Strategies of *Mycobacterium tuberculosis* during infection: A look at the virulence factor PtpA and its role as a modulator of macrophage lipid metabolism

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BACKGROUND

The protein tyrosine phosphatase PtpA is a demonstrated virulence factor of *Mycobacterium tuberculosis*

Bach, H. et al. (2008), Cell Host and Microbe; Wang, J. et al. (2015) Nature immunology

During infection, PtpA translocates to the cytosol and nucleus of macrophages

Sullivan *et al.* (2012); Wong *et al.* (2013) *Nature Commun.* ; Wang, J. et al. (2017)

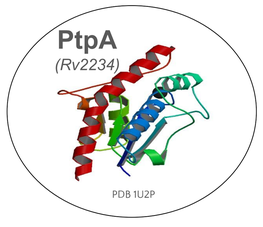
and interacts with various eukaryotic proteins, modulating different cellular responses:

Inhibition of phagosome maturation:
V-ATPase/VPS33B
(Bach et al. Cell Host Microbe., 2008, Wong et al. Proc Natl Acad Sci., 2011)

Inhibition of innate immune response:
MAPK p38/ Jnk: ↓ TNF**α**, IL-1β, IL-12 y NF-**κ**β
(Wang et al. Nat Immunol., 2015, Wang et al. Nat Commun., 2017)

Inhibition of apoptosis:
GSK3**α**
(Poirier et al. J. Biol. Chem., 2014)

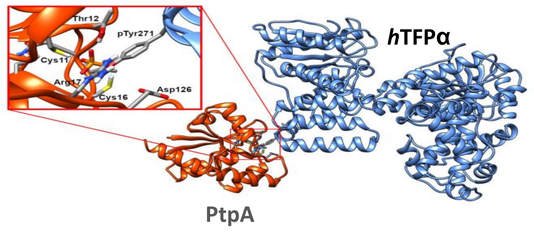
Potential modulation of host-lipid metabolism: *h*TFP$_{\alpha/\beta}$
(Margenat et al. Sci Rep., 2015)

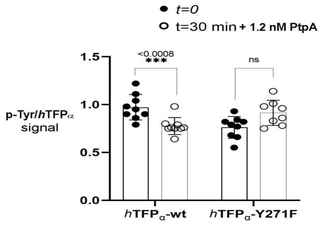


In vitro the *hTFP*$_{\alpha}$ is a substrate of PtpA

Margenat et al. Front Cell Infect Microbiol, 2023

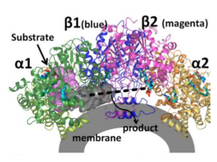
- *h*TFPα interacts with the active site of PtpA
- PtpA specifically dephosphorylates *h*TFPα in the p-Tyr271
- Tyr-271 is absent in TFPα of bacteria and is present only in more complex eukaryotic organisms



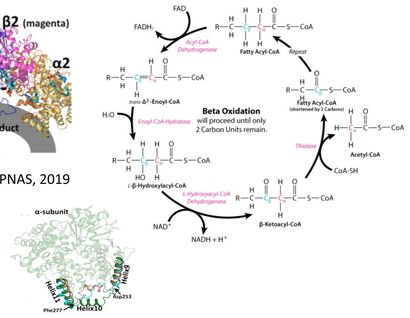


Human Trifunctional Protein - *h*TFP$_{\alpha/\beta}$

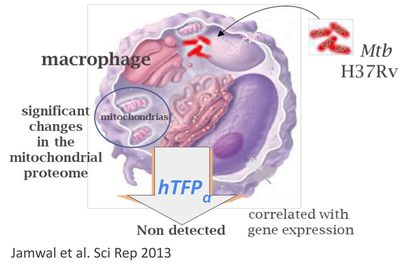
- *A key enzyme of the fatty acid β-oxidation*
- *The hTFP is synthesized in the cytosol and then translocated to the inner mitochondrial membrane, where it catalyzes 3 of the 4 reactions of the ß-oxidation of long-chain fatty acids*



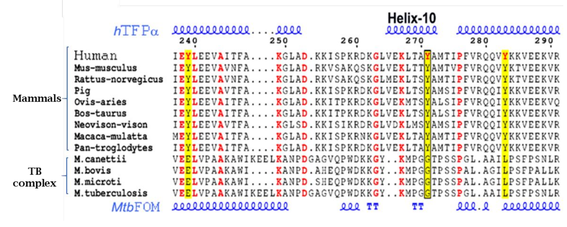
Xia et al, PNAS, 2019



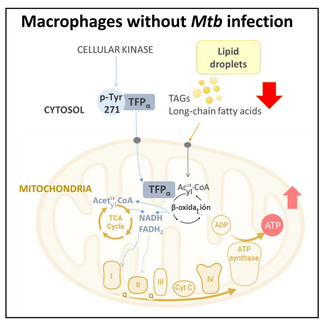
The hTFP$_{\alpha}$ *was no longer detected in the mitochondria of macrophages infected with the virulent Mtb H37Rv*

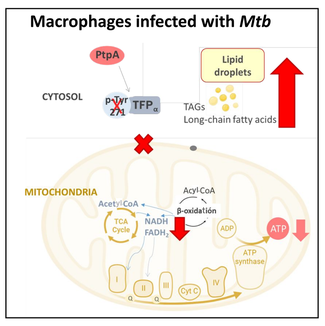


Jamwal et al. Sci Rep 2013



WORKING HYPOTHESIS

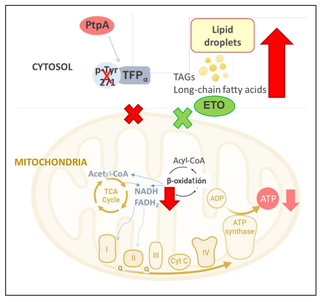




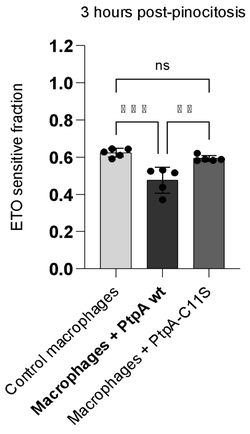
Mycobacterial PtpA affects *h*TFPα's subcellular localization and/or activity, decreasing macrophage ß-oxidation activity and promoting the accumulation of long-chain fatty acids in lipid droplets during infection

RESULTS of *in cellulo* approximations

- **We assessed the effect of PtpA activity on macrophage ß-oxidation by introducing either PtpA-wt or the inactive mutant PtpA-C11S into these cells**
- In these assays, long-chain fatty acids were provided as the primary carbon source for macrophages

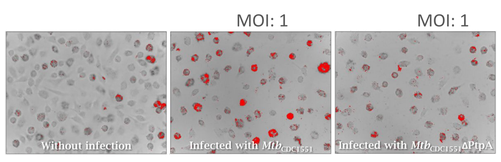


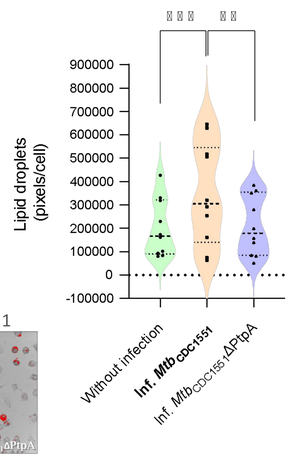
We determined oxygen consumption and the proportion of macrophages sensitive to Etomoxir, an inhibitor targeting the ß-oxidation pathway



The results showed that the proportion of macrophages responding to the inhibitor was lower only when PtpA was active, suggesting that the dephosphorylation of hTFP by PtpA was already affecting ß-oxidation

- **We evaluated the lipid droplet content of human macrophages derived from monocytes of healthy donors after infection with the virulent Mtb-CDC1551 and the mutant *Mtb*-CDC1551ΔPtpA**





We demonstrated that lipid droplet content increased only when PtpA was present

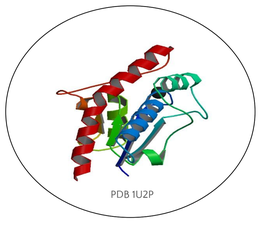
CONCLUSION

During infection, mycobacterial PtpA modulates lipid metabolism in human macrophages

Inhibits phagosome maturation

Inhibits innate immune response

Inhibits apoptosis



PtpA
(Rv2234)

PtpA promotes the persistence of M. tuberculosis within human macrophages

ACKNOWLEDGMENTS

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