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Characterization of the *Tc*AQPα Aquaporin in *Trypanosoma cruzi* and Its Impact on H_2O_2 Tolerance

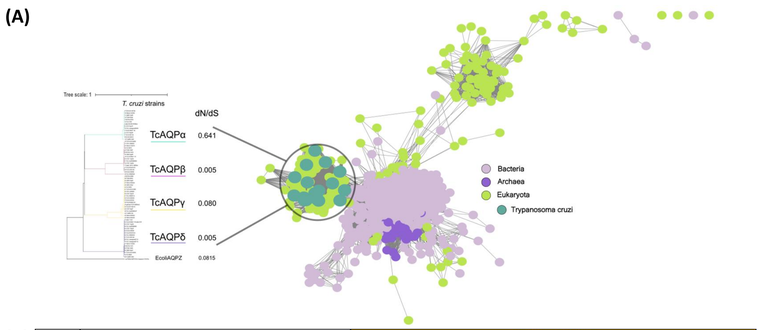
Ramoa U L[1,2], Bussolini Lizundia R[1], Tomasina R[4,5], Tinaglini R[3] , Alleva K[1,2], Chiribao M L[3,4], Vitali V[1,2].

[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Fisicomatemática, Cátedra de Física, Buenos Aires, Argentina.
[2]Universidad de Buenos Aires-CONICET, Instituto de Química y Fisicoquímica Biológicas "Prof. Alejandro C. Paladini", Buenos Aires, Argentina.
[3]Unidad Académica/Departamento de Bioquímica, Facultad de Medicina. Universidad de la República, Montevideo, Uruguay.
[4]Laboratorio de Interacciones Hospedero Patógeno, Instituto Pasteur de Montevideo, Montevideo, Uruguay.
[5]Unidad Académica de parasitología y micología, Instituto de Higiene, Facultad de medicina, Universidad de la República, Montevideo, Uruguay.

uramoa@docente.ffyb.uba.ar

Introduction

The AQPX subfamily: the case of *Tc*AQPα

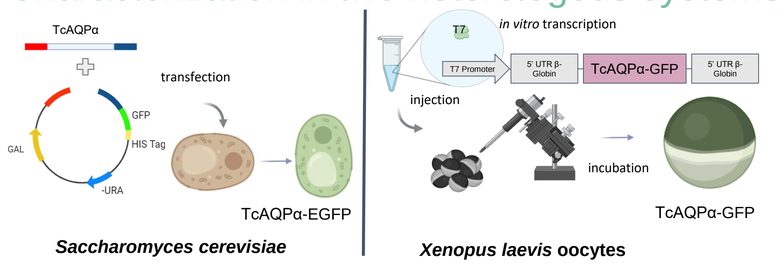


TcAQPX	Selectivity Filter						Distintive residues								
TcAQPα	NPA	NPA	I	S	G	V	T	A	I	I	Y	N	T	T	A
TcAQPβ	NPA	NPA	I	S	G	A	V	G	V	L	F	A	S	L	L
TcAQPγ	NPS	NPM	V	G	G	V	V	G	V	L	F	A	S	L	L
TcAQPδ	NPA	NPA	E	R	G	V	V	G	V	L	F	A	S	L	L

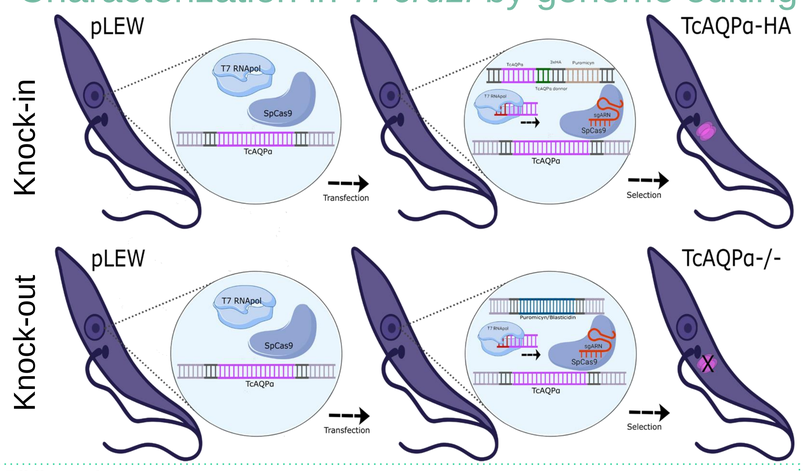
Figure 1 - Structural determinants of TcAQPα in the AQPX subfamily[1]. (A) Sequence Similarity Network (SSN) for AQPX (total sequences :1034) generated with EFI-EST[2]. The dN/dS values , calculated with PAML, indicate purifying selection in the four TcAQPX paralogues (B) Distinctive residues of TcAQPα. The motifs responsible for the unique selectivity filter of AQPX are presented in Table I, along with the specific structural determinants characteristic of TcAQPα, identified through Multi-Harmony[3] analysis of 68 different trypanosomatid strains. (C) Schematic representation of the TcAQPα residues identified B. (D) TcAQPα AlphaFold2 model highlighting residues identified in panel C.

Methods

Characterization in two heterologous systems



Characterization in *T. cruzi* by genome editing



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Acknowledgements

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for the modeling performed with AlphaFold2

Results

*Tc*AQPα-GFP localizes intracellularly in heterologous systems

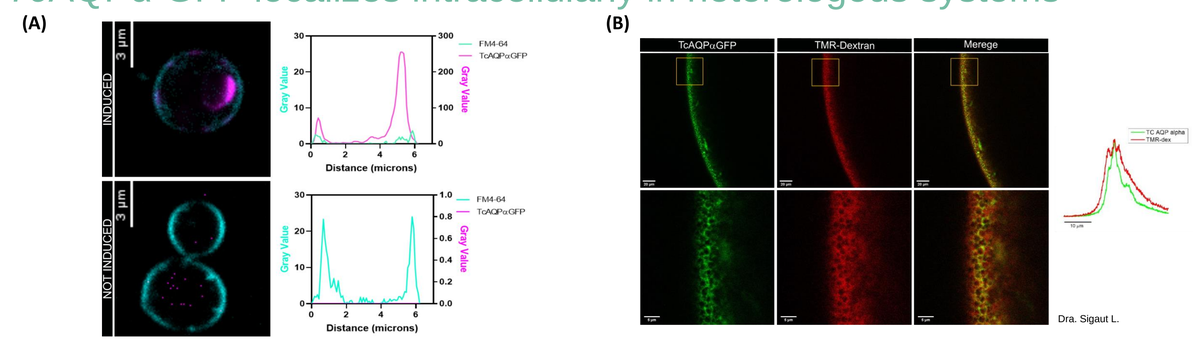

Figure 2 - In vitro characterization of TcAQPα. (A) Localization of TcAQPα-EGFP in *Saccharomyces cerevisiae* cells under inducing (2% galactose) and non-inducing (2% dextrose) conditions. Merged confocal images show TcAQPα-GFP (magenta) and yeast plasma membrane stained with FM4-64 (cyan). Both merged images and fluorescence intensity profiles (right panel) reveal that TcAQPα-GFP is predominantly localized intracellularly. (B) Confocal images of *Xenopus laevis* oocytes displaying TcAQPα-GFP (green) and TMR-dextran-labeled internal membranes (red). Merged images and fluorescence intensity profiles (right panel) indicate intracellular, non-membrane localization of TcAQPα-GFP, with intensity profiles shown for both the green and red channels. Analysis was performed using Fiji (version 1.54f). (C) Osmotic water permeability of *Xenopus* oocytes injected with TcAQPα-GFP RNA (8 ng), *MtPIP2;3* RNA (2 ng) as a positive control, and non-injected oocytes as a negative control.

*Tc*AQPα-HA is localized near the flagellar pocket in the epimastigote and trypomastigote stages, but is absent in amastigotes

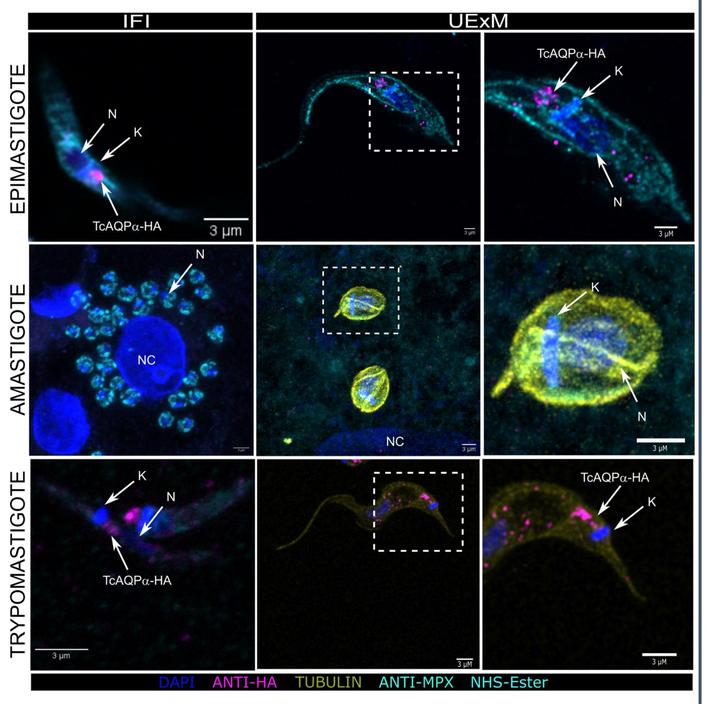

Figure 3 - **Immunolocalization of TcAQPα in *Trypanosoma cruzi* in different life stages**. The comparative panel shows indirect immunofluorescence (IFI, left column) and ultrastructure expansion microscopy (UExM, right column) images of a *Trypanosoma cruzi* edited line expressing TcAQPα-HA at different life stages: epimastigotes, amastigotes, and trypomastigotes. IFI samples were stained with DAPI (blue), anti-TcMPX (cyan), and anti-HA (magenta). UExM samples were stained with DAPI (blue), anti-TcMPX (cyan), and anti-HA (magenta) in epimastigote stage. For amastigote and trypomastigote stages, UExM samples were stained with NHS ester (cyan) and tubulin (yellow). No detectable signal for TcAQPα-HA was observed in the amastigote stage. K: kinetoplast; N: T. cruzi nucleus; NC: macrophage nucleus.

Growth rate and viability of *Tc*AQPα knockout strain resemble those of the wild type

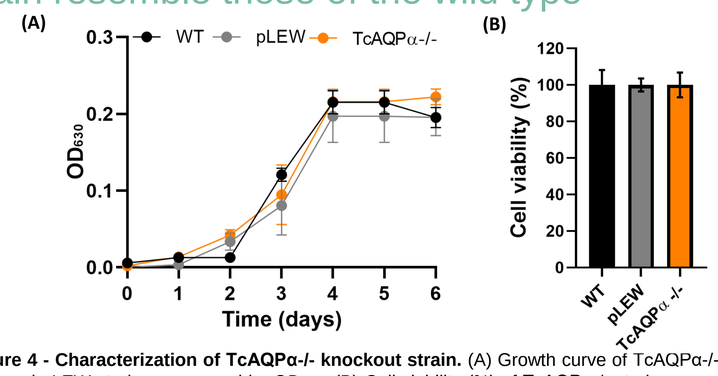

Figure 4 - Characterization of TcAQPα-/- knockout strain. (A) Growth curve of TcAQPα-/- (orange), WT, and pLEW strains, measured by OD_{630}. (B) Cell viability (%) of TcAQPα-/- strain assessed by the resazurin reduction assay[4].

The *Tc*AQPα-/- strain shows improved viability, and *Tc*AQPα-HA maintains its localization under oxidative stress

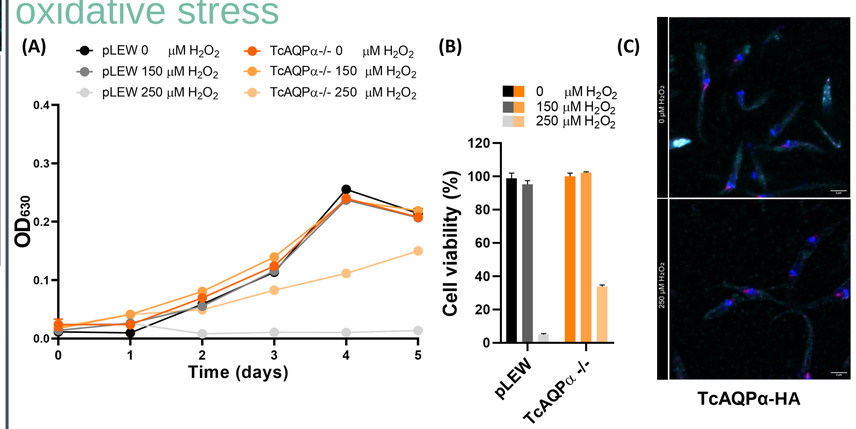

Figure 5 - Characterization of TcAQPα-/- and TcAQPα-HA strains under H_2O_2-induced stress. Parasites were treated with H_2O_2 (0, 150, or 250 µM) for 30 minutes. (A) Growth curve of epimastigotes pLEW and TcAQPα-/- by OD_{630}. (B) Cell viability (%) in response to oxidative stress.(C) Immunolocalization of TcAQPα-HA in *T. cruzi* epimastigotes treated with H_2O_2 (0 or 250 µM). Immunolabeling shows DAPI (blue), anti-HA (magenta), and anti-TcMPX (cyan), indicating TcAQPα-HA remains in its original location under oxidative stress.

Discussion

Our findings demonstrate that ***Tc*AQPα is not essential for parasite survival**, as evidenced by the viability of the *Tc*AQPα -/- strain. The enhanced tolerance to oxidative stress observed in this mutant strain strongly supports the hypothesis that *Tc*AQPα plays a pivotal role in maintaining redox balance within *Trypanosoma cruzi.* Interestingly, **the absence of *Tc*AQPα expression during the amastigote** stage may be linked to structural changes in the flagellar pocket, which occur at this life cycle stage and could influence the localization or function of aquaporins, including TcAQPα. Furthermore, ***Tc*AQPα-GFP localizes intracellularly in heterologous systems**, similar to its localization in the parasite, suggesting that structural motifs may also play a role. Specific motifs within the *Tc*AQPα sequence may regulate its membrane expression; however, further studies are required to elucidate the molecular determinants driving this process. While the precise permeability profile of *Tc*AQPα remains unknown, our results suggest that **H_2O_2 is a likely permeant candidate.**