Studying two complementary infection models to identify common mechanisms of intracellular parasite survival: The roles of Leishmania and Eimeria exo-kinases in subversion of host cell signalling

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Studying two complementary infection models to identify common mechanisms of intracellular parasite survival: The roles of *Leishmania* and *Eimeria* exo-kinases in subversion of host cell signalling.

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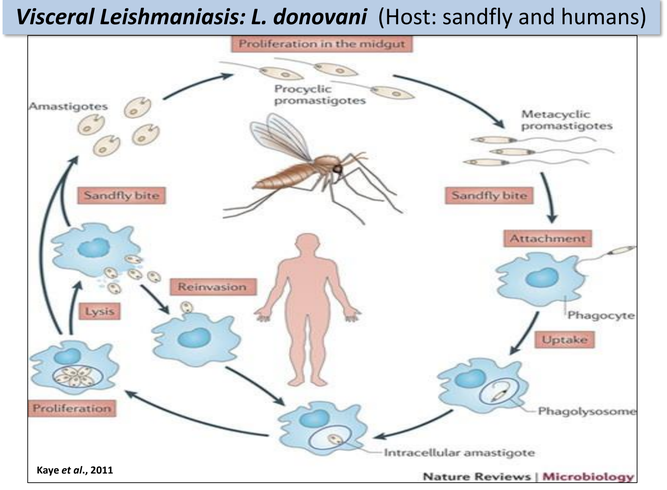
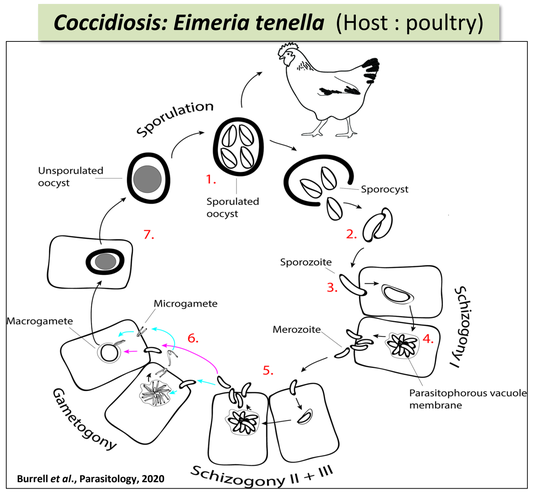
ABSTRACT: Upon infection, parasites secrete effector molecules particularly kinases, that modify their host cells to create a permissive environment. For instance, *Leishmania* secretes casein kinase 1.2 (L-CK1.2) into the host cell via exosomes. Previous studies in the lab demonstrated that L-CK1.2 interacts with and phosphorylates more than 200 host proteins *in vitro* and when expressed ectopically alters the phosphorylation of 771 host proteins involved in numerous host pathways, including a few known to be modified during *Leishmania* infection. During *Eimeria tenella* infection, rhoptry kinase 1 (*Et*ROP1) inhibits host cell apoptosis by interacting with host p53. It appears that these two exo-kinases from divergent parasites, impact similar host pathways. Our hypothesis states that there might exist convergence in the host pathways modified by *Leishmania* and *Eimeria*. To investigate this hypothesis, we will perform phosphoproteomic and transcriptomic studies. For *Leishmania*, we have just established the first ever phosphoproteome for *L. donovani* infected mouse BMDMs. We quantified about 12,000 phosphosites comparing infected versus uninfected, including 663 sites with a p-value below 0.05. In order to uncover L-CK1.2 specific effects, we also had infected cells treated or untreated with the L-CK1.2 inhibitor, D4476. Further analysis is ongoing. For *E. tenella*, chicken lung epithelial cells (CLEC213) were infected with either wild type or *Et*ROP1 overexpressing parasites; the samples were collected and will soon be sent for transcriptomic analysis. Once we have the complete set, including the phosphoproteome for *Eimeria*-infected CLEC213 cells and the transcriptome for *L. donovani*-infected macrophages, we will then assess which pathways are similarly or differently modified during both infections and decipher their role by interfering with important genes of these pathways using CRISPR-Cas knockout, RNAi or overexpression.

1) *Leishmaniasis and Coccidiosis*

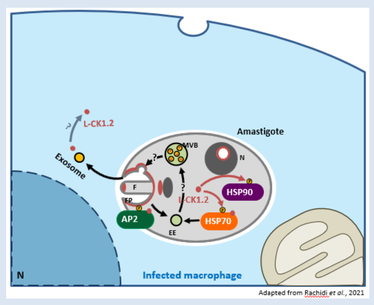
Visceral Leishmaniasis: L. donovani (Host: sandfly and humans)



Coccidiosis: Eimeria tenella (Host : poultry)

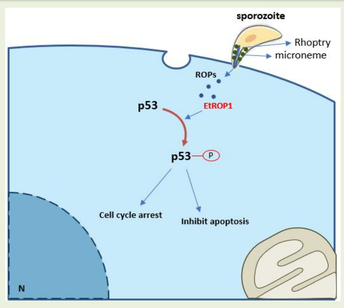


2) *Leishmania* casein kinase 1.2 (L-CK1.2)



- L-CK1.2 is a signalling protein kinase.
- Released via extracellular vesicles into the host macrophage (Silverman *et al*., 2010)
- Plays an essential role in intracellular parasite survival.
- It is a validated drug target.

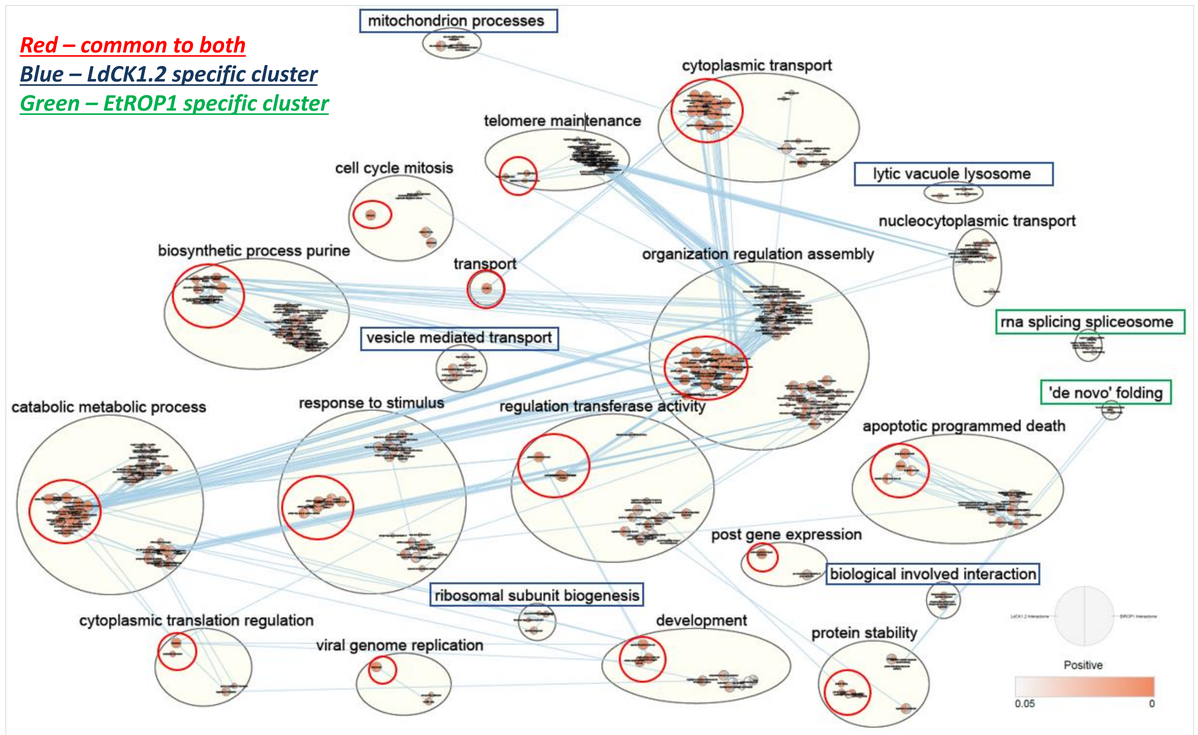
3) *Eimeria tenella* rhoptry kinase 1 (*Et*ROP1)



- *Et*ROP1 is an active kinase
- Protein family secreted into host cells
- Interacts with many host proteins *in vitro*
- *Et*ROP1 inhibits apoptosis and induces cell cycle arrest in host (MA Diallo *et al*., 2019)

4) Common host pathways might be targeted by LdCK1.2 and *Et*ROP1

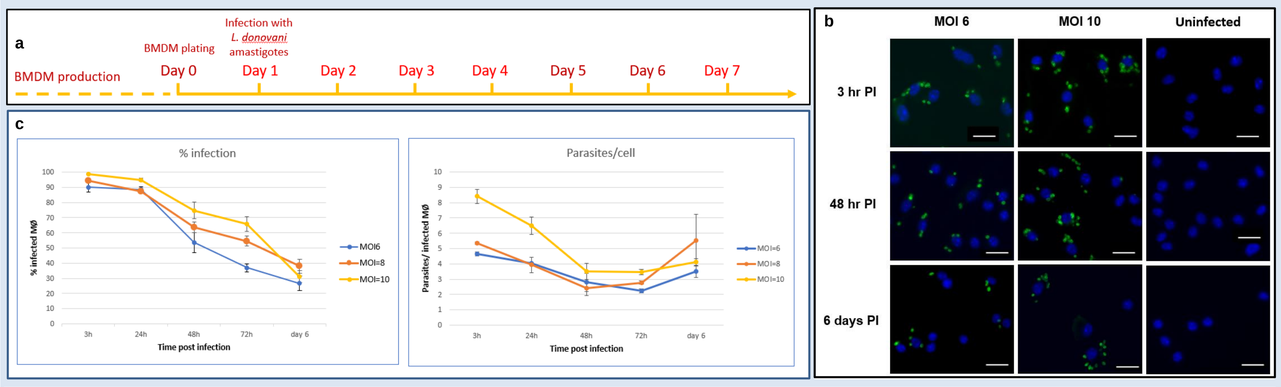
Using Cytoscape, the *in vitro* interactome data for the two kinases was used to compare the enriched pathways. The clusters affected by both kinases were identified.



5) Identification of host pathways affected by LdCK1.2 in infected BMDMs

A) Optimization of infection conditions

To obtain the best infection conditions (>70% infection at the chosen timepoint) for phosphoproteomic analysis, optimization of the infection conditions was performed. Bone marrow derived macrophages were plated in 24 well plates with 1.5x10^5 MØ/ well and infected with *L. donovani* amastigotes (MOI 6, 8 or 10) 24 hrs after plating.



a) Experimental plan b) MOI 6 and 10 at 3h, 48h and 6 days post infection. The fixed cells were stained with Hoechst (Blue) for nuclei and hamster immune serum (Green) for parasites. The cells were imaged with the Andor camera of Zeiss Axiophot fluorescence microscope at 63X magnification. c) 100-200 MØ per condition were analysed. The percentage of cells infected per field and the number of parasites per infected cell for different timepoints post infection was calculated and the mean was plotted. Error bars = SEM.

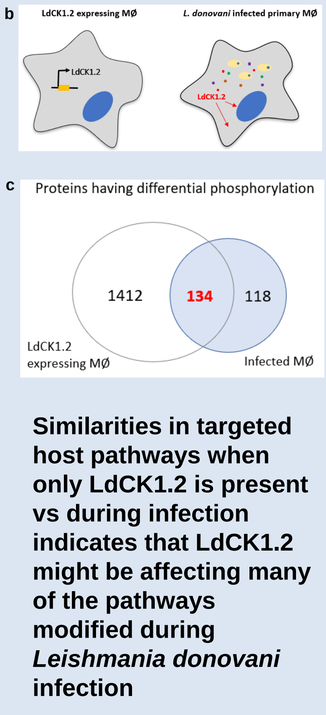
For phosphoproteomic analysis, BMDMs were infected with *L. donovani* amastigotes at MOI 10 and collected at 48h post infection.

B) Phosphoproteomics

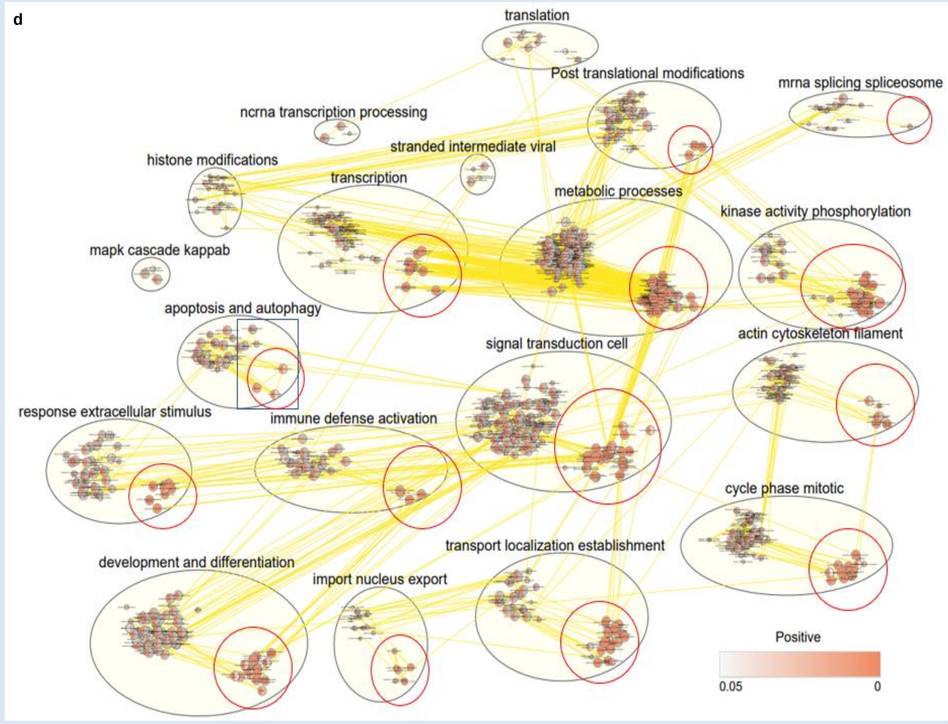
To validate the *in vitro* results in *L. donovani* infected macrophages, we established the phosphoproteome of infected BMDMs versus that of uninfected BMDMs. BMDMs were plated in 6 well plates with 3x10^6 MØ/ well (Day 0) and the workplan (fig a) was followed. 48 hrs post infection (Day 3), the lysates were sent for phosphoproteomic analysis. We identified 12000 phosphosites (663 with p<0.05) and 252 proteins with an altered phosphorylation (>2 fold change, p < 0.05) were identified.

Comparison of host pathways enriched during infection and in macrophages expressing LdCK1.2

We compared the phosphoproteome for MØ expressing LdCK1.2 and that of infected MØ (b) and found 134 proteins in common with modulated phosphorylation (c). We also looked at enriched pathways and found 14 common clusters (circled in red in d) such as apoptosis, cell cycle, signalling, response to stimuli, metabolism, transcription.



Similarities in targeted host pathways when only LdCK1.2 is present vs during infection indicates that LdCK1.2 might be affecting many of the pathways modified during *Leishmania donovani* infection



CONCLUSIONS

LdCK1.2 and *Et*ROP1, although belonging to very different parasites, seem to target common host pathways *in vitro*. Some of the LdCK1.2 targets are also modulated during *Leishmania* infection. We established the first phospho-proteome of *L. donovani* infected versus non infected macrophages to confirm the *in vitro* results. Over 50% of the differentially phosphorylated proteins upon infection were found in the phosphoproteome of macrophages expressing LdCK1.2. We also found that the pathways enriched in LdCK1.2 expressing macrophages contained all the enriched pathways found during infection. This indicates that LdCK1.2 might play a pivotal role in host modification during infection.

PERSPECTIVES

- **We will validate our *in vitro* observations for the two kinases in an infection model and compare them to find similarities or unique signatures of host modulation during infection.**
- **Using tools such as CRISPR-Cas system, overexpression, RNAi, we will assess the essentiality of these modulations during infection**
- **The 134 common proteins found between infected macrophages and LdCK1.2 expressing macrophages will be assessed by MOTIF analysis and for shortlisted proteins *in vitro* kinase assay will be performed to confirm the involvement of LdCK1.2**
- **We have identified a few candidates that seem to be affected by infection or are substrates of both the kinases, but this is being currently investigated.**