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Intracellular Parasite Toxoplasma Exploits the Unfolded Protein Response to Acquire Mitochondrial Metabolites

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Intracellular parasite *Toxoplasma* exploits the unfolded protein response to acquire mitochondrial metabolites

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Toxoplasma infection causes host ER stress

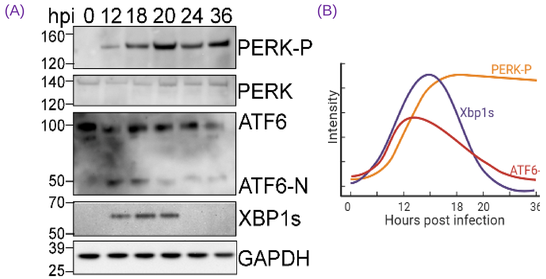

Figure 1. *Toxoplasma* infection activates unfolded protein response (UPR) in infected cells. (A and B) At the indicated times post infection (hpi), cells were harvested and the activation of each UPR sensory protein was measured by immunoblot using specific antibodies [1].

Toxoplasma recruits host mitochondria and ER

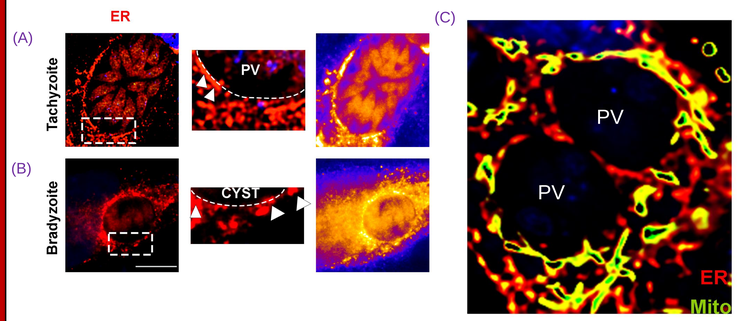

Figure 2. Host ER and mitochondria are recruited to *Toxoplasma* PV. (A) Cells were infected for 24 hours or (B) cells were infected for 24 h, then the cyst formation was induced for 48h using a standard protocol (CO_2 deprivation and media pH 8.3) to determine the host ER localization during acute (tachyzoite) and chronic (bradyzoite) stages, respectively. After fixation, cells were probed with ER marker antibody (KDEL-RED). ER (KDEL) localization is shown as a heat map, with yellow showing the highest ER-KDEL intensity and blue showing the lowest intensity. (C) Cells were infected for 24 h, and after fixation, cells were probed with ER marker (IRE1) and mitochondria markers (MitoAF488-Millipore) to determine colocalization of two organelles and *Toxoplasma*-PV PV. Representative image from Z-stack after deconvolution. PV: parasitophorous vacuole. Bar: 10um.

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NIH

Abstract and Background

- *Toxoplasma gondii* is an obligate intracellular parasite that can infect virtually all warm-blooded animals.
- It is estimated that nearly **2 billion people** globally and approximately **25% of the US population** have been infected.
- *Toxoplasma* recruits the host cell's ER and mitochondria into close proximity to the parasitophorous vacuole (PV).
- Unpublished data suggests that *Toxoplasma* induces mitochondrial elongation in order to acquire fatty acids and establish a niche for itself.
- It has been shown that PERK, a protein that is part of the unfolded protein response (UPR), coordinates mitochondria elongation [2].
- We hypothesize that *Toxoplasma* uses PERK to induce mitochondrial elongation, giving it access to the fatty acids it needs.
- Our data shows that *Toxoplasma* induces the UPR, activating PERK and subsequently inducing mitochondrial elongation.
- Using immunofluorescence in a procedure similar to [2][3], we found that this elongation could be prevented using a pharmacological approach to inhibit PERK.
- This discovery could lead to new strategies for treatment of toxoplasmosis.

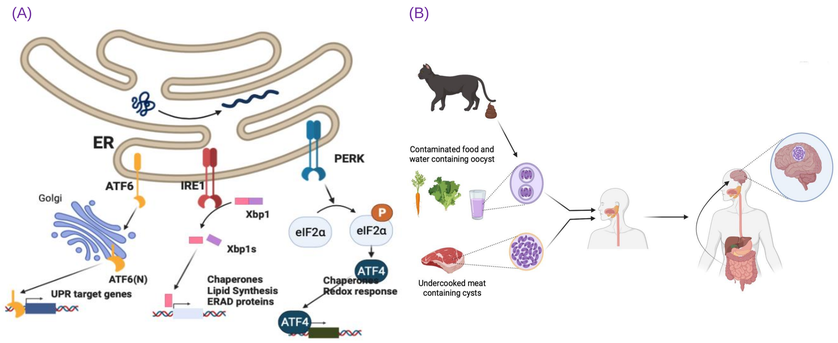
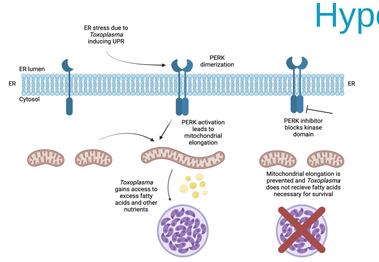


Figure 3. Background
(A) Model of the unfolded protein response (UPR) [4]. ATF6, IRE1, and PERK, the three arms of the UPR, work together to eliminate stress in the ER. (B) Humans get infected after ingestion of chronic forms oocyst or tissue cyst in contaminated food or water supplies [4]. Typically associated with felines including cats, the definitive host in which occur *Toxoplasma* sexual cycle, *Toxoplasma* can infect virtually all warm-blooded animals.

Hypothesis



- We hypothesize that *Toxoplasma* infection leads to mitochondrial elongation so that the parasite can access fatty acids and other nutrients.
- We also hypothesize that this process is mediated by PERK and that by inhibiting PERK, the infection can be fought against.

Figure 4. Model of Hypothesis [4]

Toxoplasma induces host mitochondrial elongation through PERK activation

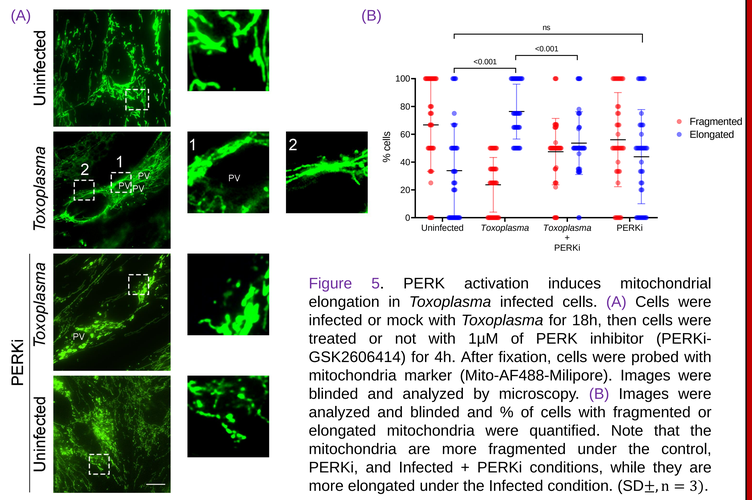


Figure 5. PERK activation induces mitochondrial elongation in *Toxoplasma* infected cells. (A) Cells were infected or mock with *Toxoplasma* for 18h, then cells were treated or not with 1μM of PERK inhibitor (PERKi-GSK2606414) for 4h. After fixation, cells were probed with mitochondria marker (Mito-AF488-Milipore). Images were blinded and analyzed by microscopy. (B) Images were analyzed and blinded and % of cells with fragmented or elongated mitochondria were quantified. Note that the mitochondria are more fragmented under the control, PERKi, and Infected + PERKi conditions, while they are more elongated under the Infected condition. (SD±, n = 3).

Conclusion and Future Directions

- *Toxoplasma* infection induces mitochondrial elongation.
- Elongation due to *Toxoplasma* infection can be suppressed with the introduction of a PERK inhibitor.
- An investigation into whether treatment with PERK inhibitor prevents the acquiring of fatty acids by the *Toxoplasma* will be the next step.
- A bacterial hybrid system may be used to see if ROPp18, a protein secreted by *Toxoplasma* known to interact with ATF6, interacts with PERK as well.
- We can test if administration of a PERK inhibitor is able to combat toxoplasmosis in murine models.

References

[1] Augusto et al. "*Toxoplasma gondii* Co-opts the Unfolded Protein Response To Enhance Migration and Dissemination of Infected Host Cells", 2020.
[2] Perea et al. "PERK Signaling Promotes Mitochondrial Elongation By Remodeling Membrane Phosphatidic Acid", 2022.
[3] Lebeau et al. "The PERK Arm of the Unfolded Protein Response Regulates Mitochondrial Morphology during Acute Endoplasmic Reticulum Stress", 2018.
[4] Figure made in BioRender