3.) It is interesting that authors do not see a reduction in TFAM levels in PERM1-/- mutants, considering that TFAM interacts with MICOS complex and MICOS complex levels are reduced (at least in SSM).
4.) It is difficult to differentiate between the effects of MICOS-MIB reduction and the changes in mitochondrial positioning on mitochondrial respiratory ability. The loss of PERM1 affects both, but the reduction in MICOS-MIB will inevitably affect respiration. Therefore, further analysis of ANKB deficient cells would be interesting. Does ANKB loss affects PERM1 stability or just the abundance of SSM, and how does it reflect on mitochondrial respiration?
5.) The authors speculate that mitophagy does not play a role in the reduction of the number of SSM upon PERM1 knockout. Have the electron microscopy pictures or staining with mitophagy-specific markers corroborated this speculation?
6.) What happens in physically active muscles in terms of MICOS-MIB composition, and ANKB levels and localization? Whereas this is a complex question out of the scope of the present study, it is still an important one and will be hopefully addressed in the future by the authors.

This study is very thorough and detailed, and the results strongly support the conclusions of the authors. It will be interesting to see in the future if similar mechanisms of mitochondrial positioning, mediated by perhaps other proteins, and involving MICOS-MIB complex, can be identified in other tissues. The presented paper opens a possibility that this might be the case.