Mutation of *Rozella* ATP transport protein

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1. Introduction

Rozella allomyces is an obligate intracellular parasite that infects the water mold *Allomycis*. Like its close microsporidian parasitic relatives, *Rozella* has lost metabolic pathways for making the building blocks (nucleotides) of DNA and RNA. In order to compensate for these missing pathways, *Rozella* is thought to use putative nucleotide transport (NTT) proteins (as has been shown for microsporidia) to steal nucleotides needed for its replication from the infected host cell.

2. Aims

I will study the transport proteins of *Rozella* using *E. coli* to express the mutant proteins, and will formulate and test hypotheses of their function using computational analyses as well as lab experiments involving a variety of molecular biology techniques.

3. Methodology

1. The NTT mutations fused to a His affinity tag (5 in total) were cloned into an expression vector (pET-16b) and a combination of PCR and agarose gel electrophoresis were used to confirm that the mutations were present in the plasmids
2. *E. coli* (Rosetta (DE3)pLysS) were transformed with the plasmids. The expression of the mutant proteins was either induced with IPTG or not induced for each mutation
3. The expression of mutant proteins was tested for using SDS-PAGE and then a Western blot
4. An IFA (immunofluorescence assay) was carried out the on infected rabbit kidney cells to visualise presence of NTT

4. Results - Western blot following SDS-PAGE

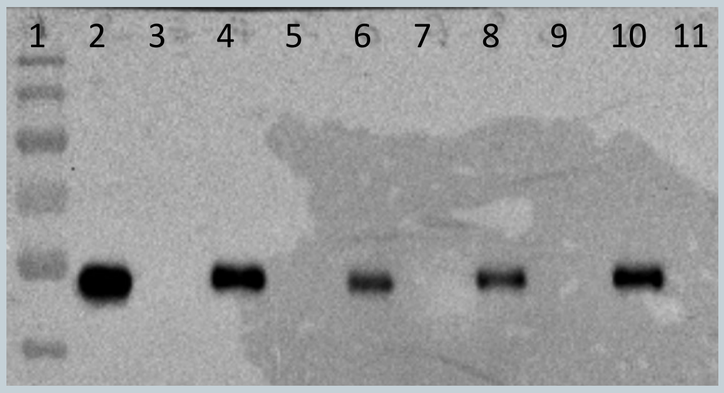


Figure 1:
Merge of Western blot following induction/ non-induction of mutant NTTs with IPTG

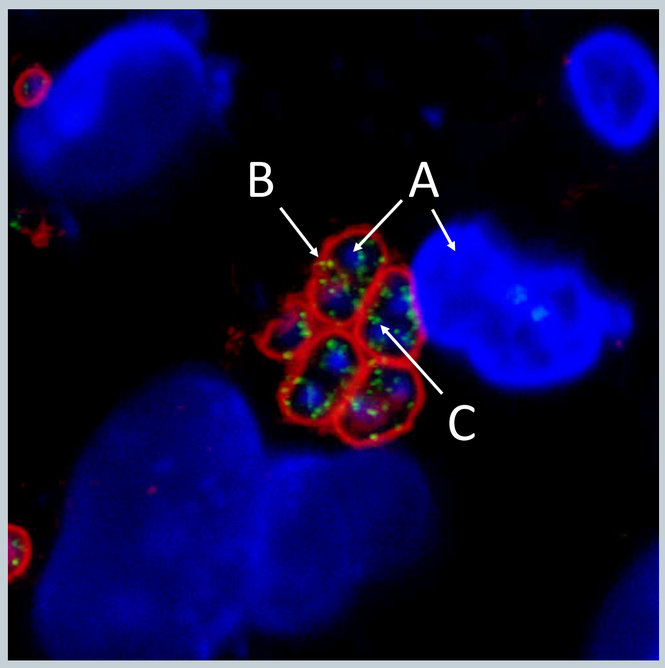
1: Molecular weight marker

2, 4, 6, 8, 10: Expression of mutant protein induced

3, 5, 7, 9, 11: Not induced

4. Results - IFA

Figure 2:
Staining of rabbit kidney cell infected by parasite. **A** shows the staining of nuclear material with DAPI (Both the rabbit nuclei and the nuclei of the parasite) in blue. **B** shows the NTTs stained in red. **C** is labelling the Hsp70



4. Discussion and Conclusions

- The mutant proteins were all expressed successfully in the Rosetta *E. coli* cells as can be seen by **Figure 1**. The addition of IPTG induced the expression of the proteins, and the cells that were not introduced to IPTG act as controls
- The **B** label in **Figure 2** demonstrates that the NTT protein localises to the plasma membrane of the parasite in order to steal nucleotides from the host
- The **C** label in **Figure 2** is showing the mitosomes of the parasite, where the Hsp70 protein is localised to

References

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2. Trentmann, O., Decker, C., Winkler, H. H., and Neuhaus, H. E. (2000) Charged amino-acid residues in transmembrane domains of the plastidic ATP/ADP transporter from arabidopsis are important for transport efficiency, substrate specificity, and counter exchange properties. *European journal of biochemistry / FEBS* **267**, 4098-4105

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