Identification and functional characterisation of zinc transporters in the human fungal pathogen *Candida parapsilosis*

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INTRODUCTION *Candida parapsilosis* is a diploid fungus belonging to CUG-clade of Ascomycota. It is a member of the normal human microbiota but can cause mild or severe infections particularly amongst low birthweight neonates under specific circumstances (1). During host - pathogen interaction microbes need to acquire not only carbon and nitrogen sources, but essential minerals as well. Indeed fighting for essential ions is so important that the host employs energy demanding processes to keep the concentration of free minerals as low as possible. This is known and characterised as nutritional immunity (2). Successful pathogens have evolved specific mechanisms by which they become capable of acquiring growth limiting minerals upon interaction with the host. One of these trace elements is zinc, that plays a very important role as a cofactor of proteins (3). The way of zinc acquisition is a described process in *Candida albicans*, where a secreted zinc-binding protein Pra1 uptakes zinc ions from host zinc-chelator/binding proteins and delivers them to the fungal high affinity plasma membrane importer Zrt1 (4). Pra1 ortholog is not present in *C. parapsilosis* that suggests that this pathogen utilises a different mechanism for zinc acquisition during the interaction.

Zinc transporters in <i>Candida albicans</i>	Function of orthologs in <i>S. cerevisiae</i>	Orthologs in <i>C. parapsilosis</i>
<i>CaZRT1</i>	High affinity zinc ion transporter	CPAR2_500170
<i>CaZRT2</i>	Low affinity zinc ion transporter	<i>CpZRT2</i> (CPAR2_210740)
	(Paralog)	CPAR2_806710
	(Paralog)	CPAR2_806720
<i>CaZRT3</i>	Vacuolar zinc exporter	CPAR2_212080
orf19.1536 (<i>ZRC1</i>)	Vacuolar zinc importer	<i>CpZRC1</i> (CPAR2_212100)

To reveal the key components of the zinc uptake in *C. parapsilosis* an *in silico* analysis was performed to identify potential zinc transporters.

Knock-out mutants were generated and sentenced to phenotypic analysis under different stress conditions as well as various zinc ion concentrations (5). The viability of only two out of six mutants differed from the control. At lower zinc ion concentration *cpzrt2Δ* mutant while at higher concentration *cpzrc1Δ* strain showed growth deficiency.

Kinetics of uptake by J774.2 macrophages

Mutant strains were incubated with J774.2 murine macrophages to record uptake kinetics with Amnis® FlowSight® flow cytometer. The *cpzrt2Δ* mutant was more, while *cpzrc1Δ* was less resistant to phagocytosis compared to the control in our model.

We also analysed the percentage of phagocytes associated with one or two (empty symbols) and more than two (solid symbols) fungal cells. The population of macrophages associated with at least three yeast cells formed more rapidly in the presence of *cpzrc1Δ* - and slower upon challenging with *cpzrt2Δ* mutants compared to the control.

Killing efficiency of J774.2 macrophages 3h post-infection

This was in line with our findings regarding fungal elimination by J774.2 cells. Killing of *cpzrt2Δ* was less effective, while in the case of *cpzrc1Δ* was more prominent, than the one of the control.

SUMMARY AND HYPOTESIS We found that the lack of the low affinity (fast) zinc transporter resulted in less viability during zinc starvation (0.05 mM) that coupled with higher resistance to uptake and elimination by J774.2 phagocytes. At the same time the absence of the vacuolar zinc importer decreased the viability of *C. parapsilosis* at high zinc concentration (5 mM) and the deletion strain was less resistant to phagocytosis and killing. This suggests that J774.2 macrophages might increase the zinc concentration in phagolysosomes to eliminate *C. parapsilosis*. To prove our hypothesis, we are planning to apply fluorescent dyes to measure phagolysosomal zinc concentration.