Characterisation of a cation diffusion facilitator from the malaria parasite *Plasmodium falciparum*

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Malaria

- 229 million annual cases
- 409,000 deaths – mainly in children under five
- Resistance to almost all available drug classes
- New drugs with novel modes of action are required

Transition metal homeostasis

Transition metals such as zinc and iron are essential to the survival of the malaria parasite *Plasmodium falciparum*, but become toxic at high concentrations. Hence, the careful regulation of transition metals is required as the parasite progresses through its complex life cycle. With that considered, the perturbation of transition metal homeostasis is an attractive approach for novel malaria control strategies. Cation Diffusion Facilitators (CDFs) are a family of membrane transporters that enable the detoxification of various divalent transition metal ions from cells. These include, iron, zinc, manganese, cobalt and nickel.

Here we study the function of the sole predicted CDF from *P. falciparum*, using a *Saccharomyces cerevisiae* (yeast) heterologous expression system.

PfCDF confers partial zinc tolerance to a zinc-sensitive yeast line

The yeast expression vector p415GPD featuring the codon-optimised, full-length PfCDF coding sequence was transfected into a zinc-sensitive yeast strain (Δzrc1cot1), resulting from a lack of endogenous Zn^{2+} transporters Zrc1 and Cot1 **(Fig 2)**. In parallel, Δzrc1cot1 were transfected with empty p415GPD and p415GPD containing full-length *zrc1*, these were used as negative and positive controls respectively. Growth assays were undertaken to determine if PfCDF could rescue the growth phenotype of wildtype yeast at high concentrations of supplementary zinc.

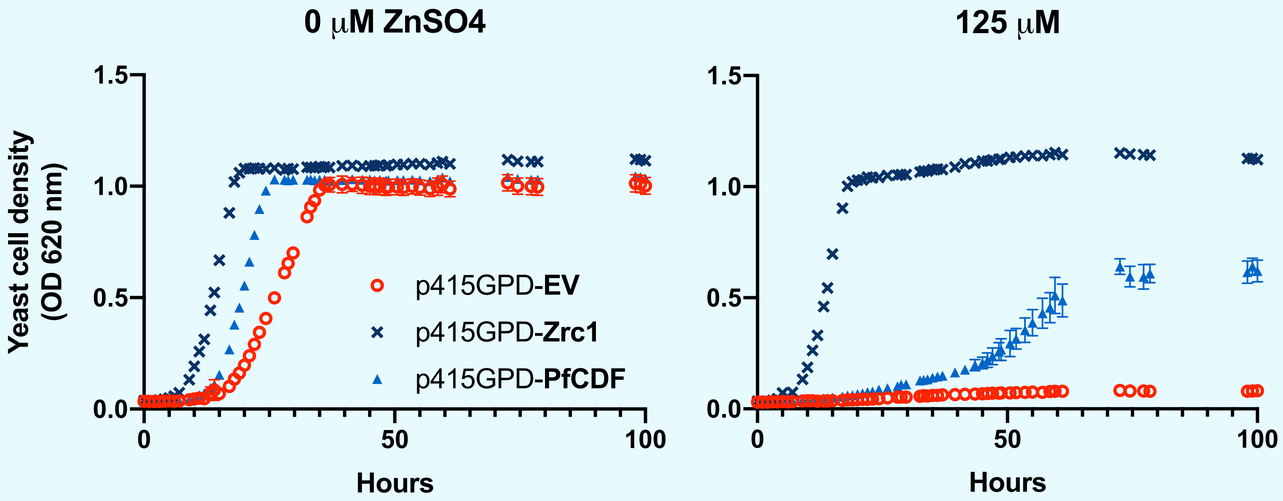

Figure 1. Growth assays in liquid media reveal that PfCDF confers partial zinc tolerance to Δzrc1cot1. Δzrc1cot1 transfected with p415GPD-PfCDF, p415GPD-Zrc1 (positive control), and p415GPD-EV (negative control) were cultured for 100 h ± 125 μM $ZnSO_4$. Yeast cell densities were determined by absorbance measurements at 620 nm. The data points shown are means (± SD) of five technical replicates. Growth curves are representative of three independent experiments.

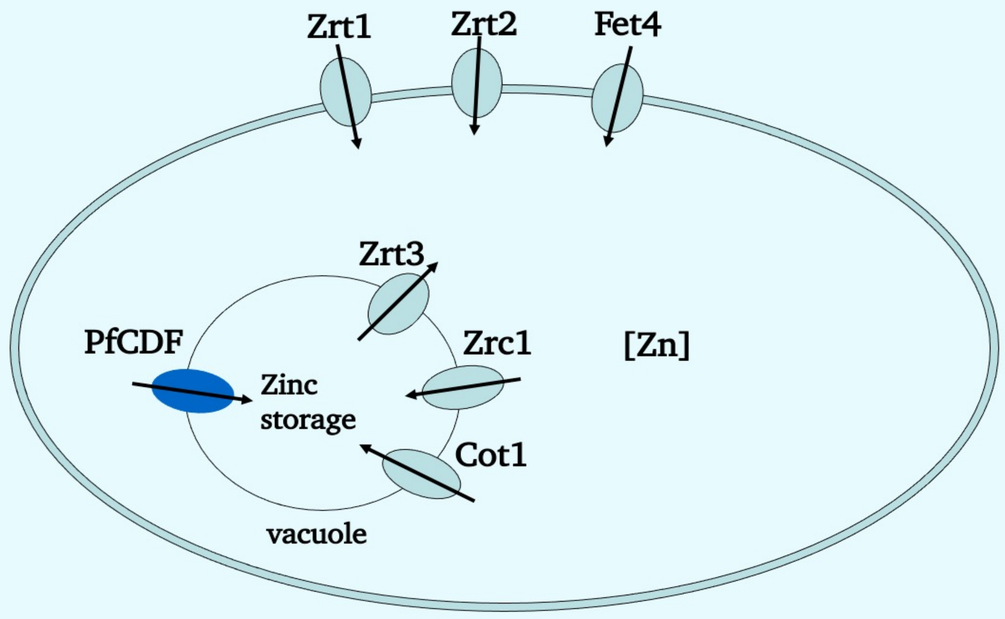

Figure 2. Diagrammatic representation of zinc transporters in *S. cerevisiae* yeast. Zrt1, Zrt2 and Fet4 mediate extracellular uptake of zinc into the cytosol. Zrc1 and Cot1 facilitate detoxification by zinc efflux into the vacuole. Zrt3 transports stored zinc back into the cytosol. Arrows indicate direction of zinc transport. The zinc sensitive yeast line used in these experiments - Δzrc1cot1 – lacks functional vacuolar zinc uptake by Zrc1 and Cot1, hence we recomplemented with PfCDF (blue). Sub cellular localisation of PfCDF in yeast is still to be confirmed.

When cultured in low zinc conditions, the growth of all three yeast strains was comparable **(Fig 1).** However, when cultured in high zinc conditions (125 μM), yeast expressing PfCDF achieved approximately half the yeast cell density of those that were recomplemented with Zrc1. Moreover, there was no detectable growth in yeast that were transfected with empty p415GPD. Together these data show that PfCDF expression conferred partial zinc tolerance to the zinc sensitive yeast. This suggests that PfCDF likely transports Zn^{2+} out of the cytosol, thus potentially implicating PfCDF as an important mediator of zinc tolerance in the parasite.

Substrate specificity of PfCDF

Family-wide phylogenetic analyses of CDFs have identified three major groups with each featuring different substrate specificities including zinc, zinc/iron and manganese[1, 2]. Our analysis shows that PfCDF sits within the defined zinc group, alongside orthologues from other apicomplexans and the yeast zinc transporters Zrc1 and Cot1 **(Fig 3)**. Also within the zinc group, the *Toxoplasma gondii* CDF has been characterised as an important mediator of zinc tolerance[3].

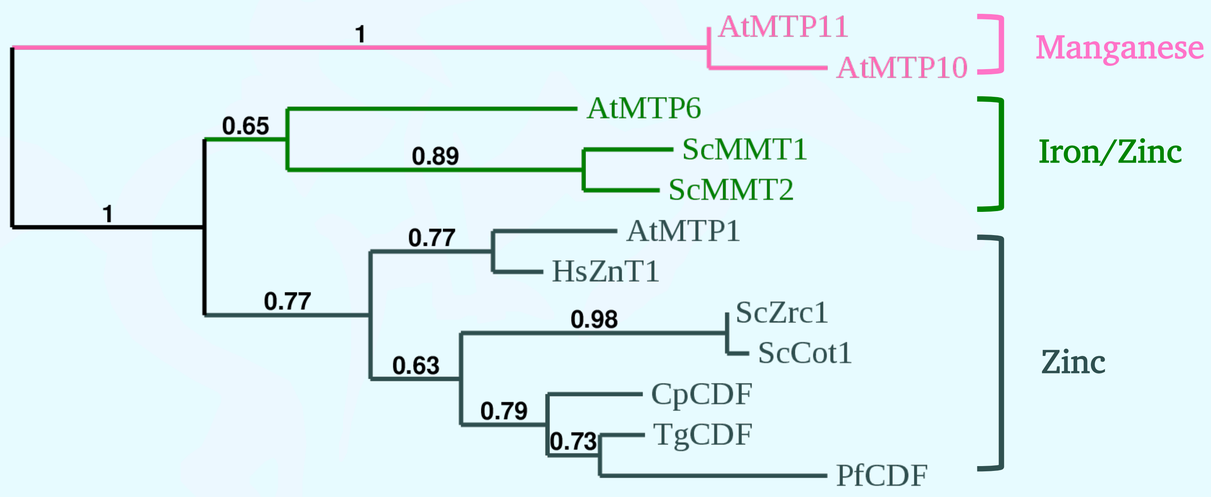

Figure 3. Phylogenetic analysis of PfCDF and functionally characterised orthologues shows three distinct groups with different substrate specificities. The tree was constructed using Phylogeny.Fr[4]. CDFs from the following organisms are represented; Tg = *T. gondii*; Cp = *Cryptosporidium parvum*; Sc = *S. cerevisiae*; Hs = *Homo sapiens*; At = *Arabidopsis thaliana*.

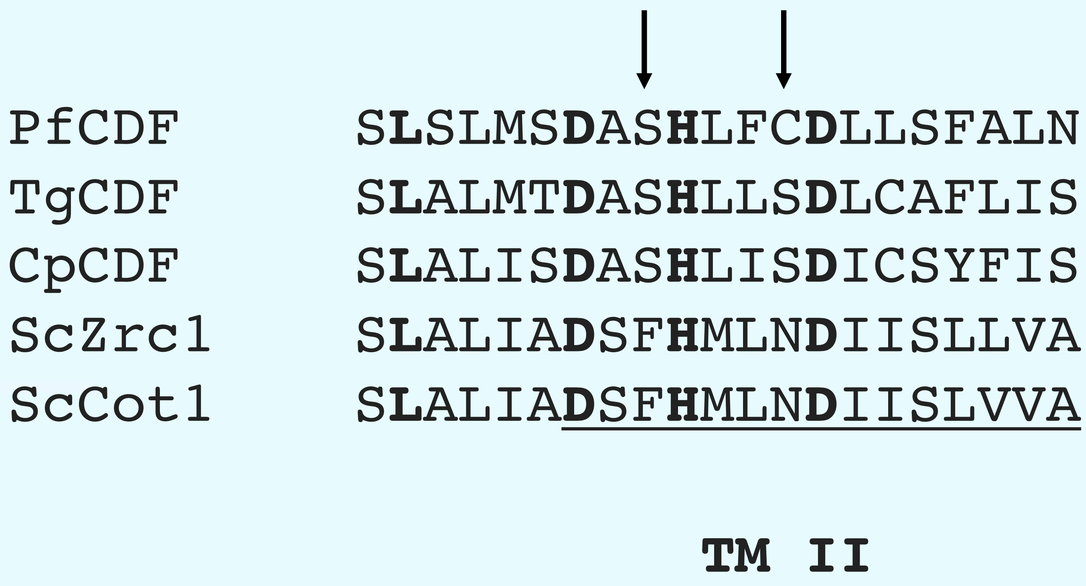

Figure 4. Protein sequence alignment of the second predicted transmembrane domain (TM II) of PfCDF with related apicomplexans (*T. gondii* & *C. parvum*) and functionally characterised yeast CDFs. Mutations in Zrc1 (F40S & N44I – relative positions indicated with arrows) shown to alter substrate specificity to include Fe^{2+}. The homologous serine (40) in PfCDF could enable iron transport function. Conserved residues required for zinc transport are in bold.

We have identified an amino acid residue (S40) in the PfCDF sequence that might enable the transport and perhaps therefore detoxification of iron (**Fig 4**). Mutant Zrc1 (F40S) was shown to gain iron transport function, but also retain zinc specificity as a result of this single mutation[5]. This hypothesis will form the basis of future study utilising an iron-sensitive yeast line.

References: **1**. Montanini et al., (2007) *BMC Genomics.* 8(107). **2.** Ibuot et al., (2020) *Metallomics.* 12(4). **3.** Chasen et al., (2019) *mSphere.* 4(3). **4.** Dereeper et al., (2008) *Nucleic Acids Res.* 36 (Web Server Issue). **5.** Lin et al., (2009) *Biochem J.* 422(2)