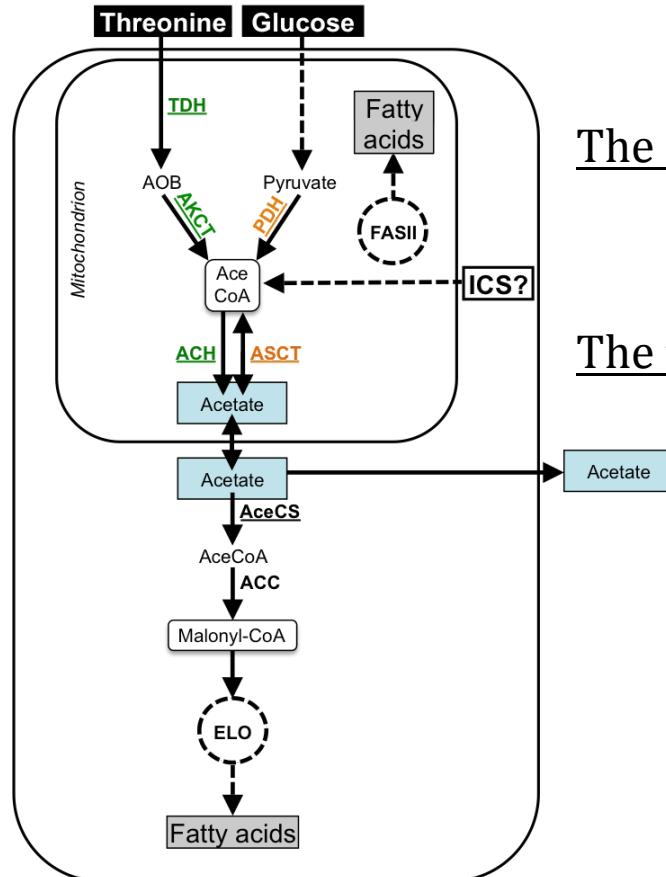


# The key enzymes of acetyl-CoA metabolism targetable for inhibitor development against *Trypanosoma brucei*



The question: How is acetate production from threonine and glucose separated and what is the internal carbon source?

The technologies: combining reverse genetics and NMR spectroscopy  
 BioID and MS to show protein interactions

**Schematic depiction of the acetate metabolism in *T. brucei*.**  
 Enzymes involved in the hypothesized metabolic channeling are highlighted in orange or green. It is hypothesized that PDH and ASCT interact and AKCT with ACH and possibly with TDH.

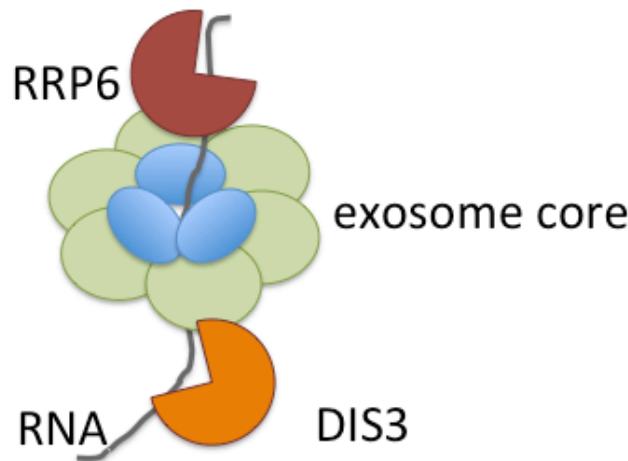
PI: Prof. Arthur Scherf (Institut Pasteur, Paris)

Co-PI: Dr. Stanislas Tomavo (Centre d'Infection et Immunité de Lille, CNRS)

# The RNA exosome complex in *Plasmodium falciparum*

The question: What are the roles of DIS3 and RRP6 exonucleases in gene regulation?

The technologies: genome modification using CRISPR/Cas9,  
IFA, RNAseq, CHIP



## **The RNA exosome complex**

The RNA exosome is a multi-subunit protein complex, responsible for 3' to 5' RNA decay and processing. The main architecture is conserved in eukaryotes. The two catalytic subunits, Dis3 and Rrp6 can be found in different subcellular compartments, and can also have exosome-core independent functions.

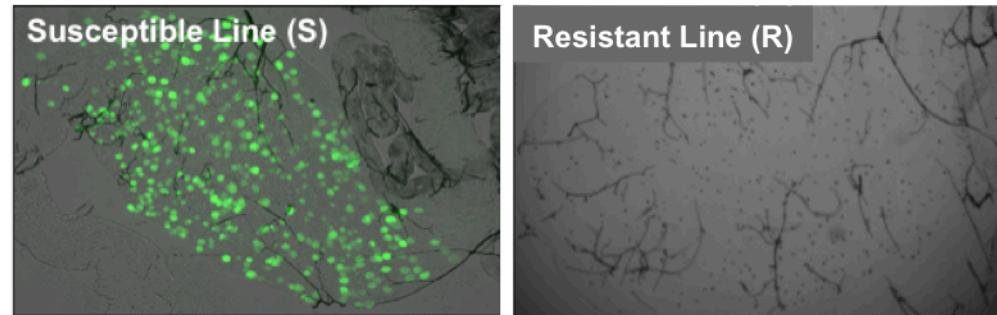
# Identification of malaria parasite virulence factors that counteract mosquito natural resistance

The question: Which genetic factors enable *Plasmodium* parasites to counteract natural resistance of *Anopheles gambiae* mosquitoes?

The technologies: Forward genetic approaches

## *A. gambiae* midguts infected with *Plasmodium* parasites

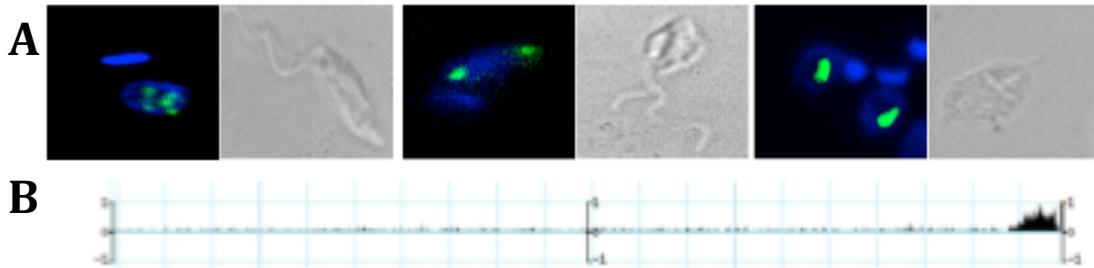
The midgut of a susceptible line is highly infected with viable, GFP-expressing oocysts, while the midgut of a resistant line is cleared from viable parasites.



# Chromosomal segregation and aneuploidy in the protozoan parasite *Leishmania*: the quest for centromeres

The question: Are centromeres and kinetochore in *Leishmania* unconventional elements distinctive of these parasites?

The Technologies: ChIP-Seq , microscopy and proteomics



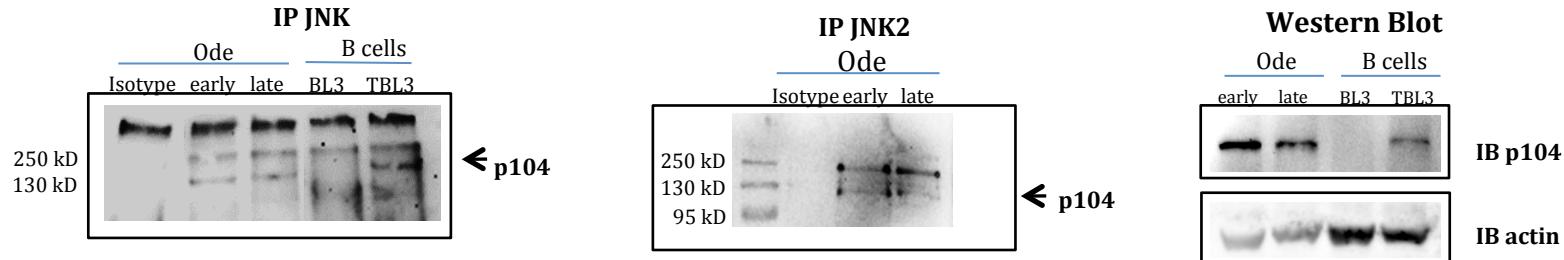
**Example of *L. major* putative Kinetochore Protein chromosome binding profile obtained by ChIP-Seq.** A. Localization of *L. major* kinetochore protein (green) by immunofluorescence during cell cycle. B. ChIP peak obtained for one chromosome.

PI: Dr. Gordon LANGSLEY (INSERM U1016/Institut Cochin/Paris-Descartes University, Paris)  
 Co-PI: Dr. Mohamed Ali HAKIMI (CNRS/ Joseph Fourier University, Grenoble)

# *Theileria*-encoded JNK-binding proteins and leukocyte transformation

The question: How *Theileria* induces constitutive phosphorylation of JNK ?

The technologies: Penetrating competitive peptides, Immunoprecipitation,  
CRISPR Cas9 System.



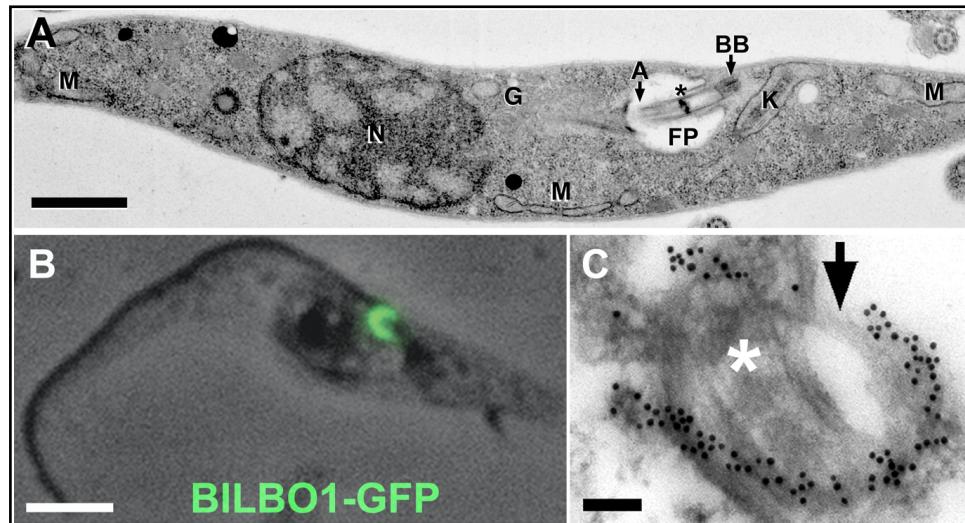
**p104 the most promising parasite protein encoding 2 putative JNK-binding motifs interacts with JNK:** P104 is a surface protein of *Theileria annulata* macroschizont possesing predicted JNK binding motifs.

**IP:** immunoprecipitation, **Ode:** macrophage cell line infected by *Theileria*, **Early:** virulent macrophage cell line, **Late:** attenuated macrophage cell line, **BL3:** bovine B-lymphosarcoma cell line, **TBL3:** BL3 infected by *Theileria*

# Identification and analysis of *Trypanosome* and *Leishmania* kinetoplastid flagellar pocket proteins

The question: Does BILBO1, the only identified flagellar pocket protein (FPC), influence cell survival/biogenesis alone or *via* a complex of FPC proteins?

The technologies: RNAi-knockdown, Gene tagging, Proximity-dependent biotin identification (BioID), Proteomics, Electron and fluorescent microscopy.



**BILBO1 localization in *T. brucei* procyclic cells (A)** A micrograph showing the morphology of a trypanosome using thin section electron microscopy. Scale bar is 1µm. (M - Mitochondrion, N - Nucleus, G - Golgi apparatus, FP - Flagellar pocket, BB - Basal body, A - Axoneme, \* - Transition zone).

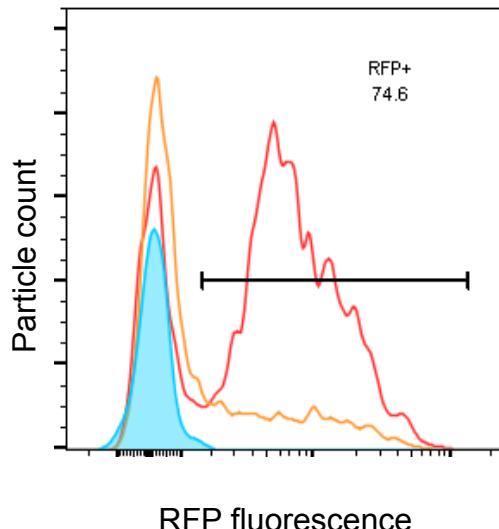
**(B)** Immunofluorescence micrograph of a cytoskeleton from a cell expressing BILBO1-GFP tagged protein. Scale bar is 2.5µm.

**(C)** BILBO1 localization at the FPC using immunoelectron microscopy. Scale bar 100nm.

# Biogenesis of the intravacuolar network of *Toxoplasma gondii*

The question: What is the contribution of host and parasite to the lipids and proteins of the IVN?

The technologies: Proteomics, lipidomics, BioID, cell fractionning and organelle sorting



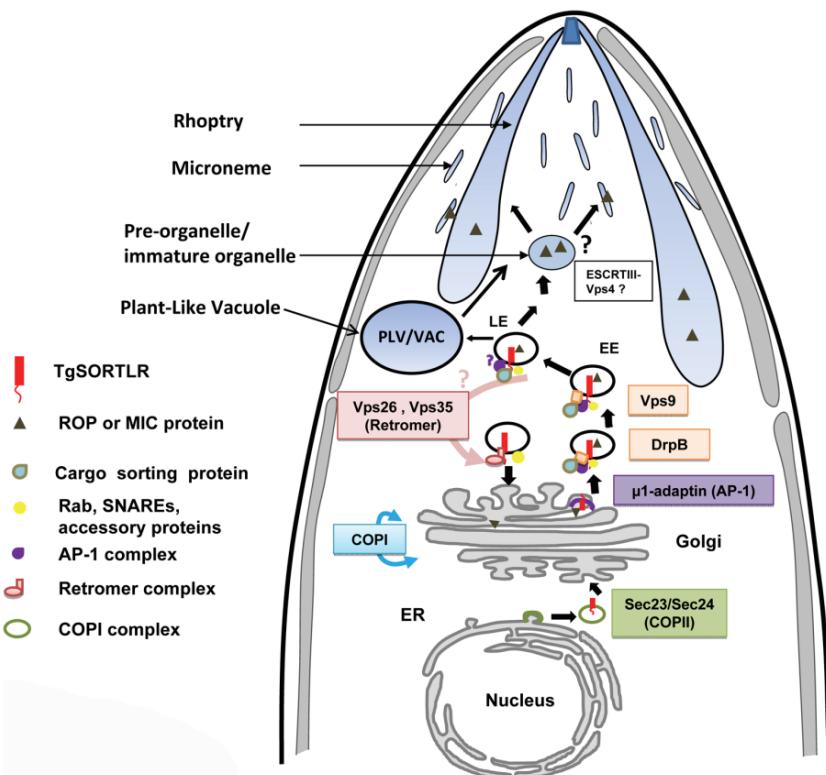
**Purification of *T. gondii* dense granules.** Cytometry analysis of organelles in a French press lysate of parasites expressing a protein marker of the dense granules fused to RFP, before (yellow) and after (red) fluorescence-activated particle sorting. Background from buffers was also recorded (blue).

The purified dense granules will be submitted to proteomics and lipidomics. Protein data will be compared to BioID results from IVN; lipid candidates will be sought at the IVN using specific probes.

PI: Dr Stanislas TOMAVO

Co-PI: DR Gordon Langsley (Institut Cochin, Paris), Dr Ludger Johannes (Institut Curie, Paris)

## Exploring functions of proteins that cooperate with TgSORTLR in intracellular trafficking of *Toxoplasma gondii*



### The question:

How *Toxoplasma* manages proper protein trafficking to apical secretory organelles?

### The technologies:

Reverse genetics, Proteomics, and Microscopy

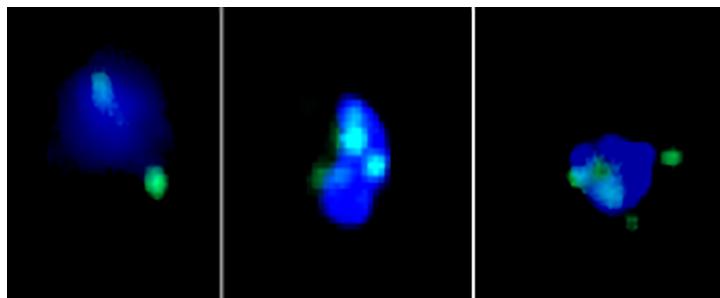
### A model for TgSORTLR functions in protein sorting and the biogenesis of apical secretory organelles.

The proteins of apical secretory organelles are transported through the endoplasmic reticulum (ER) and Golgi, also require the parasite endosome-like system to access their respective organelles.

# Transcription factors and novel histone marks in chromatin structure in *Toxoplasma* and *Plasmodium*

The question: Do Pioneer transcription factors play a role in regulating chromatin structure to induce gene expression for parasite differentiation?

The technologies: Our study involves use of CRISPR technology, combined with microscopy, CHIP and biochemical experiments



**Localization of histone mark H4K31me in *Plasmodium*.** Blue staining represents the *Plasmodium* nucleus and the modification of histone H3 at lysine 31 position by methyl group is represented in green. The histone modification is observed at the nuclear periphery and localizes to heterochromatin region of *Plasmodium* nucleus.