Investigating Msp1 in *T. brucei* Mitochondrial Quality Control

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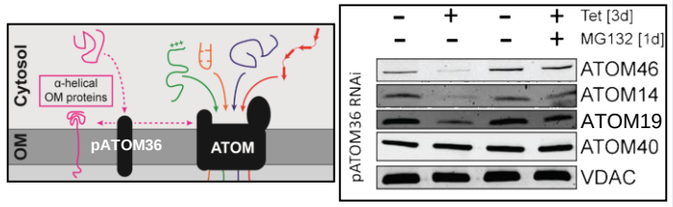
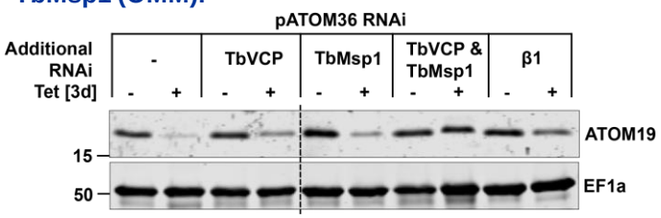
Introduction

Proteasome degrades outer mitochondrial membrane (OMM) proteins facilitated by AAA-ATPase orthologs



Whole cell lysates analyzed on SDS page, adapted from (1). Destabilized OMM proteins are degraded by the cytosolic proteasome.

How are these proteins recognized and removed from the membrane?
Orthologs of proteins with this function in yeast can be found in trypanosomes: TbVCP (cytosolic) and TbMsp1 (OMM).



Whole cell lysates analyzed on SDS page. Only simultaneous knockdown of TbVCP and TbMsp1 restores levels of pATOM36 substrates.

=> TbMsp1 & TbVCP have a synergistic relationship

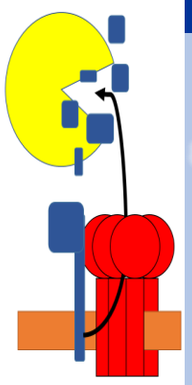
Mitochondrial Sorting of Proteins 1 (Msp1)

Hydrolyses ATP to remove defective or mislocalized c-terminally anchored proteins from membranes, allowing degradation by the proteasome

Localizes to OMM and peroxisomes in yeast

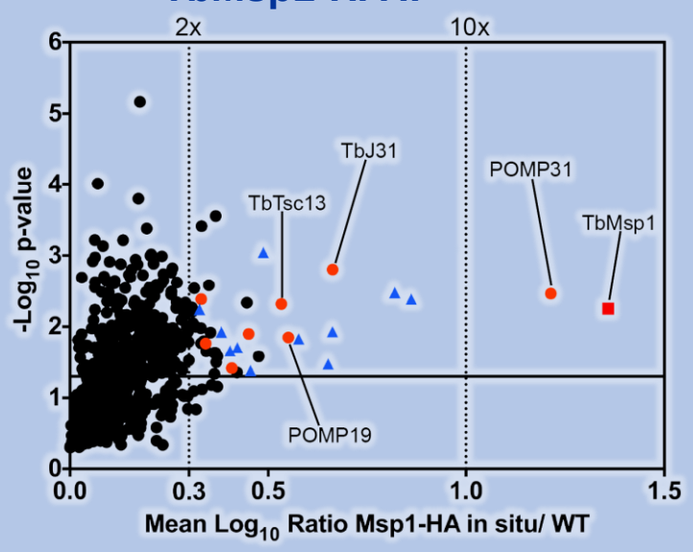
Functions in a hexameric complex

Yeast Msp1 is capable to remove proteins from a membrane without any substrate modifications or cofactors



Results

TbMsp1 stably interacts with OMM and glycosomal proteins

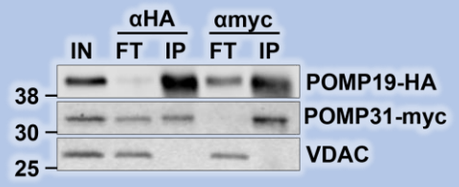


Accession number	Protein	Localisation
Tb927.5.960	TbMSP1	
Tb927.6.3680	POMP31	
Tb927.11.11520	PEX11	
Tb927.11.3130	GAT2	
Tb927.7.990	TbJ31	
Tb927.4.4050	GAT1	
Tb927.10.10610	protein tyrosine phosphatase	
Tb927.3.4500	fumarate hydratase	
Tb927.10.510	POMP19	
Tb927.3.1840	TbTsc13	
Tb927.1.720	PGKA	
Tb927.5.1710	p18	
Tb927.11.2380	phosphoglycerate kinase	
Tb927.2.2520	VDAC	

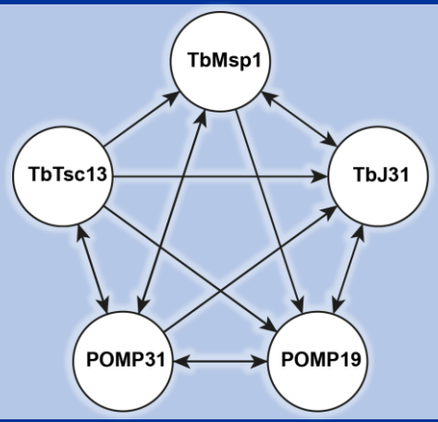
Mass spectrometry identified interactors in TbMsp1-HA SILAC IP with organelle-enriched fractions.
Localisation: Orange = Mitochondrial proteins; Blue = Glycosomal proteins; Red = Msp1

Interactions between Msp1 and identified OMM interaction partners confirmed by reciprocal CoIPs

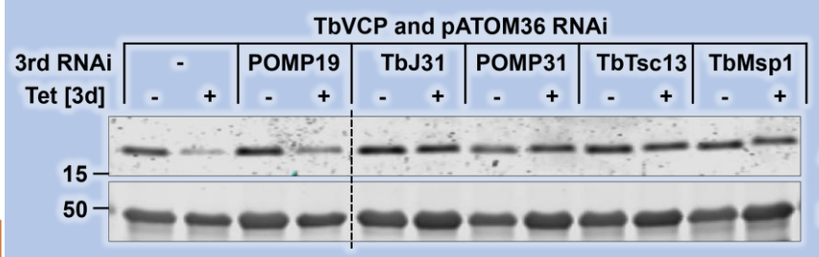
CoIPs were performed in dually tagged cell lines with one protein myc and one protein HA tagged. One exapmple is shown below. All pulldowns are summarized to the right.



=> Identified OMM interaction partners stably interact not only with Msp1 but also with each other



Msp1 function depends on the presence of TbTsc13, POMP31 and TbJ31 to remove pATOM36 substrates



When pATOM36 and TbVCP are both knocked down, the degradation of destabilized ATOM19 depends on TbMsp1

The knockdown of TbJ31, POMP31, and TbTsc13 inhibits the degradation of destabilized ATOM19 in the background of pATOM36 and TbVCP RNAi.

Outlook

Characterization of Msp1 function and interactions in bloodstream form trypanosome OMM

IP with substrate trap mutant Msp1

Investigate the role of Msp1 in the removal of foreign proteins

References:
(1) Sandro Käser, Silke Oeljeklaus, Jiří Týč, Sue Vaughan, Bettina Warscheid and André Schneider.
Outer membrane protein functions as integrator of protein import and DNA inheritance in mitochondria, PNAS, 2016

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