Ankyrin-B interacts with fission machinery in skeletal muscle to modulate mitochondrial dynamics

Trevor Pharr[2], Kayleigh M Voos[2], and Damaris Lorenzo[2]
[2]Department of Cell Biology and Physiology, UNC-Chapel Hill School of Medicine

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

UNC SCHOOL OF MEDICINE

ABSTRACT

Ankyrin-B (AnkB) is a cytoskeleton-associated protein which helps facilitate microtubule-based organelle transport globally[1] and has a role in insulin secretion, specifically in pancreatic β cells[1]. Mutations in AnkB have been linked to cell-autonomous adiposity[2], hereditary cardiac arrhythmia[1], and type 2 diabetes[1]. Mutations in AnkB have implicated metabolic syndrome in nearly one million North Americans[1]. Unpublished studies in the Lorenzo Lab have shown that mice lacking AnkB only in skeletal muscle (SKM) exhibit decreased exercise capacity, glucose mishandling, and elongated mitochondria **(Fig 1A)**. Previous proteomic data showed that Mitochondrial Fission Factor (MFF) interacts with AnkB **(Fig 1B)**. This project investigates the interaction of AnkB with mitochondrial fission protein MFF, Dynamin-related protein 1 (DRP1), and Mitochondrial Fission 1 protein (FIS1) endogenously in mitochondria-rich (soleus, SOL) and mitochondria-poor (gastrocnemius, GC) SKM. MFF was shown to interact with AnkB endogenously, while FIS1 and DRP1 do not appear to have a prolonged interaction with AnkB. Overexpression of fluorescently tagged AnkB with fluorescently tagged MFF and DRP1 in HEK293 cells confirms interaction between AnkB and MFF, but does not suggest sustained interaction between AnkB and DRP1; further experiments are needed to confirm transient interactions between AnkB and DRP1.

BACKGROUND

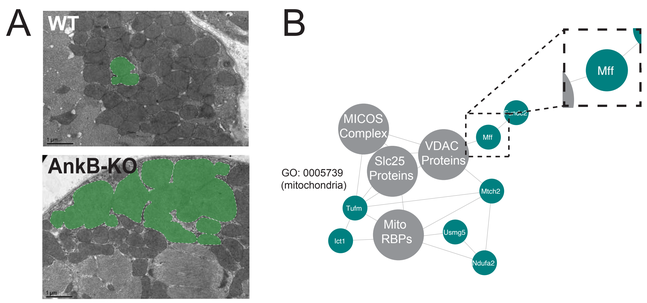


Figure 1: (A) Enlarged mitochondria phenotype is observed in SKM lacking AnkB. (B) Previous proteomics data showed that AnkB is an interactor of MFF in SKM, an important protein in mitochondrial fission.

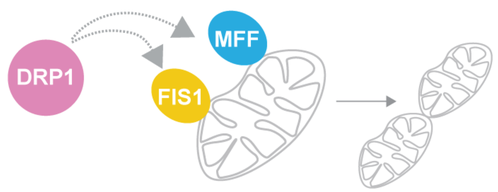
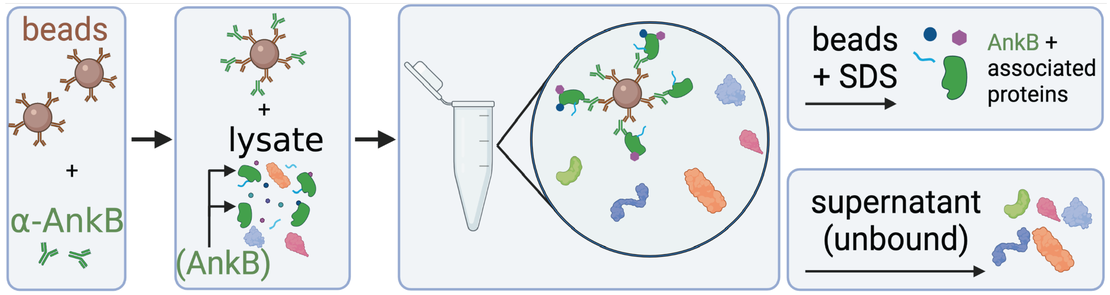


Figure 2: DRP1, a cytosolic protein, is recruited to the mitochondrial membrane via FIS1 and MFF to induce mitochondrial fission in healthy cells.

OBJECTIVE

To validate interactions between AnkB and mitochondrial fission proteins MFF, FIS1, and DRP1.

METHODS



RESULTS

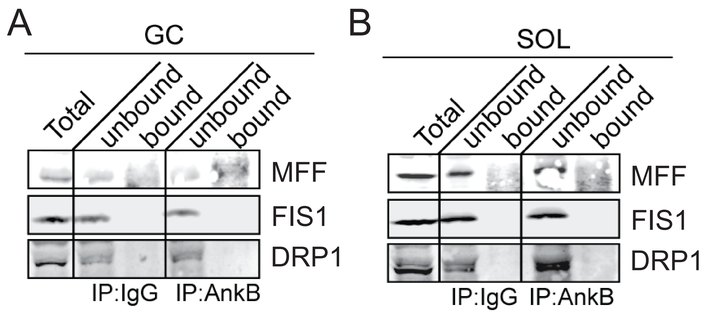


Figure 3: IP results pulling down AnkB and associated proteins from mitochondria-poor GC and mitochondria-rich SOL SKM. An α-rabbit IgG antibody is used as a negative control.

- The MFF band is enriched in the bound fraction of the AnkB-IP in both muscle types
- Mitochondrial fission proteins FIS1 and DRP1 are not enriched in the AnkB-IP for either muscle type, suggesting no strong interactions between AnkB and FIS1 or DRP1

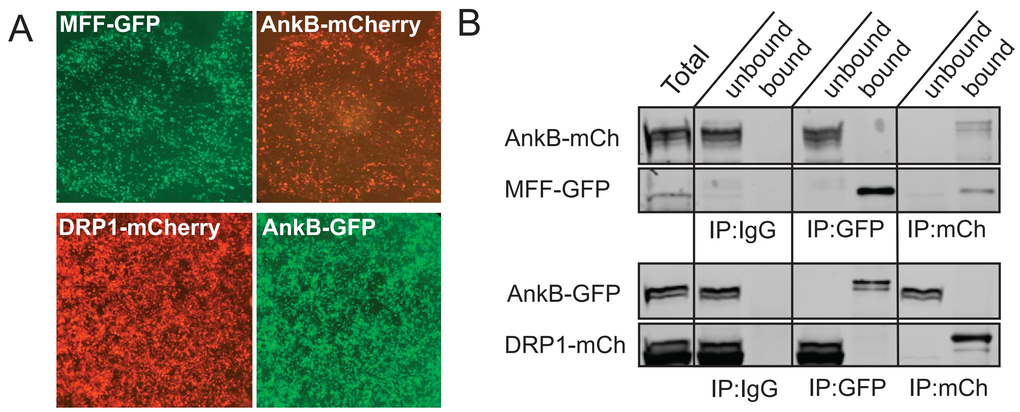


Figure 4: (A) Transfection of HEK293 cells with fluorescently tagged AnkB, MFF and DRP1 constructs is successful. Fluorescence expression patterns are similar between the labeled MFF and AnkB, as well as the labeled DRP1 and AnkB, suggesting similar expression levels. (B) IP results of AnkB interactions with MFF and DRP1 in over-expression system. IP pulling down GFP, mCherry, and anti-rabbit IgG antibody as a control.

CONCLUSIONS

- I have validated the MFF-AnkB interaction in SKM and in an over-expression system
- AnkB does not appear to interact with FIS1 endogenously, as expected
- AnkB-DRP1 interaction was not detected at endogenous levels in SKM or in over-expression system in HEK293 cells

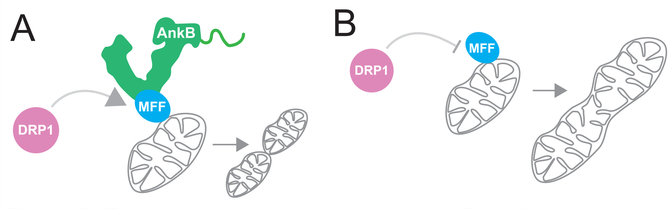


Figure 5: Proposed mechanism by which AnkB mediates the fission of mitochondria; (A) DRP1 is able to interact with MFF through AnkB-scaffolding, mediating mitochondrial fission. (B) In the absence of AnkB, scaffolding between MFF and DRP1 does not occur, and mitochondrial fission is inhibited. This leads to the enlarged mitochondria phenotype observed in AnkB-deficent SKM and energetic deficits.

FUTURE DIRECTIONS

- Purify mitochondria from control and AnkB-KO SKM to evaluate protein expression levels via western blotting and metabolic activity via an ATP synthase activity assay
- Investigate potential transient interaction between DRP1 and AnkB using cross-linking experiments
- Determine which domain of AnkB interacts with MFF

REFERENCES

(1) Lorenzo, D. N., Healy, J. A., Hostettler, J., Davis, J., Yang, J., Wang, C., Hohmeier, H. E., Zhang, M., & Bennett, V. (2015). Ankyrin-B metabolic syndrome combines age-dependent adiposity with pancreatic β cell insufficiency. Journal of Clinical Investigation, 125(8), 3087–3102. https://doi.org/10.1172/jci81317
(2) Lorenzo, D. N., & Bennett, V. (2017). Cell-autonomous adiposity through increased cell surface GLUT4 due to ankyrin-B deficiency. Proceedings of the National Academy of Sciences, 114(48), 12743–12748. https://doi.org/10.1073/pnas.1708865114

ACKNOWLEDGMENTS

I would like to thank PhD candidate Kayleigh Voos and Principal Investigator Dr. Damaris Lorenzo for their hard work and dedication to this project as well as their encouragement and assistance throughout the semester. I would also like to thank the Department of Cell Biology and Physiology at the University of North Carolina at Chapel Hill School of Medicine for making this research possible.