Decoding the signaling pathways modulated by phosphatases of intracellular pathogens.

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INRA SCIENCE & IMPACT

Decoding the signaling pathways modulated by phosphatases of intracellular pathogens.

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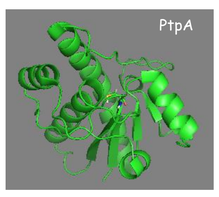
Decoding the eukaryotic signaling pathways

Modulated by protein tyrosine phosphatases (PTPs)



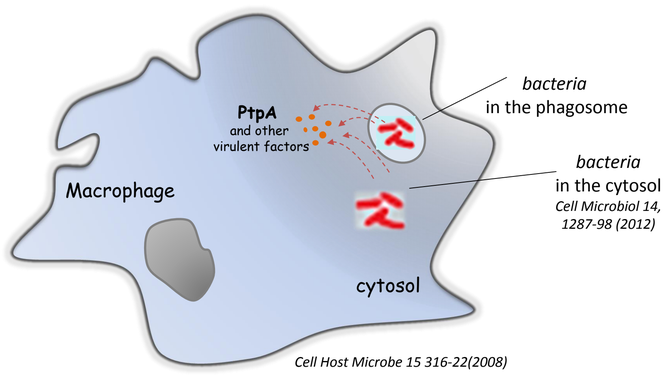
of intracellular pathogens

Mycobacterium tuberculosis

1-Which are the eukaryote substrates of these phosphatases?

2- Which are the signaling pathways modulated by these phosphatases?

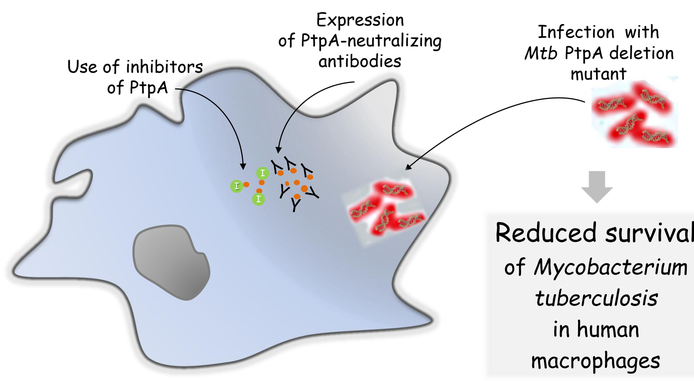
✓ The tyrosine phosphatase PtpA from *Mycobacterium tuberculosis* has been detected in the cytosol of infected macrophages



Cell Host Microbe 15 316-22(2008)

Cell Microbiol 14, 1287-98 (2012)

✓ PtpA is a key virulent factor



Reduced survival of *Mycobacterium tuberculosis* in human macrophages

Bioorg Med Chem 18, 3783-89 (2010)

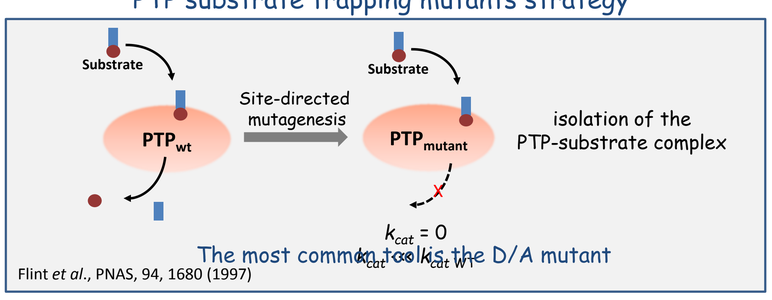
✓ Important role of mycobacterial phosphatases in the colonization of spleen and lung

MtbΔmms (**PtpA**, PtpB, SapM genes disrupted)

Plos ONE, 8, e77930 (2013)

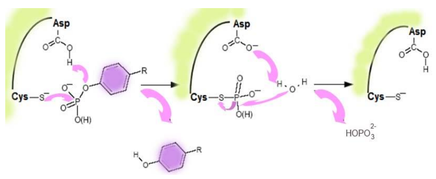
No bacilli were recovered in spleens and lungs of guinea pigs after 10 weeks of inoculation

"PTP substrate trapping mutants strategy"



The most common tool is the D/A mutant

Flint *et al.*, PNAS, 94, 1680 (1997)



We obtained the PtpA D/A mutant

The Asp 126 is a catalytic residue and is not considered crucial in defining substrate specificity

1-Which are the eukaryote substrates of these phosphatases?

"Improved PTP substrate trapping strategy"

A) Verification of the **structural and biochemical properties** of the PtpA D126A mutant to ensure its adequacy for substrate trapping

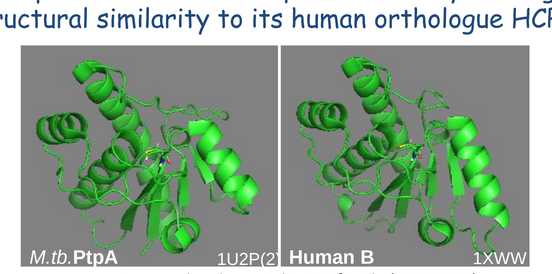
B) Preparation of **extracts** of human-macrophage-like THP-1 cells **preserving phospho-tyrosine** (P-Y) modifications

C) Analysis by SPR of **the interaction** between the phosphatase and the macrophage protein extracts, selection of conditions for washing and elution steps.

D) **Substrate trapping assay** using stringent experimental conditions defined by SPR to reduce non-specific interactions

E) **Validation** of protein candidates as substrates of PtpA

PtpA shows 37% of sequence identity and high structural similarity to its human orthologue HCPTPB.

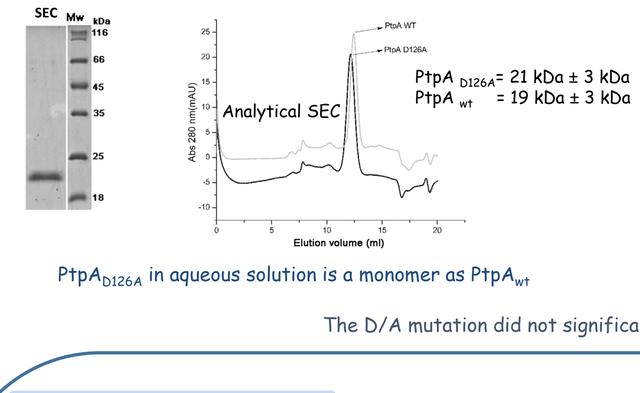


Low molecular weight PTP family (LMW-PTP)

The human genome encodes for 107 PTPs but only one belongs to the PtpA family. *Cell 117, 699-711 (2004)*

Verification of the structural and biochemical properties of the PtpA D126A mutant to ensure its adequacy for substrate trapping

$PtpA_{D126A}$ behaved similarly to the $PtpA_{wt}$ through the whole expression and purification procedure



$PtpA_{D126A}$ = 21 kDa ± 3 kDa

$PtpA_{wt}$ = 19 kDa ± 3 kDa

$PtpA_{D126A}$ in aqueous solution is a monomer as $PtpA_{wt}$

The secondary structure of $PtpA_{D126A}$ and $PtpA_{wt}$ are basically equal

Circular dichroism studies (CD)

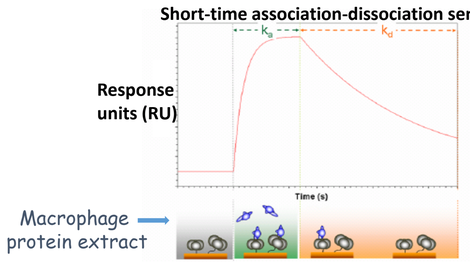
PtpA D126A
34% α-helical
19% β-strands
47% random coils

in agreement with the wt crystal structure (PDB 1U2P)

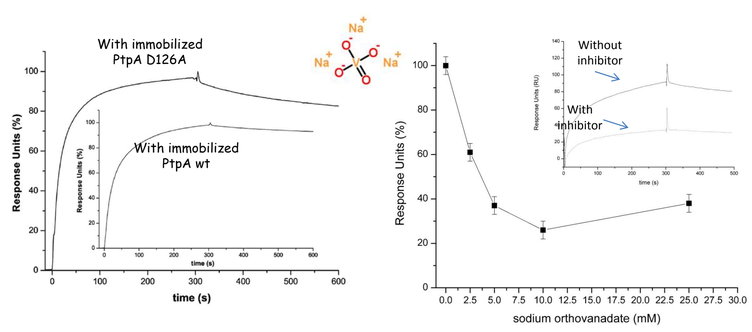
The D/A mutation did not significantly alter the enzyme structure

Study by Surface Plasmon Resonance (SPR) how PtpA interacts with macrophage protein components

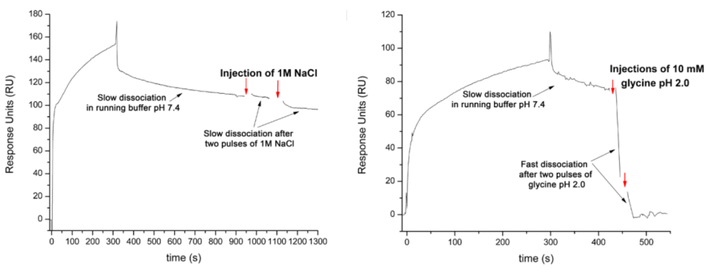
Short-time association-dissociation sensogram



Immobilization of PtpA wt or PtpA D126A on a sensor chip



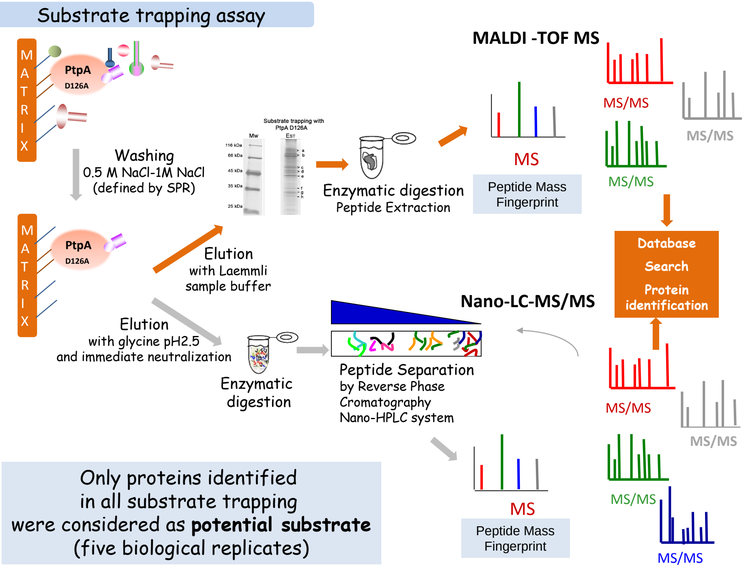
The macrophage extract contained components which were capable of binding to PtpA mutant through the active site



High salt concentration seems to be a good choice for washing steps to reduce non-specific binding

and low pH would be useful for eluting the proteins bound to PtpAD126A.

Substrate trapping assay



Only proteins identified in all substrate trapping were considered as **potential substrate** (five biological replicates)

Four novel putative PtpA substrates

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

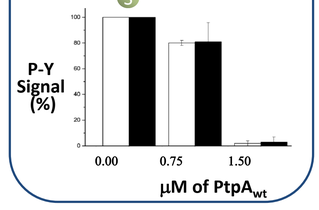
[a] Only the main biological processes with traceable author statement are shown, form UniProt (http://www.uniprot.org) database (released on January 2014)[58].
[b] The value of score, number of matched ions and peptide sequences is the best value obtained.

By different approaches we are addressing the validation of these proteins candidates as PtpA substrates.

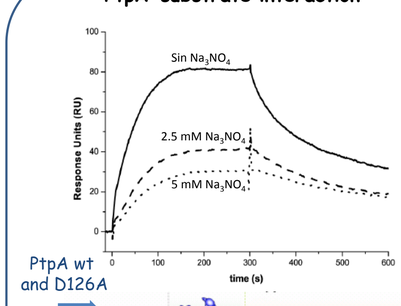
Immunopurification of each potential substrate

Dynabeads

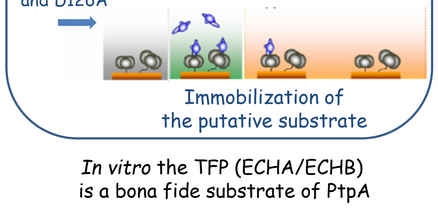
Phosphatase activity *in vitro*



PtpA-substrate interaction



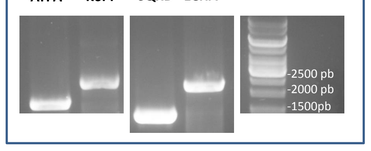
PtpA wt and D126A

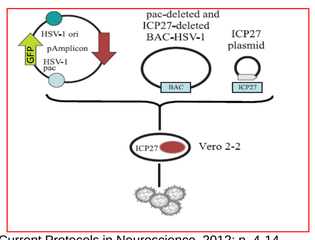


Immobilization of the putative substrate

In vitro the TFP (ECHA/ECHB) is a bona fide substrate of PtpA

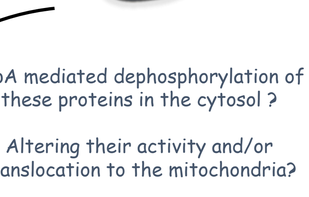
DNAc



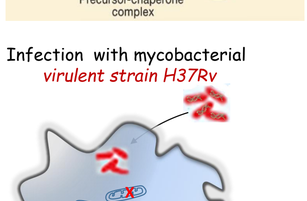


Current Protocols in Neuroscience, 2012: p. 4-14.

PtpA has been localized in the cytosol of infected macrophages



ECHA, ATPA, SQRD are synthesized in the cytosol



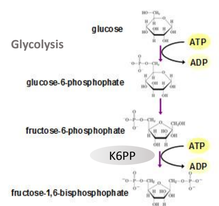
Infection with mycobacterial *virulent strain H37Rv*

Scientific Reports 3:1328(2013)

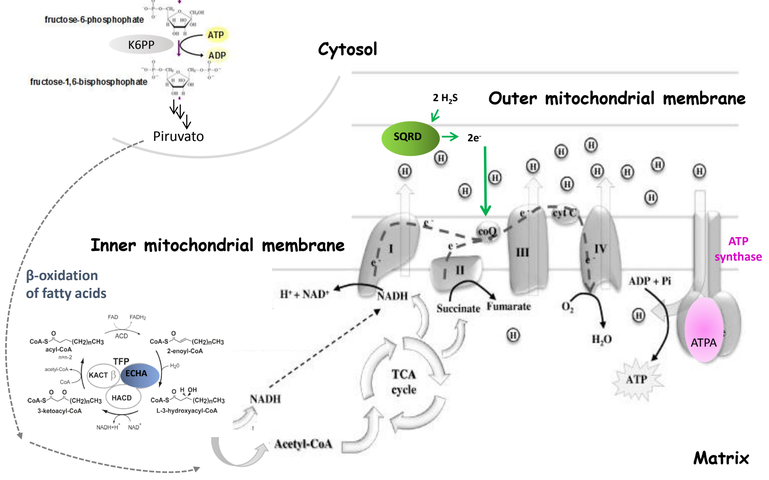
PtpA mediated dephosphorylation of these proteins in the cytosol ?

Altering their activity and/or translocation to the mitochondria?

Affecting pathways involved in cell energy metabolism?

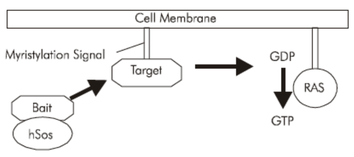


All putative substrates related to energy metabolism



Interaction at a cellular level

Two –hybrid system

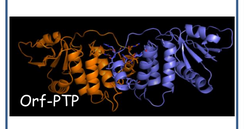


1. Target protein becomes anchored to cell membrane
2. Bait protein binds to target, localizing hSos to membrane
3. hSos activates RAS by promoting GDP/GTP exchange
4. RAS activates signaling cascade that permits mutant yeast cdc25H to grow at 37°C

CytoTrap Vector kit_Stratagene

Decoding the eukaryotic signaling pathways

Modulated by protein tyrosine phosphatases (PTPs)



of intracellular pathogens

ORF virus

1-Which are the eukaryote substrates of these phosphatases?

2- Which are the signaling pathways modulated by these phosphatases?

Project ECOS-SUD-France-Uruguay-ANII-CSIC-Uruguay