SLAMTB
SOCIEDAD LATINOAMERICANA DE TUBERCULOSIS Y OTRAS MICOBACTERIOSIS

XI Reunión de la Sociedad Latinoamericana de Tuberculosis y otras Micobacteriosis

Characteristics of *Mycobacterium tuberculosis* PtpA interaction and activity on the alpha subunit of human mitochondrial trifunctional protein, a key enzyme of lipid metabolism

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Background

PtpA interacts with numerous eukaryotic proteins modulating cell signaling pathways relevant to bacterial persistence

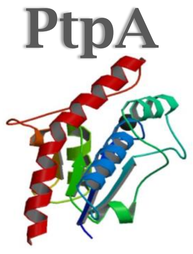
Inhibition of innate immune response

MAPK p38/ Jnk

↓ TNFα, IL-1β, IL-12 y NF-κβ

(Wang et al. 2015, 2017)

PtpA



Promotes bacterial survival in infected cells

Inhibition of apoptosis

GSK3α

(Bach et al. 2008, Wang et al. 2011 y 2015)

Potential modulation of host-lipid metabolism

***h*TFPα/β**

(Margenat et al. 2015)

Inhibition of phagosome maduration

VPS33B

(Bach et al. 2008, Wang et al. 2011 y 2015)

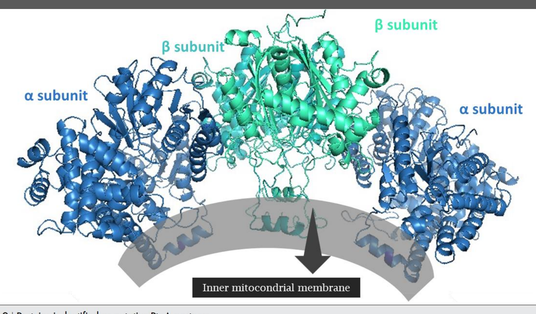


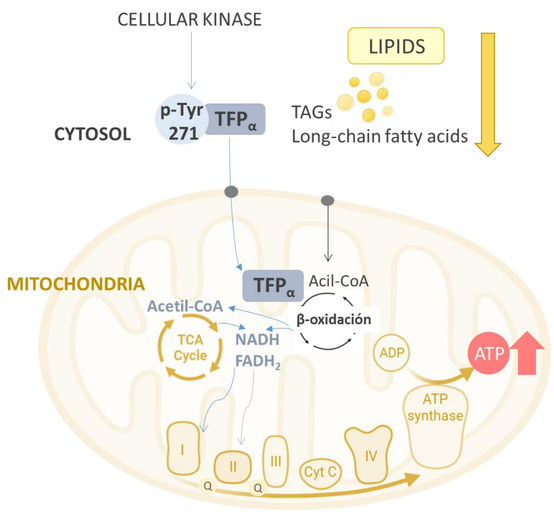
Table 2 | Proteins indentified as putative PtpA partners

Biological process[a]	Protein accession	Protein name	Mascot score[b]	Mass (Da)	No. of matched ions[b]	No. of peptide sequences[b]
Lipid metabolism, fatty acid beta-oxidation	ECHA_HUMAN	Trifunctional enzyme subunit alpha, mitochondrial	2422	82947	63	23
Sulfide oxidation, using sulfide: quinine oxidoreductase	SQRD_HUMAN	Sulfide:quinone oxidoreductase, mitochondrial	705	49929	28	10
Respiratory electron transport chain	ATPA_HUMAN	ATP synthase subunit α, mitochondrial	531	59714	16	7
Glycolysis	K6PP_HUMAN	6-phosphofructokinase, platelet type	922	85542	47	15

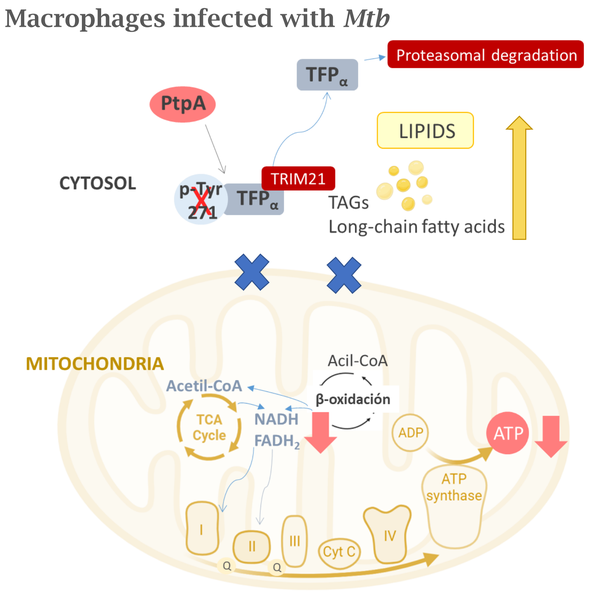
[a] Only the main biological processes with traceable author statement are shown, form UniProt (http://www.uniprot.org) database (released on January 2014)[38].
[b] The value of score, number of matched ions and peptide sequences is the best value obtained.

Working hypothesis

Macrophages without *Mtb* infection

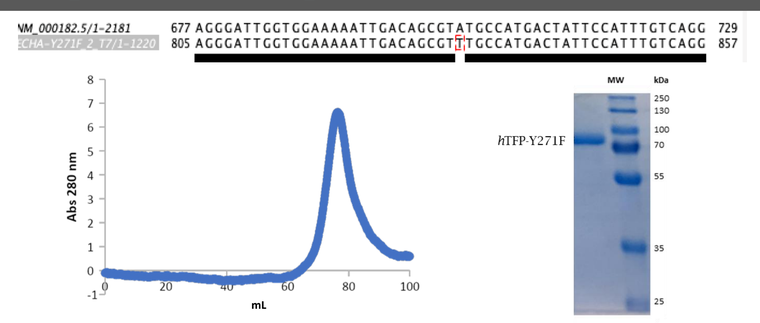


Macrophages infected with *Mtb*



Results

We produced and purified the recombinant *h*TFPα

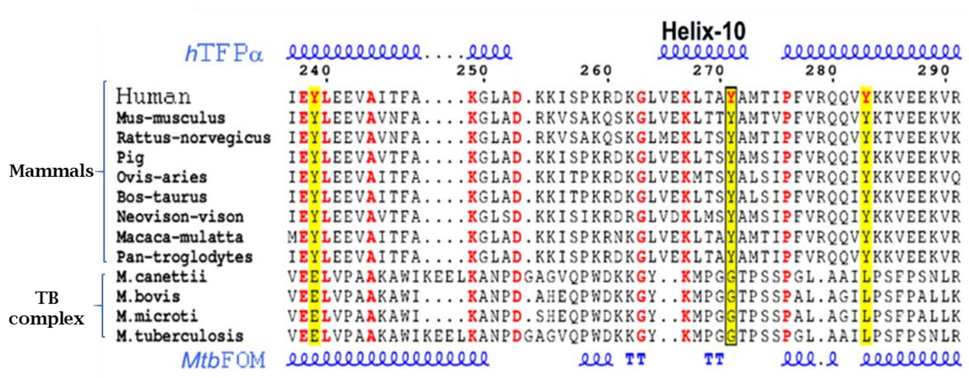


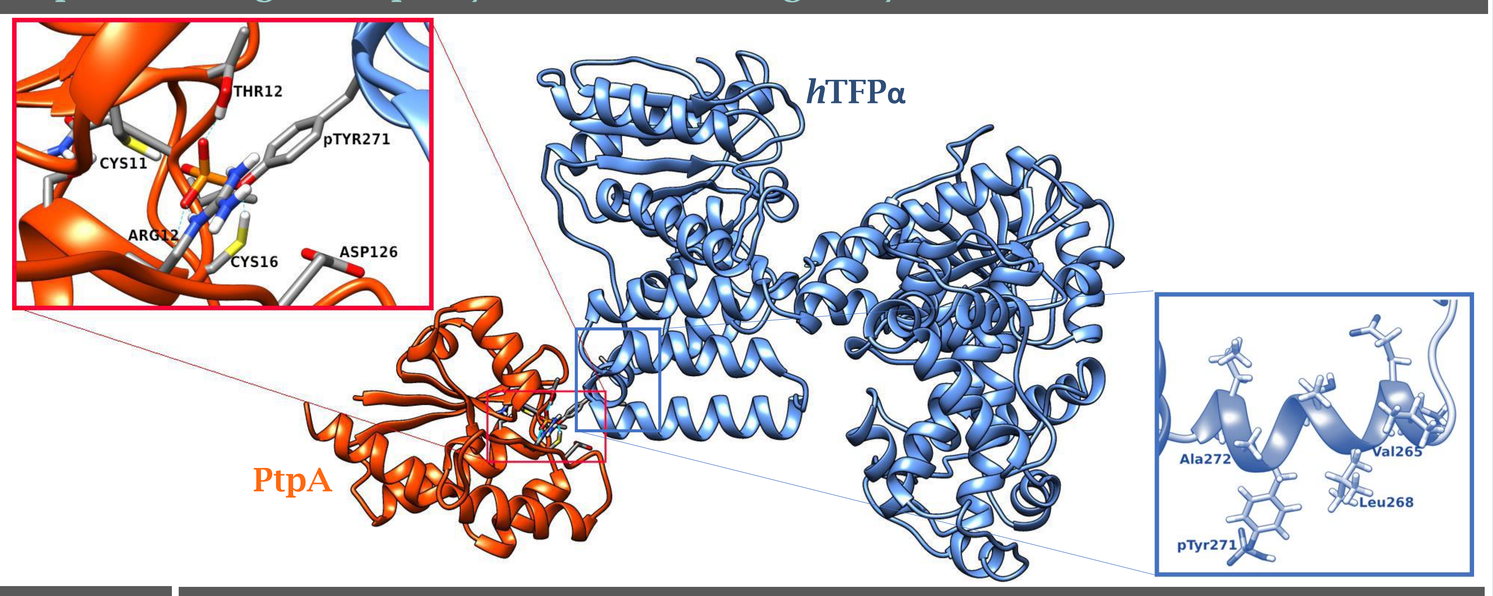
We demonstrated that *h*TFPα wt could be phosphorylated *in vitro* by Jak in the p-Tyr43 of the predicted signal peptide and in the p-Tyr-271 of helix-10 described as relevant for its anchorage to the inner mitochondrial membrane and activity

Detected p-Tyr	Peptide sequences	m/z	Measured MH	Theoretical MH	PPM	Primary Score	Secondary Score	Delta CN	Peaks Matched	Red Time	Classification Score
Tyr435	ALTSFERDSIFSNLTGQLDY**[79.9663]**QGFEKAD	1045,4754	3134,4115	3132,419737	-4,7655	2,0501	7,42192	0,4108	9	68,89	0,210116272
Tyr271	LTAY**[79.9663]**AMTIPFVR	731,867	1462,7267	1462,716369	7,0628	1,3658	7,420249	0,5141	5	71,87	0,64719594
Tyr499	KVIGMHY**[79.9663]**FSPVDKMQLLEIITTEK	968,489	2903,4525	2900,470121	-9,5304	2,0332	13,222184	0,5616	9	69,03	0,363273411
Tyr43	THINY**[79.9663]**GVKGDVAVVR	569,9602	1707,8659	1707,857765	4,7633	3,1363	21,506338	0,6802	15	46,35	0,780111649

We define the p-Tyr-271 as the potential target of PtpA by molecular docking assays

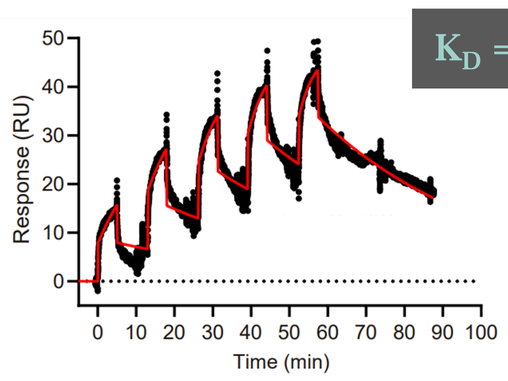
- Tyr-271 is absent in TFPα of bacteria and is part of the helix-10 of hTFPα, relevant for the activity and mitochondrial localization of the *h*TFP.

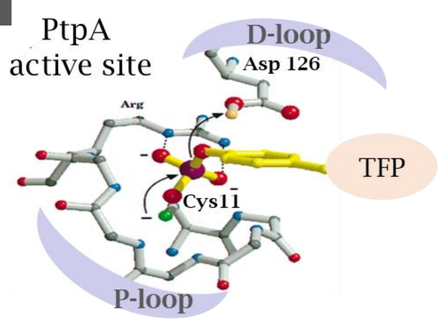


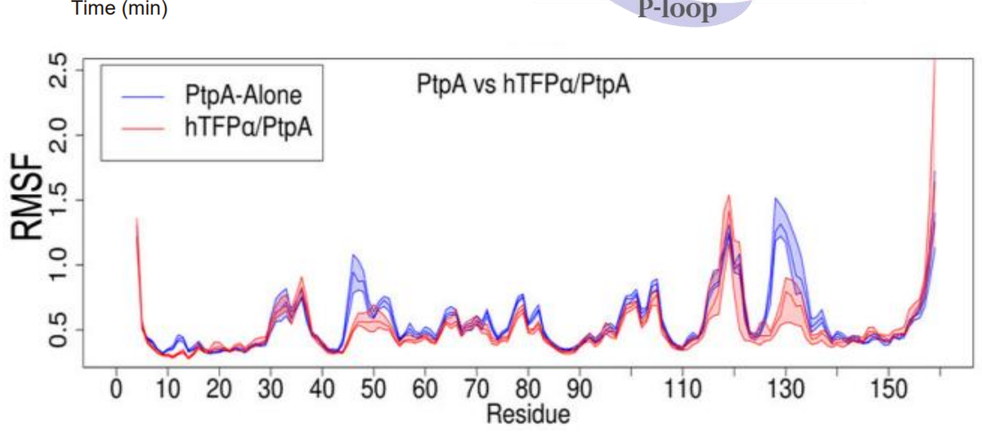


PtpA and *h*TFPα form a stable complex that involves PtpA active site

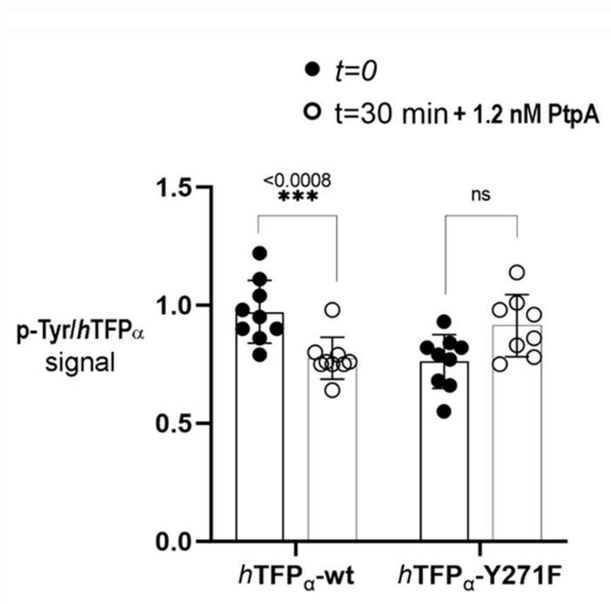
- Evaluated by surface plasmon resonance and molecular dynamics



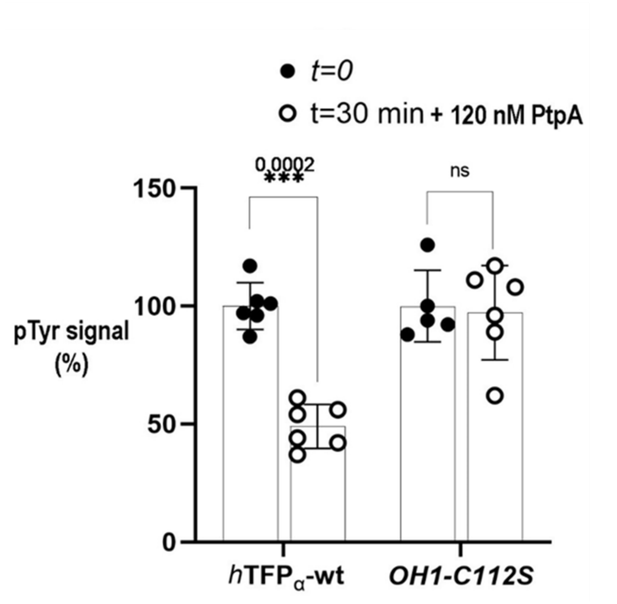




PtpA specifically dephosphorylates the recombinant hTFPα



Evaluation of dephosphorylation of *h*TFPα-wt and *h*TFPα-Y271F by PtpA



Evaluation of the specificity of PtpA using *h*TFPα-wt and the inactive mutant rOH1-C112S phosphorylated with the JaK kinase as substrates.

Challenge

We are analyzing the samples obtained after macrophages infections with $Mtb_{CDC1551}$ wt and $Mtb_{CDC1551}$ΔPtpA to evaluate whether potential changes in macrophage metabolism can be correlated with PtpA activity on TFP.

Acknowledgments

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