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# Functional characterization of the Rab11A-dependent secretory pathway in *Toxoplasma gondii.*

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# Résumé

*Toxoplasma gondii* possède une armada d'effecteurs parasitaires qui permettent l'invasion et la survie des parasites dans la cellule hôte. Ces facteurs sont contenus dans des organites sécrétoires spécifiques, les rhoptries (ROP), les micronèmes (MIC) et les granules denses (DG) qui libèrent leur contenu lors de l'adhésion et l'invasion active de l'hôte. Les protéines des DG (GRA) sont également sécrétées de manière dite « constitutive » lors de la réplication du parasite et jouent un rôle crucial dans la modulation de la réponse de l'hôte, assurant la survie et la dissémination du parasite. Alors que les mécanismes moléculaires régulant la libération des protéines ROP et MIC lors de l'invasion parasitaire ont été bien étudiés, la sécrétion constitutive des DG reste un aspect totalement inexploré du trafic vésiculaire de *T. gondii*. Au cours de cette thèse, nous avons d'abord étudié le rôle de la petite GTPase Rab11A, un régulateur de l'exocytose connu dans les cellules eucaryotes. Nous avons démontré que lors de la réplication parasitaire, *Tg*Rab11A régule le mouvement des DG dépendent de l'actine et stimule l'étape finale de leur l'exocytose au niveau de la membrane plasmique du parasite et donc la libération des protéines GRA dans l'espace vacuolaire et dans le cytosol de la cellule hôte. En outre, nous avons démontré une nouvelle fonction pour *Tg*Rab11A dans les premières étapes d'adhésion du parasite aux cellules hôtes et dans la motilité du parasite, et donc dans l'invasion des cellules hôtes. En accord avec ces résultats, la sécrétion de l'adhésine MIC2 est altérée chez les parasites extracellulaires inhibés dans l'activité de *Tg*Rab11A. De manière surprenante, les parasites extracellulaires mobiles envahissants la cellule hôte présentaient une accumulation apicale polarisée et focalisée des vésicules Rab11A-positives, suggérant un rôle pour *Tg*Rab11A dans les premiers évènements sécrétoires précoces déclenchés lors de l'invasion parasitaire. Collectivement, nos données ont révélé *Tg*Rab11A comme un régulateur crucial de la voie de sécrétion constitutive chez *T. gondii*. Dans une deuxième partie de cette thèse, nous avons caractérisé un nouveau partenaire de *Tg*Rab11A, contenant un domaine HOOK unique, que nous avons appelé *Tg*HOOK. Nous avons constaté que cette protéine forme un complexe stable avec un homologue de la protéine Fused Toes (FTS) et une protéine interagissant avec HOOK (appelée HIP) nouvellement identifiée spécifique aux parasites coccidiens. HOOK et FTS sont deux régulateurs conservés du trafic endosomal connus pour favoriser le transport et/ou la fusion des vésicules chez d'autres eucaryotes. In *T. gondii*, nous avons constaté que le complexe *Tg*HOOK-*Tg*FTS-HIP s'accumule à l'extrémité apicale du parasite et favorise la sécrétion des protéines MIC, contribuant ainsi à l'invasion du parasite.

# Abstract

*Toxoplasma gondii* possesses an armada of secreted virulent factors that enable parasite invasion and survival into the host cell. These factors are contained in specific secretory organelles, the rhoptries (ROP), micronemes (MIC) and dense granules (DG) that release their content upon host adhesion and active invasion. DG proteins (GRA) are also secreted in a so called « constitutive manner » during parasite replication and play a crucial role in modulating host responses, ensuring parasite survival and dissemination. While the molecular mechanisms regulating ROP and MIC protein release during parasite invasion have been well studied, constitutive secretion of DG remains a fully unexplored aspect of *T. gondii* vesicular trafficking. During this thesis, we first investigated the role of the small GTPase Rab11A, a known regulator of exocytosis in eukaryotic cells. We demonstrated that during parasite replication, *Tg*Rab11A regulates actin-dependent DG motion and stimulates the final step of their exocytosis at the parasite plasma membrane and therefore GRA protein release in the vacuolar space and host cytosol. Moreover, we demonstrated a novel function for *Tg*Rab11A in the early steps of parasite adhesion to host cells and parasite motility, and thus host cell invasion. In agreement with these findings, the secretion of the MIC2 adhesin was severely perturbed in extracellular *Tg*Rab11A-defective parasites. Strikingly, extracellular adhering and invading parasites exhibited an apically polarized and focalized accumulation of *Tg*Rab11A-positive vesicles, suggesting a role for *Tg*Rab11A in early secretory events triggered during parasite invasion. Collectively, our data revealed *Tg*Rab11A as a crucial regulator of the constitutive secretory pathway in *T. gondii*. In a second part of this thesis, we functionally characterized a novel *Tg*Rab11A-binding partner, containing a unique HOOK-domain, that we called *Tg*HOOK. We found that this protein forms a stable complex with a homologue of the Fused Toes (FTS) protein and a newly identified HOOK Interacting Protein (HIP) specific to coccidian parasites. HOOK and FTS are two conserved endosomal trafficking regulators known to promote vesicle trafficking and/or fusion in other eukaryotes. In *T. gondii,* we found that the *Tg*HOOK-*Tg*FTS-HIP complex accumulates at the apical tip of parasites and promotes microneme secretion, thereby contributing to parasite invasion.

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# Scientific Output

## Article:

- **Rab11A regulates dense granule transport and secretion during *Toxoplasma gondii* invasion of host cells and parasite replication.**

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# List of Abbreviations

**ABA** Abscissic Acid

**ACT** Actin

**ADF** Actin Depolymerization Factor

**AIP** Armadillo Interacting Protein

**AKMT** Apical Complex Lysine Methyltransferase

**ALD** Aldolase enzyme

**AMA1** Apical Membrane Antigen 1

**AP** clathrin Adaptor Protein complex

**APH** Acetylated-pleckstrin-homology domain-containing protein

**APR** Apical Polar Ring

**ARF** ADP Ribosylation Factor

**ARO** Armadillo Repeats Only Protein

**ATc** Anhydro Tetracycline

**BFD1** Myb-like transcription factor

**CAM** Calmodulin-like Proteins

**CAMLG** Calcium Modulating LiGand

**CAT** Chloramphenicol AcetylTransferase

**Cb** Chromobodies

**CBL** Chitin-binding like domain

**CCV** Clathrin-Coated Vesicles

**CD** cytochalasin D

**CDA-1** Cell Division Autoantigen 1

**CDPK** Calcium-Dependent Protein Kinase

**CDPK** Calcium-dependent Protein Kinases

**CIE** Clathrin-independent Endocytosis

**CME** Clathrin-mediated Endocytosis

**COG** Oligomeric Golgi Complex

**COP** Coat Protein Complex

**CORVET** Class C core Vacuole / Endosome Tethering

**CPL** Cathepsin-like Protein

**CRISPR/Cas9** Clustered Regularly Interspersed Short Palindromic Repeats/ CRISPR associated protein 9

**CrK** Cyclin related Kinase

**DAG** Diacylglycerol

**DAPI** 4', 6-Diamidino-2-Phenylindole

**DC** Dendritic cells

**DD** Destabilization Domain

**DG** Dense Granule

**DGK1** Diacylglycerol Kinase-1

**DHFR** Dihydrofolate Reductase-thymidylate synthase gene

**DLC** Dynein Light Chain

**DOC** Deoxycholate

**DrpA** Dynamin-related Protein A

**EE** Early Endosomes

**EGF** Epidermal Growth Factor

**ELC** Enodosomal like Compartment

**ELISA** Enzyme-Linked ImmunoSorbent Assay

**ER** Endoplasmic Reticulum

**ERC** Endosomal Recycling Compartment

**ERGIC** ER-Golgi intermediate compartment

**ESCRT** Endosomal Sorting Complexes Required for Transport

**FASII** Fatty Acid Synthesis type II

**FBS** Fetal Bovine Serum

**FHIP** FTS and HOOK-Interacting Protein

**FTS** Fused Toes

**GAC** Glideosome Associated Connector

**GAP** Glideosome-associated Proteins

**GAP** GTPase Activating Proteins

**GDI** GDP Dissociation Inhibitors

**GDP** Guanosine Diphosphate

**GEF** Guanine Nucleotide Exchange Factors

**GFP** Green Fluorescent Protein

**GPI** Glycosylphosphatidylinositol

**GRA** Dense GRAnules Proteins

**GST** Gluthatione S-Transferase

**GT1** Glucose Transporter 1

**GTP** Guanosine Triphosphate

**H.O.S.T** Host Organelle Sequestring Tubulostructures

**HAUST** Herpes Virus-Associated Ubiquitin Specific Protease

**HCE1** Host Cyclin E1

**HFF** Human Foreskin Fibroblasts

**HOPS** Homotypic Vacuolar Protein Sorting

**HSC** Heat Shock Cognate Protein

**HXGPRT** Hypohanthine Xanthine Guanine PhosphoRibosylTransferase

**ICAM-1** Intracellular Adhesion Molecule 1

**IFA** Immunofluorescence Assay

**IFN-γ** Interferon γ

**Ig** Immunoglobulin

**IL** InterLeukin

**IMC** Inner Membrane Complex

**IMP** Intermembranous particles

**IP** Immunoprecipitation

**IP3** Inositol Triphosphate

**IRG** Immune-related GTPases

**ISAGA** Immunosorbent Agglutination Assay

**ISP** IMC Subcompartment Proteins

**IST** Inhibitor of STAT1 transcriptional activity

**IVN** Intravacuolar Network

**KD** Knock-down

**KI** Knock-in

**KO** Knock-out

**LE** Late Endosomes

**LT** Lymphocytes T

**M2AP** MIC2-associated protein

**M6P** Mannose-6-Posphate

**MAF1** Mitochondrial Associating Factor 1

**MAP** Microtubule-associated Proteins

**MAR** Microneme Adhesive Repeats domain

**MIC** Microneme proteins

**MJ** Moving Junction

**MLC** Myosin Light Chain

**MT** Microtubules

**MTOC** Microtubule Organizing Center

**Myo** Myosin

**MYR1** MYc Regulation 1

**NFAT4** Nuclear Factor of Activated T cell 4

**NK** Natural Killer cells

**NO** nitric oxide

**NSF** N-ethylmaleimide Sensitive Factor

**PA** Phosphatidic Acid

**PAGE** Polyacrylamide Gel Electrophoresis

**PAN** Plasminogen Apple Nematode

**PBS** Phosphate Buffered Saline

**PCR** Polymerase Chain Reaction

**PFA or PAF** Paraformaldehyde

**PI-(4,5)-P2** Phosphatidylinositol-4,5-biphosphate

**PI-PLC** Phosphoinositide Phospholipase C

**PLP1** Perforin-like Protein 1

**PLV** Plant Vacuole-like

**PM** Plasma Membrane

**PP2C** Protein Phosphatase 2C

**PRF** Profilin

**PV** Parasitophorous Vacuole

**PVM** Parasitophorous Vacuole Membrane

**PYR** Pyrimethamine

**RAB11FIP** Rab11 Family-Interacting Proteins

**RASP** Rhotry Apical Surface Protein

**RE** Recycling Endosomes

**RILP** Rab-interacting Lysosomal Protein

**RNG1** Ring-1

**ROM** Rhomboid-like Protease

**RON** Rhoptry Neck Proteins

**ROP** Rhoptry Bulb Proteins

**SAG** Surface Antigen Glycoprotein

**SAS6L** SAS6 (centriole associated)-like protein

**SDZ** Sulfadiazine

**SEM** Scanning Electron Microscopy

**SFA** Striated Fiber Assemblins

**SNARE** Soluble NSF Attachment protein Receptor

**SORTLR** Sortilin-Like Receptor

**SPN** Subpellicular Network

**SRP** Signal Recognition Particle

**SRS** SAG1 related sequence

**STAT** Signal Transducer and Activator of Transcription

**SUB** Subtilisin-like

**TEM** Transmission Electron Microscopy

**TGN** Trans-Golgi Network

**TLR** Toll like Receptor

**TNF-α** Tumor Necrosis Factor α

**TRAF** TNF Receptor-Associated Factors

**TRAPP** Transport Protein Particle

**TSR** Thrombospondin 1 domain

**VAC** Vacuolar Compartment

**WB** Western Blot

# Introduction

# Chapter I - Introduction

## 1 The Apicomplexa

The phylum Apicomplexa forms a large group of unicellular protists. As obligate intracellular parasites, they infect a wide variety of hosts ranging from invertebrates to mammals. The invasive stages of Apicomplexa are characterized by the presence of a unique apical complex involved in host cell invasion and parasite survival, which consists of apically anchored secretory organelles, the micronemes and the rhoptries, the apical polar ring (APR) and the conoid (Figure 1) (Morrissette and Sibley, 2002a; Portman and Slapeta, 2014).

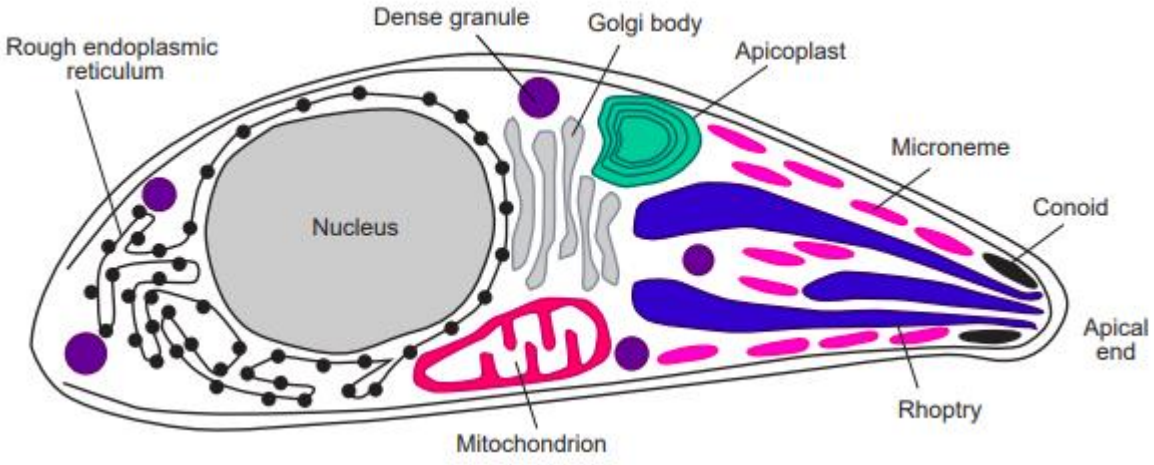



**Figure 1: The morphology of apicomplexan parasites.** Adapted from (Ajioka et al., 2001). Apicomplexa are highly polarized cells containing subcellular structures located at the apical pole of the parasite forming the apical complex. The apical complex is composed of the conoid, the apical polar ring (APR) and specific secretory organelles: the micronemes and rhoptries.

The most popular apicomplexan parasite is *Plasmodium falciparum*, the causative agent of human malaria, a devastating tropical disease caused by the bite of infected female anopheles mosquitoes. Nevertheless, other apicomplexan parasites are just as relevant due to their opportunistic nature and socio-economic impacts, such as *Theleria*, *cryptosporidium* and *Toxoplasma gondii*.

*Toxoplasma gondii* is the causative agent of one of the most common parasitic infection in the world, called toxoplasmosis. Although causing only mild symptoms in immuno-competent

adults, toxoplasmosis can cause severe life-threatening complications in immunocompromised individuals, especially in AIDS patients and those undergoing chemotherapy.

Similarly, *Cryptosporidium* causes the disease cryptosporidiosis, which can lead to severe gastrointestinal illness. *Theileria annulata and Theileria parva*, the most economically important species of tick transmitted *Theileria* parasites in cattles, cause tropical theileriosis and East Coast fever, respectively, and are responsible for mortality and important losses in production. *Babesia* and *Eimeria* are the causative agents of babesiosis and coccidiosis, respectively infecting cattles and poultry among other animals. Occasionally, unique species of *Babesia* and *Eimeria* can infect humans (Figure 2).

While most apicomplexan parasites have a limited spectra of hosts or cell types that they can infect, *Toxoplasma gondii* is considered as the world's most successful parasite, likely due to the fact that it can infect any warm-blooded animal and birds as well as all nucleated cells within these hosts (Carruthers, 2002).

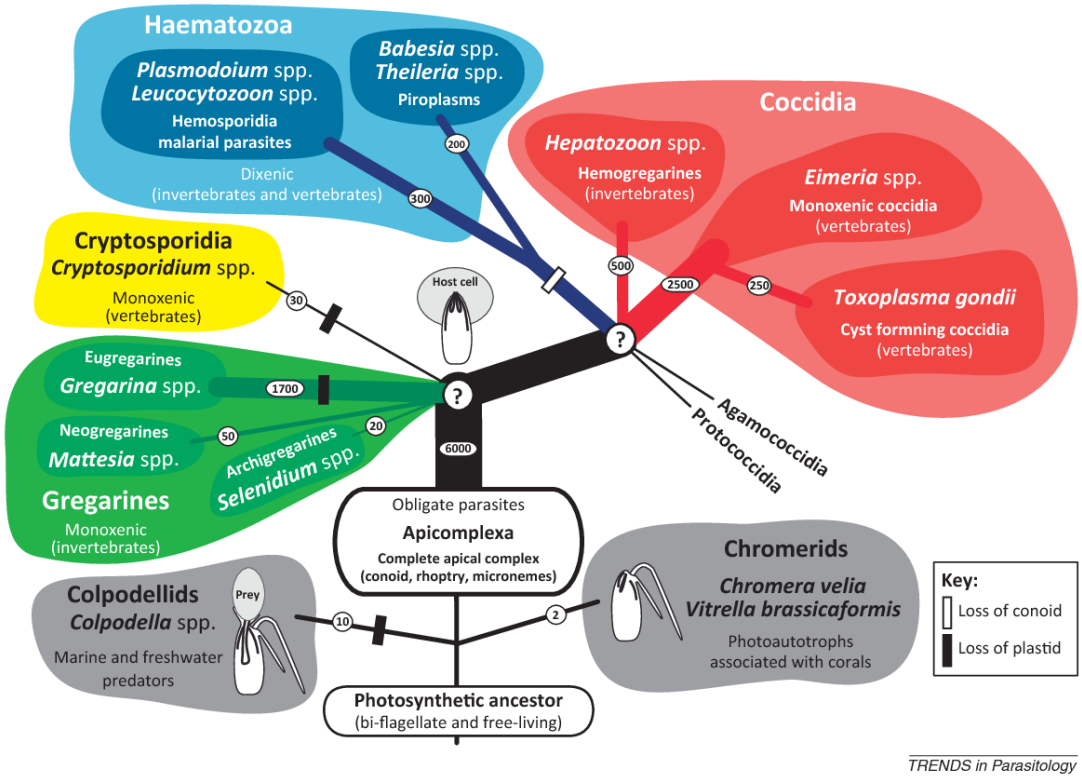



**Figure 2: Hypothetical tree of the phylum Apicomplexa**. Putative schematic representation of the different sub-classes in the phylum Apicomlexa. Adapted from (Portman and Slapeta, 2014).

# 2 *Toxoplasma gondii*

## 2.1 Discovery and history of Toxoplasma *gondii*

*T. gondii* is an obligate intracellular protozoan parasite causing the infectious disease, toxoplasmosis. It was first discovered in 1908 by two independent groups; first, in a hamster-like rodent, *Ctenodactylus gundii* by Charles Nicolle and Louis Manceaux, and later in a rabbit by Splendor in 1908 (Charles Nicolle & Louis Manceaux, 1908; Splendore, 1908). The name *T. gondii* was attributed to the isolated protozoan by Nicolle and Manceaux according to its crescent-shaped morphology; Taxon (the Greek word for arc) and plasma for form. Moreover, "*gondii"* derives from *gundii*, the organism where it was first isolated (Ferguson, 2009).

## 2.2 Taxonomic classification of *Toxoplasma gondii*

*T. gondii* belongs to the family of the Sarcocystidae in the class of coccidia and is the only species in the Toxoplasma genus. Coccidia are obligate, intracellular and cyst forming parasites that infect their host through the gastrointestinal tract.

*T. gondii* is classified according to NCBI (Taxonomy ID: 5811) as follows:

***Table 1: Taxonomic classification of Toxoplasma gondii parasite***

| | |
|---|---|
| **Domain** | Eukaryota |
| **Kingdom** | Alveolata |
| **Phylum** | Apicomplexa |
| **Class** | Conoidasida |
| **Sub-Class** | Coccidia |
| **Order** | Eucoccidiorida |
| **Sub-Order** | Eimeriorina |
| **Family** | Sarcocystidae |
| **Genus** | *Toxoplasma* |
| **Species** | *Toxoplasma gondii* |

## 2.3 Toxoplasma gondii lineages

Possibly reflecting the diversity of its natural hosts, multiple genotypes of *T. gondii* exist worldwide. Yet most isolated parasite strains fall within one of three clonal lineages: type I, II, or III, which are also the most studied in laboratory mice (Khan et al., 2009). These three clonal lineages are characterized by their distinct virulence in mice and their ability to form cysts (Howe and Sibley, 1995). When considering laboratory mice, type I strains kill their host prematurely due to hyper-inflammation and uncontrolled parasite dissemination, and thus fail to establish latent infections. Type I strains are characterized by their high virulence since the inoculation of a single parasite of this genotype is lethal (Boothroyd and Grigg, 2002). By contrast, type II and III strains exhibit a relatively low virulence during the acute phase of the infection and accordingly injection of around 1000 parasites is required to have a lethal effect (Saeij et al., 2005); Type II and III strains also display a slower growth rate compared with type I parasites (Fuentes et al., 2001; Grigg et al., 2001) and have a high cystogenic capacity (Boothroyd and Grigg, 2002; Saeij et al., 2005). Parasitic strains commonly used in laboratories are summarized in Table 2, yet genotyping techniques have identified the presence of isolates, which do not correspond to the three main clonal lines (Dardé, 2008; Khan et al., 2007; Robert-Gangneux and Dardé, 2012).

**Table 2: Different lineages of *Toxoplama gondii* strains used in laboratories.** The lethal dose (LD) corresponds to the minimum number of parasites required to cause death of the mouse. $LD_{100}$ and $LD_{50}$ represent the lethal doses required to kill 100% and 50% of the mice, respectively. These strains have been completely or partially sequenced and the genome database has been made available on http://www.toxodb.org.

| Genotypes | Type I | Type II | Type III |
|---|---|---|---|
| **Laboratory strains** | RH / GT1 | Pru / ME49 | CEP /VEG |
| **Virulence** | $LD_{100}$=1 | $LD_{50}$=$10^3$ | $LD_{50}$=$10^3$ |
| **Cysts formation in vivo** | No cysts | Cysts existence | Cysts existence |

## 2.4 *Toxoplasma gondii* life cycle

As with many Apicomplexa, *T. gondii* has a dual host life cycle, first reported in 1970 (Dubey et al., 1970; Frenkel et al., 1970). The parasite alternates between the sexual reproduction phase which is limited to the intestine of felids, its only definitive hosts; and the asexual

replication phase, which occurs in the intermediate hosts, all warm-blooded mammals including Human (Hunter and Sibley, 2012; Montoya and Liesenfeld, 2004; Robert-Gangneux and Dardé, 2012) (Figure 3). Unlike most other apicomplexan parasites, *T. gondii* does not need to go through its sexual reproduction phase to be transmitted between intermediate hosts (Su et al., 2003).

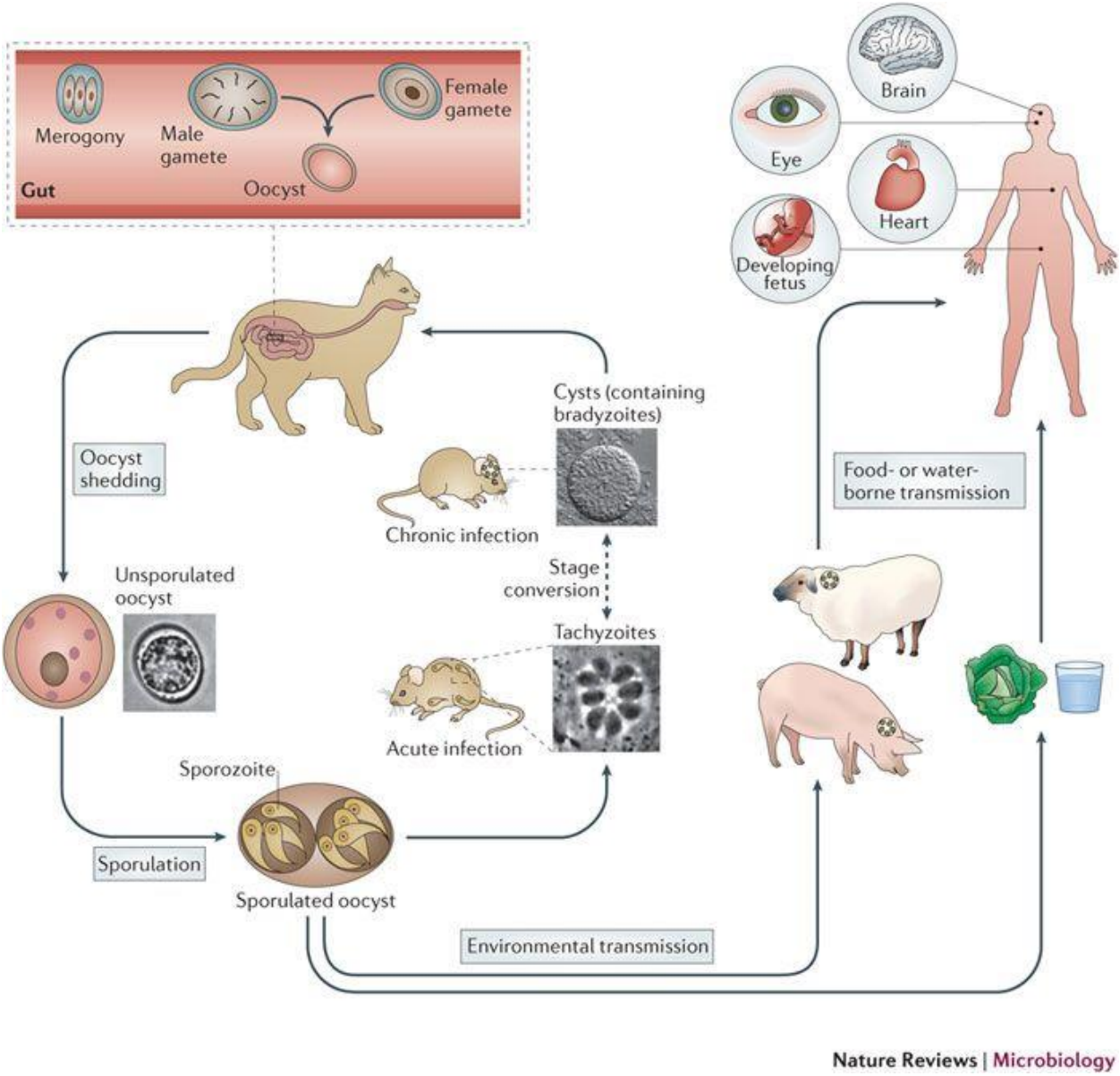



**Figure 3: The complete life cycle of *T. gondii*.** Reprinted from (Hunter and Sibley, 2012). The sexual reproduction of *T. gondii* occurs in the intestinal wall of felines. Following merozoites division within enterocytes by a process called merogony, male and female gametes are formed. These two gametes then merge to form oocysts, which are released into the environment in the animal's feces. Under the effect of environmental factors, the oocysts sporulate into sporozoites, which are then consumed by intermediate hosts (including humans). In intermediate hosts, the asexual phase takes place: the sporozoites are ingested and converted into tachyzoites which are the fast replicating and

disseminative form of the parasite responsible for the acute phase of the infection. Under the pressure of the immune system, tachyzoites can convert to bradyzoites that form quiescent intracellular cysts in certain immune-privileged tissues such as the brain, the eyes, or even cardiac and skeletal muscles throughout the life of the host. Humans can be contaminated by the ingestion of undercooked meat containing cysts. In that case, bradyzoites reach the intestine and convert into tachyzoites. Vertical transmission of tachyzoites from a primo-infected pregnant to her fetus can occur, notably during the last trimester of pregnancy.

**Sexual development in the definitive hosts**

The sexual cycle of *T. gondii* occurs exclusively in enterocytes of the small intestine of felids, the definitive host, notably domesticated cats. Once cysts or oocysts are ingested, the proteolytic enzymes in the cat's stomach and intestine digest the cyst wall, hence releasing the bradyzoites or sporozoites, which then penetrate enterocytes and undergo several cycles of asexual multiplication by endopolygeny, characterized by the development of merozoites within schizonts (Dubey and Frenkel, 1972). Two days after ingestion of tissue cysts by the cat, merozoites are released from schizonts and initiate the gametogony, resulting in micro- and macrogametes formation (Ferguson, 2002). Microgametes, using their two flagella, swim and fertilize mature macrogametes (Speer and Dubey, 2005). The zygote develops into an oocyst, which is liberated after disruption of the infected epithelial cell and excreted as an unsporulated state in cat feces. Within 1 to 5 days, depending on the surrounding aeration and temperature, the excreted oocysts sporulate generating two sporocysts, each containing four sporozoites (Dubey and Frenkel, 1972; Ferguson et al., 1979). Oocysts are infectious only after sporulation (Figure 4). Sporulated oocysts can keep their infectivity for more than a year since they are extremely resistant to chemical and physical stress conditions (Frenkel et al., 1975; Hunter and Sibley, 2012; Montoya and Liesenfeld, 2004; Robert-Gangneux and Dardé, 2012).

**Asexual replication in the intermediate hosts**

Ingestion of sporulated oocysts or bradyzoites by the intermediate hosts occurs via contaminated food and water and leads to the asexual phase of the parasite lifecycle. The asexual replication is characterized by two parasite forms, named tachyzoite and bradyzoite. During the acute phase of the infection, the sporozoites rupture from the oocyst, invade the epithelial cells of the intestinal tract and convert into tachyzoites, the active replicating form of the parasite, which expand dramatically in number and disseminate into the infected host (Dubey, 1997). If the parasite is challenged by stress conditions such as the host immune

response, the fast-growing tachyzoites convert into slow-growing bradyzoites, which remain quiescent within intracellular cysts that reside in certain immune-privileged tissues, such as neurons and skeletal muscles, for the entire life span of the intermediate host, defining the chronic phase of toxoplasmosis (Dubey, 1997) (Figure 4). Upon a lowered immune response, as in immune-compromised individuals, the reactivation of cysts may occur, leaving the bradyzoites to differentiate back to tachyzoites, causing severe tissue damage and pathogenesis (Frenkel and Escajadillo, 1987; Montoya and Liesenfeld, 2004). In the horizontal transmission route, bradyzoite cysts contained in the intermediate hosts (for example, mice) can be consumed by either a definitive or an intermediate host by carnivorism. When bradyzoites are ingested by a definitive host, the asexual phase resumes as the parasite differentiates in accordance to its new host and the sexual life cycle is initiated.

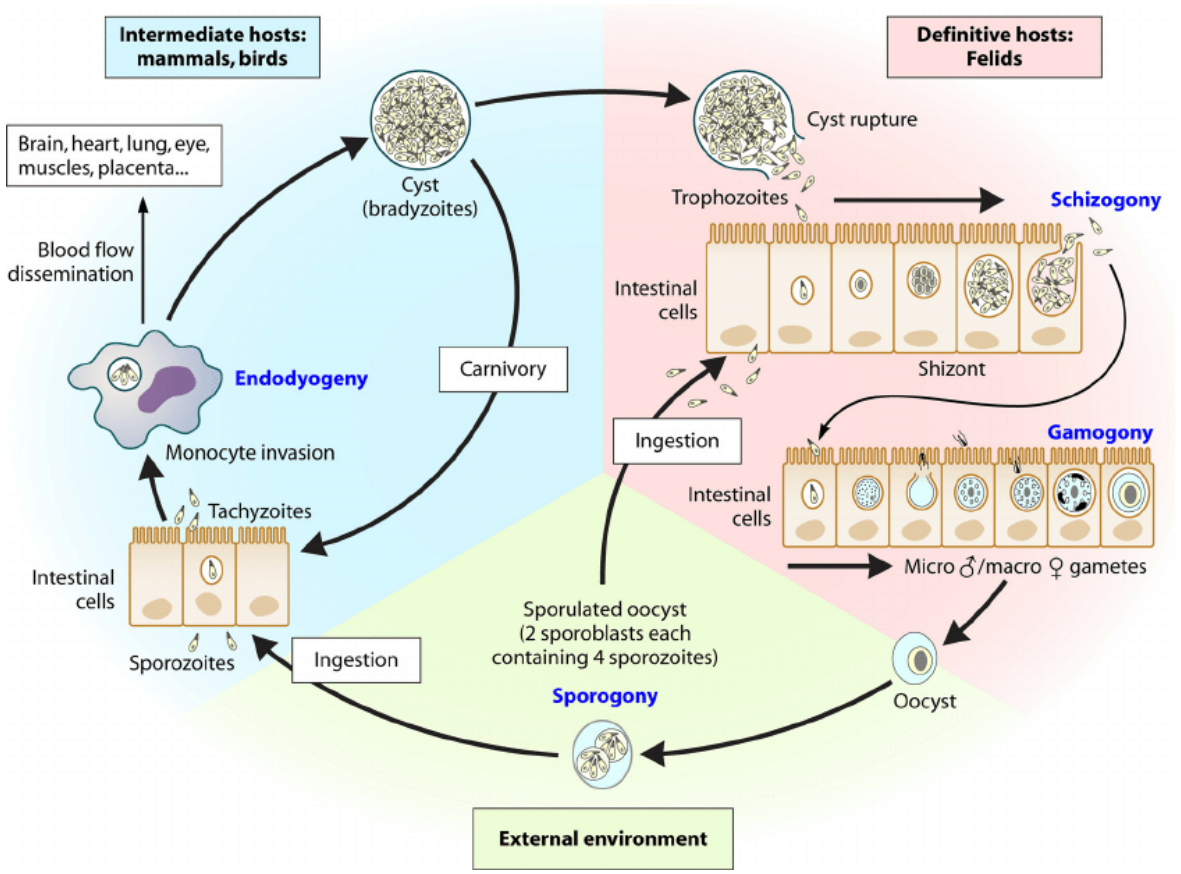



**Figure 4: Schematic representation of the three main stages of development of *Toxoplasma gondii.***

Adapted from (Robert-Gangneux and Dardé, 2012)**. Sexual phase in the definitive host.** After ingestion of tissue cysts, the cyst wall is destroyed by the gastric enzymes releasing the bradyzoites which infect the enterocytes. After a few stages of asexual multiplication, schizonts, in which the merozoites develop during schizogony, are formed. This first step is followed by a sexual multiplication or gamogony where the merozoites differentiate

into male and female gametes. After their fusion, oocysts are formed in the enterocytes and released into the outside environment in the cat's feces. **Environmental phase.** Released oocysts sporulate under the effect of environmental factors (temperature, pressure, pH…). This phase, called sporogony leads to infesting mature oocysts composed of two sporocysts each containing four sporozoites. **Asexual phase in the intermediate host.** In homeotherms, the ingestion of sporulated oocysts leads to the release of sporozoites which infect the enterocytes and differentiate into tachyzoites. The tachyzoite then replicate by endodyogeny and spread throughout the body before converting into bradyzoites to form latent tissue cysts in the intermediate or final hosts.

## 2.5 Tachyzoite to bradyzoite differentiation

Tachyzoite-bradyzoite interconversion is an important step in *T. gondii* cycle in the intermediate host. While the proliferative tachyzoite form is responsible for the acute infection, the bradyzoite form characterizes the chronic form of toxoplasmosis (Dubey et al., 1998). *T. gondii* tachyzoites and bradyzoites are similar in shape (crescent shaped) and ultrastructure, however, they also differ in size, in certain organelles and inclusion bodies (Figure 5). Bradyzoites are thinner than tachyzoites and more susceptible to proteolytic enzymes destruction. Tachyzoites have a nucleus situated towards the central area of the cell, while nucleus in bradyzoites is located towards the posterior end. The contents of rhoptries in tachyzoites are labyrinthine, whereas those of bradyzoites vary with the age of the tissue cyst: labyrinthine rhoptries are only seen in younger tissue cysts, however in older tissue cysts, rhoptries appear as electron dense. Finally, in tachyzoites, amylopectin is either found in discrete particles or absent, in contrast to bradyzoites that contain several amylopectin granules (Dubey et al., 1998)(Figure 5).

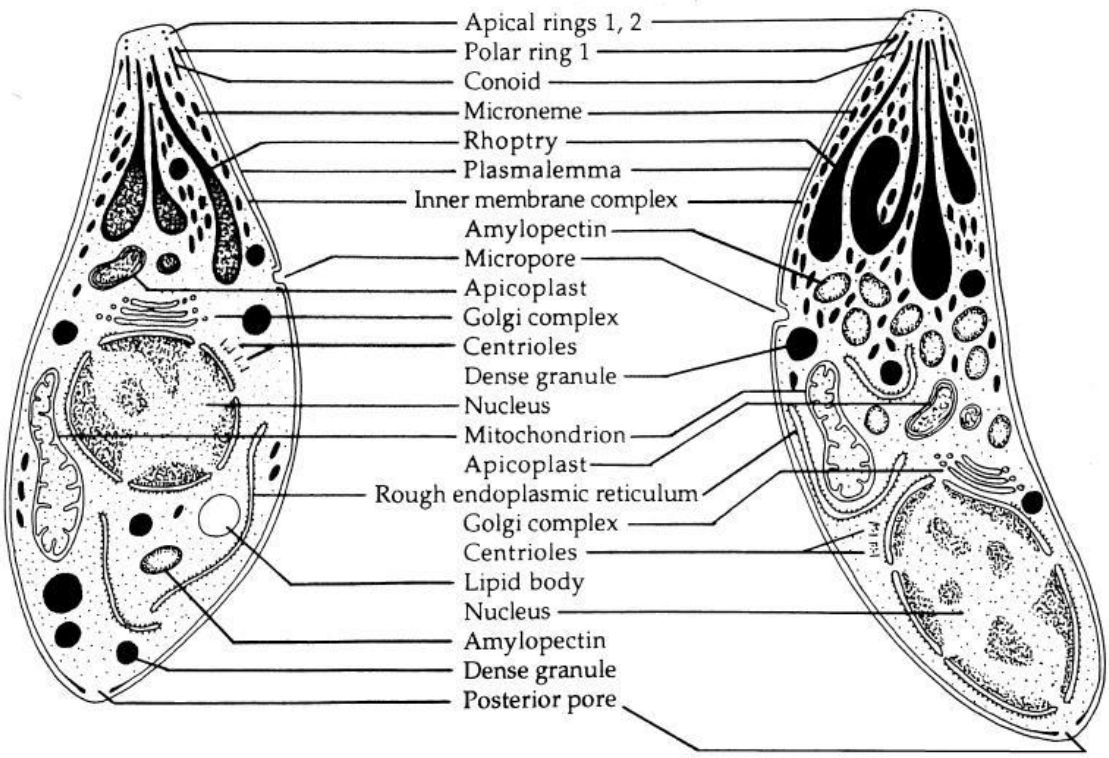



**Figure 5: Schematic drawings of a tachyzoite (left) and a bradyzoite (right) of *T. gondii.*** Adapted from (Dubey et al., 1998).

Bradyzoites (7µm long by 1.5µm wide) are the slow replicating stage of the parasite. They are found encysted in the host's tissue. In this form, the parasite can escape its detection and destruction by the immune system and lasts the entire life of the host by slowly multiplying by endodyogeny within the intracellular cyst. Tissue cysts, containing several thousands of parasites, are more prevalent in neural and muscular tissues and less prevalent in visceral organs. They are mostly found in the brain, the eyes, in skeletal and cardiac muscles (Dubey et al., 1998). The cyst is a spherical structure of 5 to 100µm in diameter consisting of a wall, derived from the PVM, composed of a compact outer layer and another more flexible layer extending into the matrix of the cyst (Tu et al., 2018). The cystic wall is only permeable to molecules of low molecular weight (10KDa maximum) suggesting a restriction of exchanges with the host cell (Lemgruber et al., 2011).

The conversion of tachyzoites (from type II low virulence strains) to bradyzoites accompanied by the transformation of the PVM into a cyst wall is relatively a fast process, starting 6 to 9 days post infection in mice (Lüder and Rahman, 2017).

This reversible process is induced by different stimuli (reviewed in (Cerutti et al., 2020)).

In vitro, variations of pH and temperature can induce the conversion of tachyzoites into bradyzoites within different cell types (Lüder and Rahman, 2017)
(Figure 6). However, in animals, this conversion is mainly triggered by the immune response and factors intrinsic to the infected host cell. Indeed, the secretion of cytokines such as IFN-γ (Interferon γ) and TNF-α (Tumor Necrosis Factor α) or oxygen species like nitric oxide (NO) promote the differentiation of tachyzoites into bradyzoites (Lüder and Rahman, 2017)
(Figure 6). Similarly, auxotrophic metabolite deprivation for *T. gondii* such as arginine or cholesterol may be sufficient to induce conversion (Lüder and Rahman, 2017; Lyons et al., 2002). In addition, two human proteins have been shown to promote differentiation: the CDA-1 protein (Cell Division Autoantigen 1) by inhibiting the growth of the host cell, and the CD73 protein by increasing the concentration of cellular adenosine (Lüder and Rahman, 2017). Bradyzoites can revert to tachyzoites following a failure of the immune system. Indeed, in immunocompromised individuals, bradyzoites can revert into tachyzoites following the reduction in LT numbers and pro-inflammatory cytokines such as IL-2 (InterLeukin-2), TNF-α, and IFN-γ (Lyons et al., 2002). The conversion of the parasite from one stage to another is accompanied by morphological, molecular and epigenetic changes following the establishment of a specific genetic program at each stage (Lyons et al., 2002; Skariah et al., 2010; Tu et al., 2018). Indeed, it has been shown that transcription factors of the ApiAP2 family are involved in the regulation of the conversion. The proteins *Tg*AP2IV-3, *Tg*AP2Ib-1, and *Tg*AP2XI-4 promote the conversion of tachyzoites into bradyzoites while the proteins *Tg*AP2IX-9, *Tg*AP2IV-4, and *Tg*AP2IX-4 suppress this process (Hong et al., 2017). Recently, a Myb-like transcription factor (BFD1) necessary for differentiation in cell culture and in mice has been identified through a CRISPR/Cas9-mediated genetic screening. Considering its function as a transcription factor, BFD1 binds the promoter of many stage-specific genes and represents a counterpoint to the ApiAP2 factors, regulating thus bradyzoite formation in *Toxoplasma* (Waldman et al., 2020). Indeed, BFD1 inactivation completely ablates bradyzoite formation, while conditional expression of BFD1 is sufficient to induce differentiation even in the absence of environmental stressors (Waldman et al., 2020). Current knowledge therefore suggests that inducing the differentiation of tachyzoites into bradyzoites is multifactorial and involves the contribution of the cell cycle and host metabolism as well as immune responses within the tissue environment. Of note, the invasion of primary neurons in culture leads to

the spontaneous conversion of tachyzoites into bradyzoites and the formation of intracellular cysts by yet unknown mechanisms.

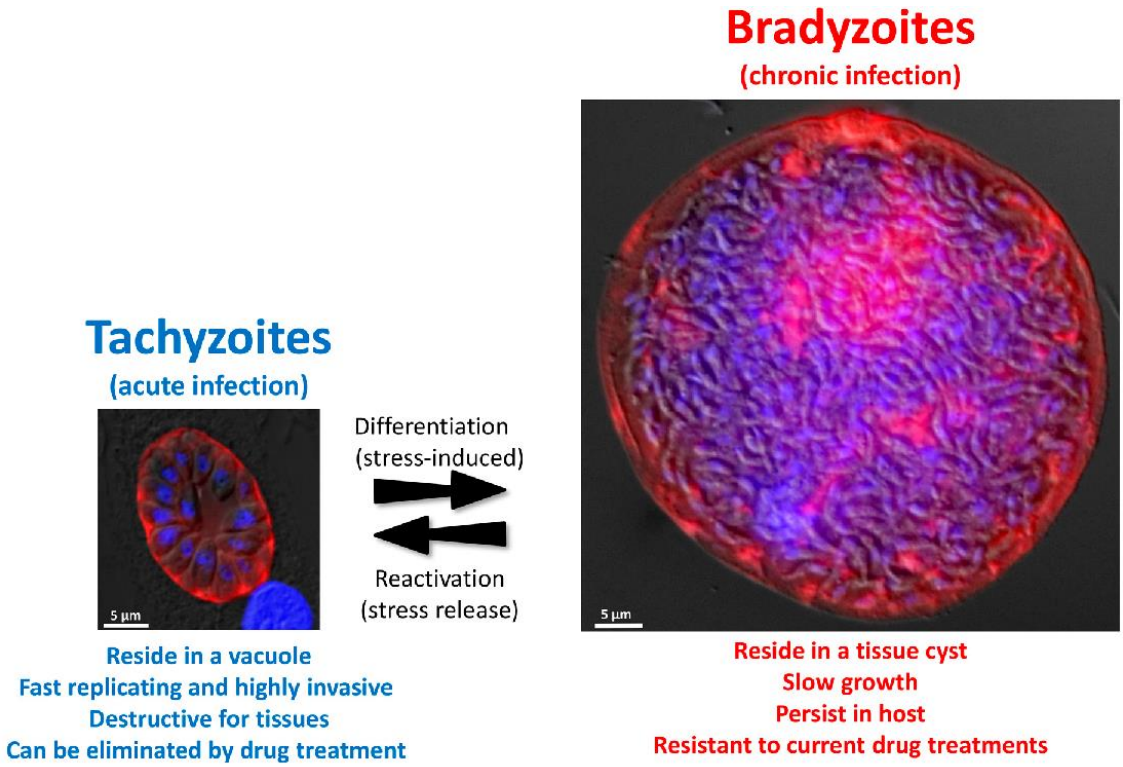



**Figure 6: Tachyzoite-containing vacuole (left) to bradyzoite-containing tissue cyst (right) inter-conversion.** Adapted from (Cerutti et al., 2020).

# 3 Toxoplasmosis

## 3.1 Modes of transmission to Humans

The mechanisms of transmission of *T. gondii* remained unknown until its lifecycle was discovered in 1970. In human, transmission occurs via two major routes (Montoya and Liesenfeld, 2004; Robert-Gangneux and Dardé, 2012).

**The vertical contamination** route corresponds to the transplacental transmission of tachyzoites from a pregnant woman to her fetus when primary infection is acquired during pregnancy. The prevalence of congenital toxoplasmosis ranges from 1 to 10 per 10,000 live births (Guerina et al., 1994; Montoya and Liesenfeld, 2004). The incidence of this pathology varies according to the trimester during which maternal infection was acquired. The rate of transmission is higher in the last trimester (65%) compared to the first and second trimester

(25% and 54% respectively) (McAuley, 2014). Inversely, the pathology is more severe when infection occurs early during gestation. **During horizontal transmission**, humans are mainly infected by consuming food or water contaminated with sporulated oocysts spread by cat feces, or undercooked meat containing tissue cysts. In very rare cases, contamination may also occur through blood transfusions and organ transplants containing cysts. Notably, due to immuno-suppressive treatment, reactivation of latent infection is the cause for disease in patients with bone marrow, hematopoietic stem cell and liver transplants (Siegel S.E. et al., 1971). Also, occupational transmission via contaminated needles, labware or animal models has been reported (Kayhoe et al., 1957; Remington and Gentry, 1970).

## 3.2 Pathogenesis

Toxoplasmosis is a cosmopolitan disease with a word seroprevalence of about 30%. The prevalence varies greatly from one country to another according to food habits, sanitary conditions, and ethnicity (Tenter et al., 2000). High seroprevalence is observed in Africa and Latin America (up to 80% for the latest) compared to North America and Southeast Asia (around 30%). In immunocompetent patients, infection by *T. gondii* is asymptomatic in 80% of cases, except in 10 to 20% of cases where a triad of mild symptoms (fever, cervical lymphadenopathy, and asthenia) is observed (Montoya and Liesenfeld, 2004). In addition to human pathology, toxoplasmosis remains an important veterinary problem resulting in heavy economic losses. It is estimated that more than 50% of cattle farms are contaminated in France, representing a potential source of contamination for humans.

**Congenital toxoplasmosis**

Congenital toxoplasmosis results from the passage of the parasite through the placental barrier during primary infection of pregnant women. As mentioned earlier, there is an inverse relationship between the rate of transmission and the severity of the infection (Dunn et al., 1999). The clinical manifestations of congenital toxoplasmosis are thus multiple. The most severe clinical outcomes include the death in utero of the fetus or the development of mental / psychomotor retardation at birth. However, in 85% of the cases, congenital toxoplasmosis is asymptomatic or leads to the development of retinochoroiditis, which may result in visual blindness in its most severe forms (McAuley, 2014).

**Acquisition in immunocompromised individuals**

Toxoplasmosis is a threat for immuno-compromised individuals following the reactivation of a chronic infection characterized by the conversion of bradyzoite-containing cysts into highly replicative tachyzoites leading to inflammation and destruction of infected tissues. Reactivation of latent cysts can lead to disseminated toxoplasmosis and affect multiple organs. However, three main pathologies are detected:

- Cerebral toxoplasmosis

It is the most common clinical manifestation in immunocompromised individuals (Lee and Lee, 2017; Luft et al., 2010). This pathology is usually accompanied by fever and symptoms such as headache, motor or sensory deficits or psychiatric disorders (Montoya and Liesenfeld, 2004). Very common in HIV-positive patients (Suzuki et al., 1988a), it was an important cause of death before the introduction of antiretroviral therapies.

- Ocular toxoplasmosis

Ocular toxoplasmosis results from local cyst reactivation at the retina level and manifests in its acute form by the appearance of floating bodies and visual blurring, which are common symptoms in diseases affecting the posterior part of the eye. Initially thought to be only associated with congenital transmissions, this pathology can be also acquired during a post-natal infection (Montoya and Liesenfeld, 2004).

- Pulmonary toxoplasmosis

In rare cases, deeply immunocompromised individuals may contract pulmonary toxoplasmosis. It is characterized by a severe form of pneumonia which can be lethal within a few days (Rabaud et al., 1996).

## 3.3 Diagnosis

Due to non-specific clinical signs or absence of symptoms, the diagnosis of toxoplasmosis is mainly established by serological, molecular, or histological techniques (Hill and Dubey, 2002; Montoya and Liesenfeld, 2004; Robert-Gangneux and Dardé, 2012).

Serological techniques allow the detection of anti-*T. gondii* IgA, IgM, and IgG antibody levels in patients' serum. The most common diagnosis is based on IgG levels, which are the only persistent immunoglobulins throughout the life of the infected host. However, the additional

detection of anti-*T. gondii* IgM reveals a recent infection (< 3 months). Serological testing includes various techniques such as the Sabin-Feldman dye test, Enzyme-Linked ImmunoSorbent Assay (ELISA), immunosorbent agglutination assay (ISAGA), indirect hemagglutination test, Western Blotting (WB), and IgG avidity test (Dard et al., 2016; Liu et al., 2015). Other diagnosis techniques are based on parasite identification in biological samples such as secretions or infected tissues that can be examined by histological tests (microscopic observation, immunohistochemistry). Finally, the detection of parasitic DNA by PCR is the most preferred technique for the diagnosis of congenital toxoplasmosis in-utero (Liu et al., 2015).

## 3.4 Treatments and Vaccination

At present, few treatments are available against toxoplasmosis due to the small number of identified active molecules against the parasite. Besides, these treatments are only administrated in limited cases, including congenital toxoplasmosis and in the most severe forms of the disease (Hill and Dubey, 2002; Montoya and Liesenfeld, 2004). They are based on the combined action of pyrimethamine (PYR) and sulfadiazine (SDZ), which act on the synthesis of folates (molecules involved in nucleic acids synthesis). Indeed, pyrimethamine is an inhibitor of folic acid synthesis acting on the parasitic dihydrofolate reductase; while sulfadiazine inhibits dihydropteroate synthetase, an essential enzyme in folate's synthesis. Of note, spiramycin (an inhibitor of protein synthesis) is also used during early pregnancy's infection until the PYR-SDZ combination can be given. Despite the effectiveness of the PYR-SDZ association, these molecules target replicative tachyzoites, thus are only efficient against acute infections (Guerina et al., 1994; Hill and Dubey, 2002). Indeed, there is no treatment available to eradicate bradyzoite-containing cysts; thus, to prevent reactivation of a latent infection. However, sulfonamides were shown to affect the enlargement of tissue cysts in mice during the chronic phase (Hill and Dubey, 2002). Moreover, an antifolate drug combination including trimethoprim and sulfamethoxazole (Schneider et al., 1992) or Dapsone plus pyrimethamine seems to be the most effective option to protect HIV positive individuals against toxoplasmic encephalitis (Girard et al., 1993). Evidence is limited on *T. gondii* prevention in organ transplantation, with possible efficacy in the use of trimethoprim and sulfamethoxazole or pyrimethamine (Baden et al., 2003; Strabelli et al., 2012).

To date, there is no vaccine suitable for human use. The one licensed vaccine against *T. gondii*, called "TOXOVAX", is only available for veterinary use. It is a live attenuated vaccine based on an S48 strain (Buxton, 1993), impaired in sexual development in cats. Recently, a *T. gondii* strain, that exhibits defective fertilization and generates oocysts that fail to produce sporozoites, was generated using a CRISPR/Cas9 technique. Vaccination of feline with this engineered parasite strain completely forestalled oocyst discharge following infection with a wild-type parasite strain, demonstrating that this mutant is an attenuated, live, transmission-blocking vaccine (Ramakrishnan et al., 2019). A challenging criterion for a potential vaccine candidate is that the antigen has to be available in all three major infectious stages namely the tachyzoites, bradyzoites and sporozoites. A study showed that using DNA vaccine boosted with recombinant adenovirus vaccine encoding ubiquitin conjugated antigens from the different infectious stages proved to be effective against type I and type II parasites (Yin, H. et al., 2015).

## 3.5 Prophylaxis

Prevention against toxoplasmosis can be achieved in different ways. The most effective measure is to prevent maternal infection during pregnancy by instructing women on how to avoid exposure to possible pathways of infection. This includes proper freezing and heating of meat-based foods, washing of vegetables and fruits, hand hygiene following gardening or soil associated chores and precautions in cleaning cat litter boxes. A second preventive measure is based on the timely treatment of an acutely infected pregnant woman to delay or prevent transplacental transmission, thus, to reduce the frequency of fetal infection or alleviate the severity of consequences. This is achieved by systematic serological screening during pregnancy to identify uninfected women who are at risk of infection and women for whom an acute infection is suspected (Gajurel et al., 2015; Opsteegh et al., 2015). A third possible intervention is mitigating the consequences of fetal infection, by treating infected fetuses and/or neonates by antibiotics to reduce the consequences of the infection (McLeod et al., 2006). Finally, cat owners should be careful about pet maintenance and make sure that they keep their cats indoors during the night and prevent them from consuming foods that carries the risk of being contaminated (Opsteegh et al., 2015).

## 3.6 Immunity against toxoplasmosis

Infection with *T. gondii* leads to the development of a robust Th1 cytotoxic immune response, which results in the rapid elimination of parasite strains of low virulence. Innate and adaptive immune responses triggered against the parasite locally and systematically, allow controlling parasitic dissemination while establishing a long-term protective immunity against secondary infections.

Enterocytes represent the first barrier against *T. gondii* (Barragan and Sibley, 2002). They play an essential role in inducing the initial innate immune response against infection by synthesizing nitric oxide (NO) as well as pro-inflammatory cytokines such as interleukins 15 and 18 (IL-15 and IL-18), which trigger the recruitment and activation of key immune cells such as neutrophils, Natural Killer cells (NKs), monocytes and dendritic cells (DCs) (Liesenfeld et al., 1996; Miller et al., 2009). DCs and monocytes secrete IL-12 that activates the secretion of interferon-γ (IFN-γ) by NK cells and T lymphocytes. IFN-γ is a major cytokine required for the resistance of the host against the infection (Gazzinelli et al., 1994; Suzuki et al., 1988b). Together with Tumor Necrosis Factor (TNFα), IFNγ activates cell-intrinsic defenses of macrophages and pro-inflammatory monocytes promoting rapid tachyzoite elimination during the acute phase of the infection (Butcher and Denkers, 2002; Sibley et al., 1991) (Figure 7). However, type I strains have developed strategies to escape cell-autonomous immunity of activated monocytes and macrophages by secreting key effectors that down-regulate IFNγ-activated signaling pathways. In the bloodstream, tachyzoites preferentially infect monocytes, which may promote parasite dissemination to distant organs including the brain (Channon et al., 2000; Silveira et al., 2011) (Ueno et al 2014). The local pro-inflammatory response in the brain, notably the induction of NO production by activated microglia and recruited monocytes, promotes the rapid conversion of tachyzoites to bradyzoites in neurons and the establishment of the chronic phase (Bohne et al., 1994; Scharton-Kersten et al., 1997).

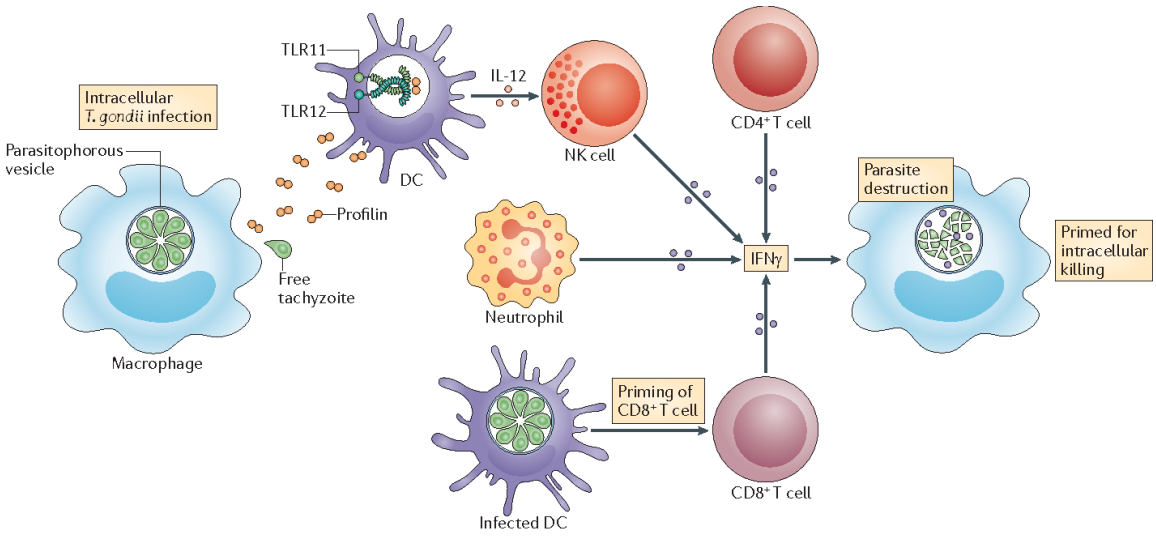



**Figure 7: Interferon γ (IFNγ) mediated immunity to *Toxoplasma gondii* infection.** Reprinted from (Yarovinsky, 2014). Toll like receptor (TLR11 and 12)-mediated activation of dendritic cells (DC) regulates their expression of stimulatory molecules and cytokines such as IL-12. IL-12 secretion triggers the production of IFNγ by different immune cells, such as the NK cells, the neutrophils, the $CD4^+$ and $CD8^+$ T cells. Infected DCs prime $CD8^+$ T cells responses against *Toxoplasma gondii* antigens, inducing the secretion of IFNγ essential for resistance to the parasite during the chronic stages of the infection.

# 4 Tachyzoite architecture and ultrastructural organization:

*T. gondii* belongs to the phylum Apicomplexa, characterized by their highly polarized ultrastructure. Notably, they are typified by the presence of an apical complex, which represents a regulated secretion gateway for invasion of host cells (Katris et al., 2014). The morphology of *T .gondii* changes depending on the parasite's life-stage (Dubey et al., 1998). For this thesis, I will focus on the most extensively studied stage in *T. gondii* lifecycle, the tachyzoite. Tachyzoites are characterized by their crescent banana-like shape of approximatively 2 by 7µm with a slightly pointed anterior end named the conoid and a rounded posterior end (Dubey et al., 1998). The name "tachyzoite" derives from the Greek word tachos, meaning speed, referring to the rapid replicative rate of the parasite within the intermediate host (Frenkel, 1973). Akin to all eukaryotes, these tachyzoites contain a nucleus encompassing a 63Mb haploid genome divided into 13 chromosomes (encoding approximatively 8000 genes) (Khan et al., 2005)(Bunnik et al., 2019; Reid et al., 2012), a single mitochondrion, an endoplasmic reticulum (ER) and a single Golgi apparatus (Pelletier et al., 2002). Tachyzoites also possess secretory organelles specific to Apicomplexa: the

micronemes, rhoptries, and dense granules that are indispensable for the parasite lytic cycle (chapter1 – Part 5). The parasite also contains a relic-like plastid termed apicoplast, as well as acidocalcisomes involved in the maintenance of cellular calcium homeostasis (Docampo, 2016; Moreno and Zhong, 1996; Waller and McFadden, 2005). The whole is structured by a cortical cytoskeleton made up of a microtubule network, completely enclosed in by a three-layered membranous structure called the pellicle, composed of the plasma membrane and the inner membrane complex (Mann and Beckers, 2001)(Figure 8).

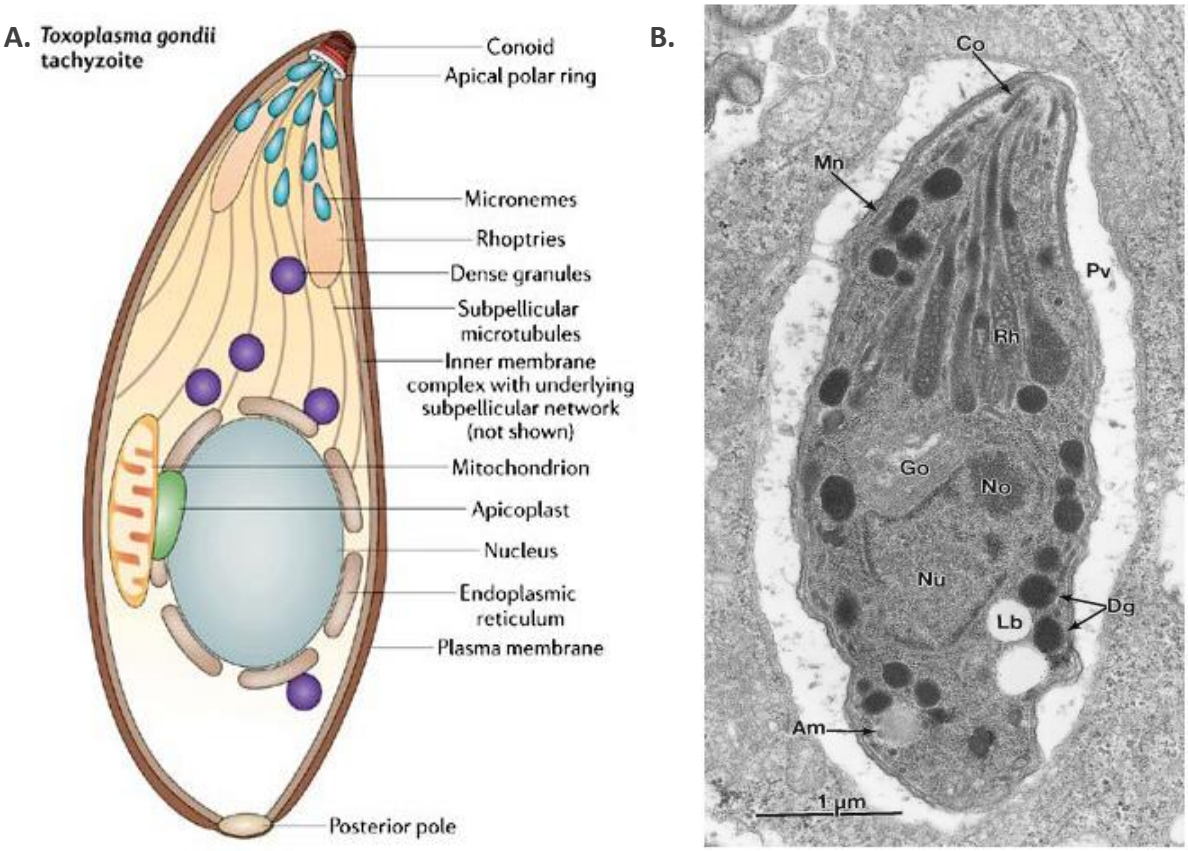



**Figure 8: The ultrastructure of *T. gondii* tachyzoite stage.** A. Schematic representation of the tachyzoite's intracellular organelle organization adapted from (Baum et al., 2006). B. Transmission electron micrograph representation of an intracellular tachyzoite adapted from (Dubey et al., 1998). Am: amylopectin granule; Co: conoid; Dg: electron-dense granule; Go: Golgi complex; Mn: microneme; No: nucleolus, Nu: nucleus; Pv: parasitophorous vacuole; Rh: rhoptry.

## 4.1 Pellicle

The pellicle is a 60nm structure composed of an external plasma membrane (plasmalemma) surrounding the parasite and two internal membranes forming an internal membrane complex called IMC (Inner Membrane Complex) (Figure 9). The superposition of these three

bilayers plays a crucial role in maintaining the parasite's structure and constitutes an important exchange zone during the stages of host cell recognition, adhesion, and invasion.

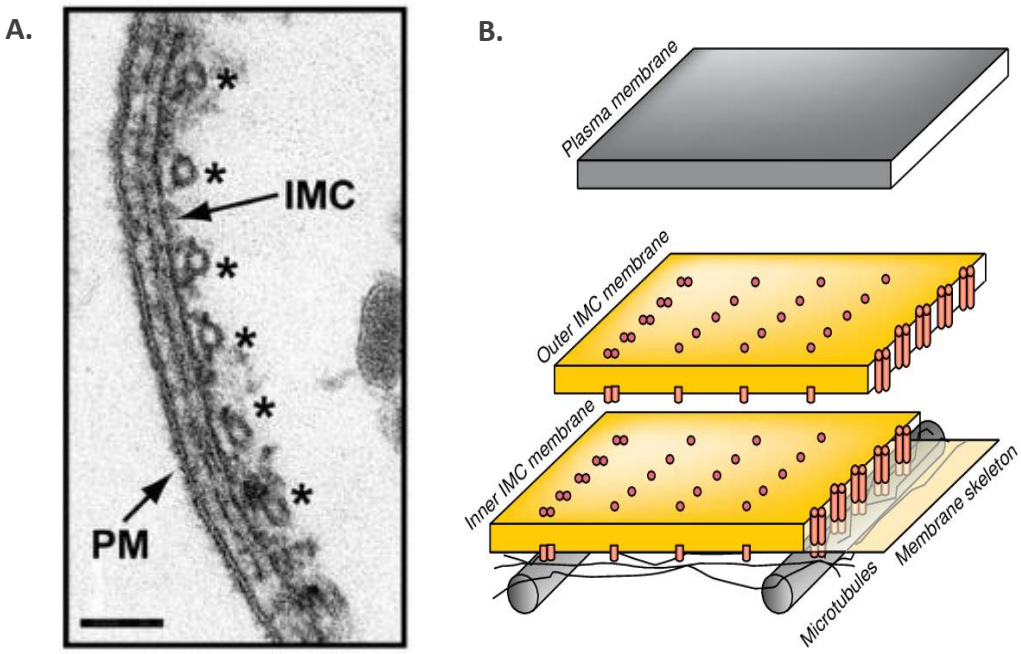



**Figure 9: The structure of *Toxoplasma gondii* pellicle. A.** Transmission electron microscopy showing an isolated fragment of *T. gondii* pellicle adapted from (Johnson et al., 2007). PM: plasma membrane; IMC: Inner membrane complex; and the asterisks the sub-pellicular microtubule network. **B.** Schematic representation of the apicomplexan pellicle containing an outer PM and an IMC anchored to the sub-pellicular microtubule network, adapted from (Keeley and Soldati, 2004).

## The external plasma membrane

The plasma membrane consists of a lipid bilayer covered with glycosylphosphatidylinositol (GPI) groups allowing the anchoring of glycoproteins (de Macedo et al., 2003; Nagel and Boothroyd, 1989; Tomavo et al., 1989) belonging mostly to the surface antigen (SAG) family. Five major antigens occupy the surface of the tachyzoite (Couvreur et al., 1988). Among them, *Tg*SAG1 is the most abundant antigen of this family which includes about twenty other proteins called SRS (SAG1 related sequence) (Lekutis et al., 2001; Manger et al., 1998). These proteins are involved in the process of attachment to the host cell membrane (Mineo and Kasper, 1994), and the modulation of the host's immune response (Dzierszinski et al., 2000; He et al., 2002).

**The inner membrane complex (IMC)**

Similarly, to all members of the group Alveolata, the IMC consists of a double membrane located 15nm below the plasma membrane. It covers the whole peripheral surface of the parasite except for the apical (at the level of the conoid) and basal pole, as well as at the level of the micropore. The micropore is located halfway-up the parasite and corresponds to an invagination of the membrane considered as a potential site of endocytosis (Nichols et al., 1994). The IMC consists of flattened membranous vesicles derived from the endoplasmic reticulum (ER)-Golgi apparatus, presenting a discontinuous structure (Morrissette et al., 1997). The strength and the stability of the parasite reside in the presence of the subpellicular network (SPN) that consists of a mesh of filamentary proteins, the alveolins (8 to 10nm in diameter) (Gould et al., 2008). In Apicomplexa, *Tg*IMC1 was the first alveolin characterized at the level of the SPN (Mann and Beckers, 2001). A total of 14 alveolin repeat-containing proteins (*Tg*IMC1 and *Tg*IMC3-TgIMC15) have been subsequently identified through systematic research (Anderson-White et al., 2011). The cytoplasmic part of the internal membrane interacts with the subpellicular microtubules allowing the stabilization of the cytoskeleton through longitudinal lines of intermembranous particles (IMP) (Morrissette et al., 1997; Morrissette and Sibley, 2002a). The outer membrane of the IMC harbors numerous proteins regulating cell division, parasitic motility, and invasion (J. M. Dobrowolski et al., 1997; Frénal et al., 2010; Ménard, 2001). These proteins include the glideosome-associated proteins (GAPs) (Gaskins et al., 2004), the GAP proteins with transmembrane domain *Tg*GAPM (Bullen et al., 2009), ISP proteins (IMC Subcompartment Proteins) (Beck et al., 2010), and SIP proteins (Stripes IMC proteins)(Lentini et al., 2015).

In addition, the parasite has a cortical actin and myosin cytoskeleton, called “glideosome” located between the plasma membrane and the IMC, which promotes parasitic motility (Opitz and Soldati, 2002). It consists of actin, myosin A, and IMC-anchoring proteins (GAPs).

## 4.2 Cortical cytoskeleton

### 4.2.1 Microtubule network

Underlying the pellicle resides a filamentous cytoskeletal structure, called the subpellicular network (SPN), that associates on its outer face with the IMC and on its inner face with 22 spiral subpellicular microtubules (MT) of 22nm diameter emanating from the apical pole and

covering 2/3 of the parasite's length (Anderson-White et al., 2012; Nichols and Chiappino, 1987). MTs are aligned in a counterclockwise direction, with their minus end anchored at the microtubule organizing center (MTOC) located at the apical polar ring (APR). Their polymerization takes place from the apical pole to the basal pole of the parasite (Cyrklaff et al., 2007; Nichols and Chiappino, 1987; Russell and Burns, 1984). The cytoskeleton is very stable and gives the parasite its shape. This stability is due to the presence of microtubule-associated proteins (MAPs) (Morrissette et al., 1997; Morrissette and Sibley, 2002a), such as the recently described ring-1 (RNG1) that localizes at the APR (Tran et al., 2010). MTs form a spiral cone structure called "conoid", located at the apical pole exclusively composed of tubulin-α. The conoid is topped by two preconoidal rings and has a pair of short adjacent intraconoidal MTs passing through the middle and ending anteriorly within the parasite cytoplasm (Morrissette et al., 1997)(Figure 10, left).

### 4.2.2 Conoid

At the extreme tip of the apical complex lies the conoid, a hollow cone-shaped structure. The conoid consists of 10 to 14 tubulin filaments that are wound spirally around two intra-conoidal microtubules that are delimited by the presence of two pre-conoidal rings at the anterior end of the conoid connecting them to the apical polar ring (APR) (Hu et al., 2002b; Morrissette, 2015; Nichols and Chiappino, 1987). The conoid is a retractable structure that extrudes during parasite's egress of the parasitophorous vacuole enabling motility and adhesion to neighboring cells and therefore the dissemination of newly egressed extracellular parasites. Conversely, the conoid retracts during the intracellular replication of the parasite. This movement is induced by calcium fluxes and regulated by calcium-binding proteins localized at the conoid (Monteiro et al., 2001; Morrissette, 2015). Besides, this extension process is thought to be actin-myosin driven (Carmen et al., 2009; Shaw and Tilney, 1999). The conoid harbor both calmodulin-like proteins (*Tg*CAM1 and *Tg*CAM2) and Dynein Light Chain (*Tg*DLC) proteins. *Tg*CAMs are involved in the extrusion of the conoid in response to a calcium flow (Anderson-White et al., 2012; Hu et al., 2006). *Tg*ICMAP-1, a novel microtubule-associated protein that binds to and stabilizes intra-conoidal microtubules, was considered as the first molecular tool used to dissect intra-conoidal microtubule biogenesis and assembly during daughter parasite construction (Heaslip et al., 2009). The intra-conoidal microtubules may anchor micronemes and rhoptries within the conoid, thus participating in the release of

secreted parasite effectors during invasion (Carruthers and Sibley, 1997; Nichols and Chiappino, 1987). SAS6 (centriole associated)-like protein (SAS6L), localizes to the preconoidal rings in tachyzoites (de Leon et al., 2013), and might play a role in the anchoring of the parasite striated fiber assemblins (SFA), which connect the conoid and the APR to the centriole in replicating parasites (Francia et al., 2012) (Figure 10, right).

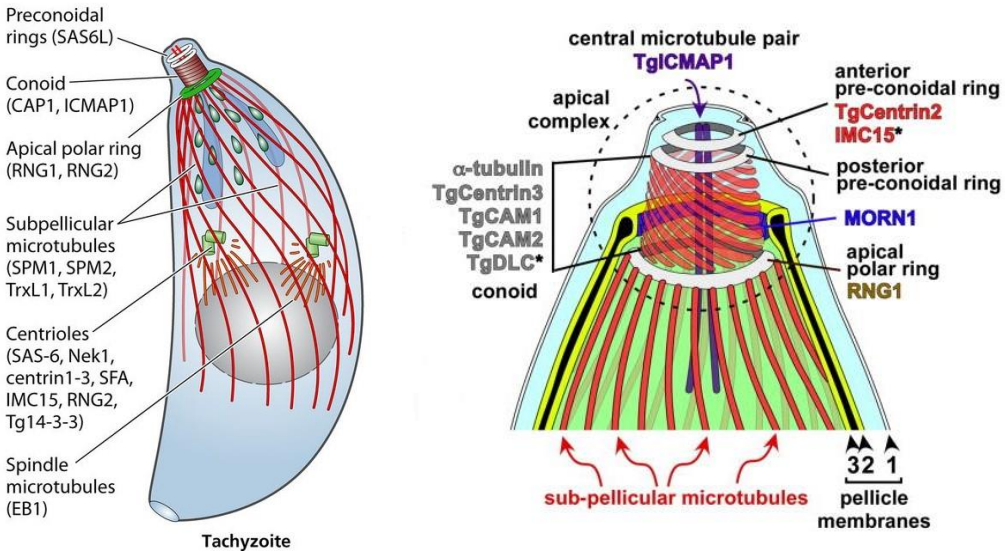



**Figure 10: *Toxoplasma gondii* cytoskeleton. A.** Schematic representation of the microtubular network of the tachyzoite, adapted from (Morrissette, 2015). The microtubule (MT) spindles and the sub-pellicular MTs represented in red are associated respectively with the centrioles and the MTOC represented in green and located at the base of the conoid. **B.** Structural representation of the apical complex and its associated protein markers, adapted from (Anderson-White et al., 2012).

## 4.3 Intracellular organelles

### 4.3.1 The apicoplast

Except for *Cryptosporidium spp.*, many apicomplexan parasites possess two endosymbiotic derived organelles, a single mitochondrion and a relic non-photosynthetic plastid known as the apicoplast (Figure 8A). The apicoplast is a relic plastid-like organelle resulting from secondary endosymbiosis (McFadden and Waller, 1997) of a cyanobacterium by a red alga, then of the red algae by an ancestor of the Apicomplexa (Lim and McFadden, 2010; Waller and McFadden, 2005). It is composed of four membranes surrounding a circular genome of 35Kb encoding about 60 genes (Wilson et al., 1996). The outermost membrane is derived

from the host phagosome with the second outermost deriving from the plasma membrane of the red algae. The inner two membranes are from the chloroplast of the original organism (Waller and McFadden, 2005). The majority of apicoplast's proteins are encoded by the nuclear genome and imported post-translation. Many features of the original plastid have been lost such as its photosynthetic ability. Nevertheless, the apicoplast remains the site of many important biosynthetic pathways, such as the synthesis of fatty acid by the FASII system (Fatty Acid Synthesis type II) (Ramakrishnan et al., 2012; Waller et al., 1998), isoprenoid precursors through the (DOXP) pathway (Nair et al., 2011; Seeber and Soldati-Favre, 2010), and the synthesis of heme and iron clustering (Gisselberg et al., 2013; Lim and McFadden, 2010; van Dooren et al., 2012). Correct segregation of the apicoplast during replication is actin and Myosin F dependent (Egarter et al., 2014; Jacot et al., 2013) and also depends on dynamin-related protein A (DrpA) (van Dooren et al., 2009). Finally, the apicoplast is considered as a privileged target for drug development since it retains a mode of functioning close to procaryotes (Striepen, 2011).

In addition, *T. gondii* possesses a specific set of secretory organelles essential to the lytic cycle enabling host cell invasion and parasite survival within the parasitophorous vacuole (PV). This includes micronemes, rhoptries, and dense granules.

### 4.3.2 Micronemes

Micronemes are small ellipsoidal shaped organelles (around 250x50nm) concentrated at the apical pole, just below the conoid. The number of micronemes is variable depending on the species, the parasitic stage of development, and the activity of the parasite; they are about a hundred at the tachyzoite stage (Carruthers and Tomley, 2008; Dubois and Soldati-Favre, 2019). Proteins stored in these organelles are essential for many processes during the parasitic life cycle, notably gliding motility and invasion (Carruthers and Tomley, 2008; Dubois and Soldati-Favre, 2019). The secretion of microneme content is induced upon parasite contact with the cytoplasmic membrane of the host cell (Carruthers and Sibley, 1997); and the chemical inhibition of this secretion strongly affects host cell invasion (Carruthers et al., 1999). The content of micronemes has been identified by different approaches (Soldati et al., 2001; Tomley and Soldati, 2001), notably by proteomics (mass spectrometry) of MIC secreted proteins (Bromley et al., 2003). Many microneme proteins (MIC) have adhesin-like domains such as the microneme adhesive repeats domain (MAR), the thrombospondin 1 domain (TSR),

the von Willebrand A domain/ Integrin inserted domain A/I domain, Apple/PAN (Plasminogen, Apple, Nematode domain), EGF like domains, and lectin domains such as the chitin-binding like domain (CBL) (Garcia-Réguet et al., 2000; Meissner et al., 2002) (Figure 11). For example, the thrombospondin-like domains found in MIC2, a major adhesive molecule for *T. gondii*, are implicated in host cell attachment (Andenmatten et al., 2013; Carruthers and Tomley, 2008).

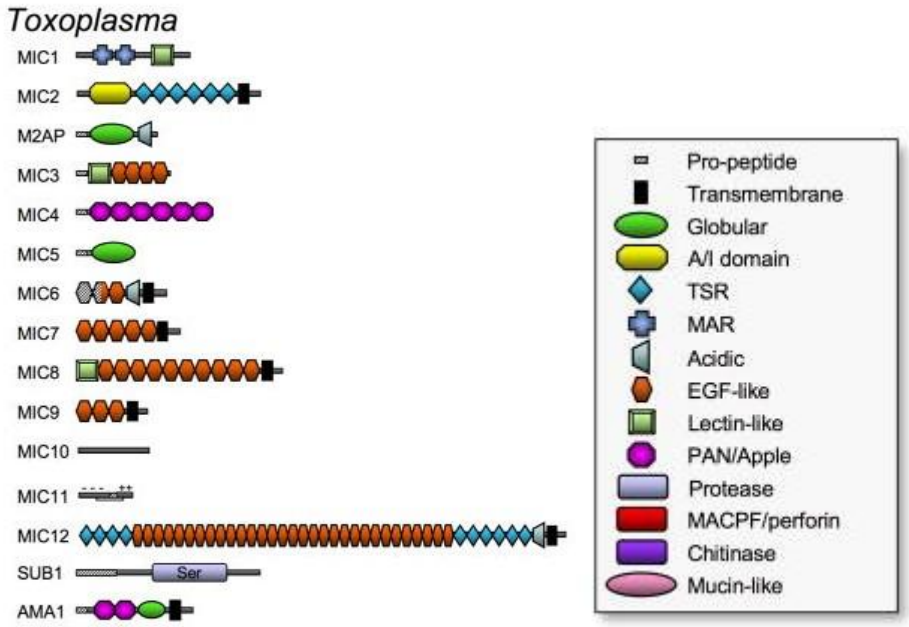



**Figure 11: *Toxoplasma gondii* microneme proteins family.** Schematic representation of the different adhesive domains of MIC proteins from *Toxoplasma* parasites involved in proteins or carbohydrate interactions; adapted from (Carruthers and Tomley, 2008).

The MIC proteins are found in two forms, soluble and transmembrane, which form complexes, such as *Tg*MIC2/*Tg*M2AP, *Tg*MIC3/*Tg*MIC8, *Tg*MIC1/*Tg*MIC4/*Tg*MIC6, which can bind receptors on the host cell (Sheiner et al., 2010)(Figure 12). For example, *Tg*MIC2, a transmembrane adhesin, and its soluble partner *Tg*M2AP (MIC2-associated protein) have been widely studied for their role as a connector between host cell attachment and the actomyosin motor (Huynh et al., 2015). *Tg*MIC2 binds to heparin and ICAM-1 protein (Intracellular Adhesion Molecule 1) (Barragan et al., 2005; Harper et al., 2004), *Tg*MIC1 recognizes sialic acid via its lectin domain (Blumenschein et al., 2007), whereas *Tg*MIC3 binds the N-acetylglucosamine of the host cell (Cérède et al., 2002).

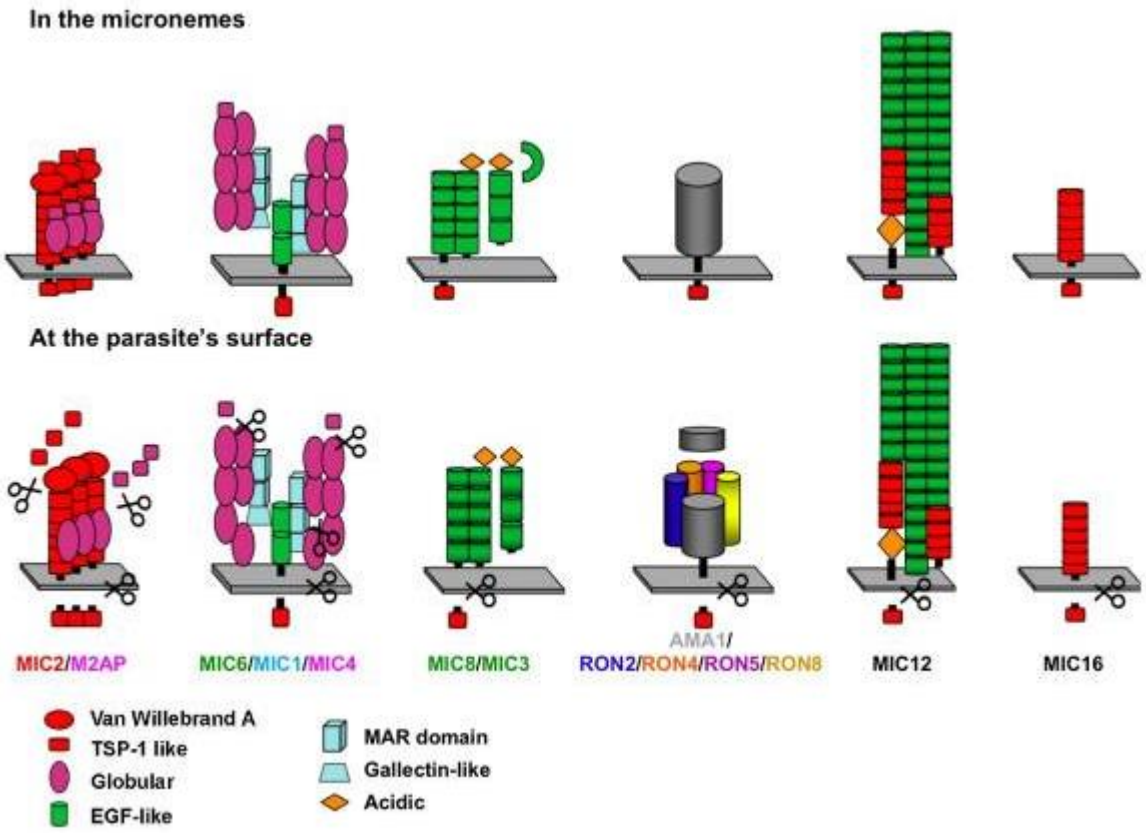



**Figure 12: *Toxoplasma gondii* MIC protein complexes.** Schematic representation of the different MIC protein complexes in *T. gondii*, as well as the individual MICs such as *Tg*MIC12 and *Tg*MIC16. Adapted from (Sheiner et al., 2010).

Similarly, by its intimate interaction with RON2 at the tight junction, apical membrane antigen 1 (AMA1) bridges the gap during host cell invasion (Bargieri et al., 2013; Lamarque et al., 2011; Mital et al., 2005). Conversely, MIC8, another MIC having adhesive domains, is not involved in host cell's attachment but is implicated in a signaling cascade that ultimately leads to rhoptry secretion, a process required for the early formation of the MJ (Kessler et al., 2008). Two types of proteases are also found in micronemes: *Tg*SUB (Subtilisin-like) and *Tg*ROM (Rhomboid). *Tg*SUB1 and *Tg*ROM1 are two serine proteases involved respectively in the maturation of certain MICs at the host cell membrane (Lagal et al., 2010) and protein recycling within the secretory pathway during cell division (Brossier et al., 2008). The rhomboid proteases *Tg*ROM4 and *Tg*ROM5 present at the parasite plasma membrane, play a role in the proteolytic cleavage of the transmembrane domain of MIC2 and MIC6 during invasion ("shedding" process) to break the adhesive interaction between the parasite and the host cell

receptor, ensuring thus the full entry of the parasite into the host cell (Brossier et al., 2005; Buguliskis et al., 2010; Dowse et al., 2005). Finally, perforin-like protein 1 (PLP1) is likewise found in micronemes. To facilitate the parasite's release from the host cell, PLP1 is secreted immediately before egress to disrupt the PVM (Garg et al., 2015; Kafsack et al., 2009).

### 4.3.3 Rhoptries

Rhoptries are 2 to 3µm club-shaped organelles anchored to the apical pole of the parasite (Boothroyd and Dubremetz, 2008; Dubey et al., 1998). Each tachyzoite contains between 8 to 12 rhoptries that can be divided into sub-compartments: the thin top duct of the rhoptries called neck which is in direct contact with the conoid and contains the rhoptry neck proteins (RONs) and a sac-like compartment below the neck called the bulb where the rhoptry proteins (ROPs) are found (Dubey et al., 1998). It has been suggested that rhoptries receive products from both the secretory and endocytic pathways, and that rhoptry biogenesis/homeostasis requires the contribution of both pathways (Ngô et al., 2004).

Rhoptries are synthesized as pre-organelles or pre-rhoptries derived from the endosomal compartment and become mature after condensation and elongation (Dubremetz, 2007; Venugopal et al., 2017). Rhoptry translocation and attachment at the apical pole are mediated by a complex consisting of the Armadillo Repeats Only Protein *Tg*ARO, the Armadillo Interacting Protein *Tg*AIP, and Myosin F (*Tg*MyoF) (Mueller et al., 2013). At present, about 40 rhoptry proteins have been identified and their discovery is constantly increasing (Boothroyd and Dubremetz, 2008; Peixoto et al., 2010). RON proteins (such as *Tg*RON2, *Tg*RON4, *Tg*RON5 and *Tg*RON8) are secreted just after the MIC proteins upon host cell adhesion and enable host cell invasion in association with TgAMA1 by forming the moving junction (MJ), a transient structure allowing the parasite to propel itself into the host cell (Alexander et al., 2005; Lebrun et al., 2005) (detailed in part 5: lytic cycle). ROP proteins contained in the bulb of the organelle are secreted after MJ formation and targeted either to the nascent parasitophorous vacuolar space, the PVM, or the cytoplasm of the host cell (Figure 13).

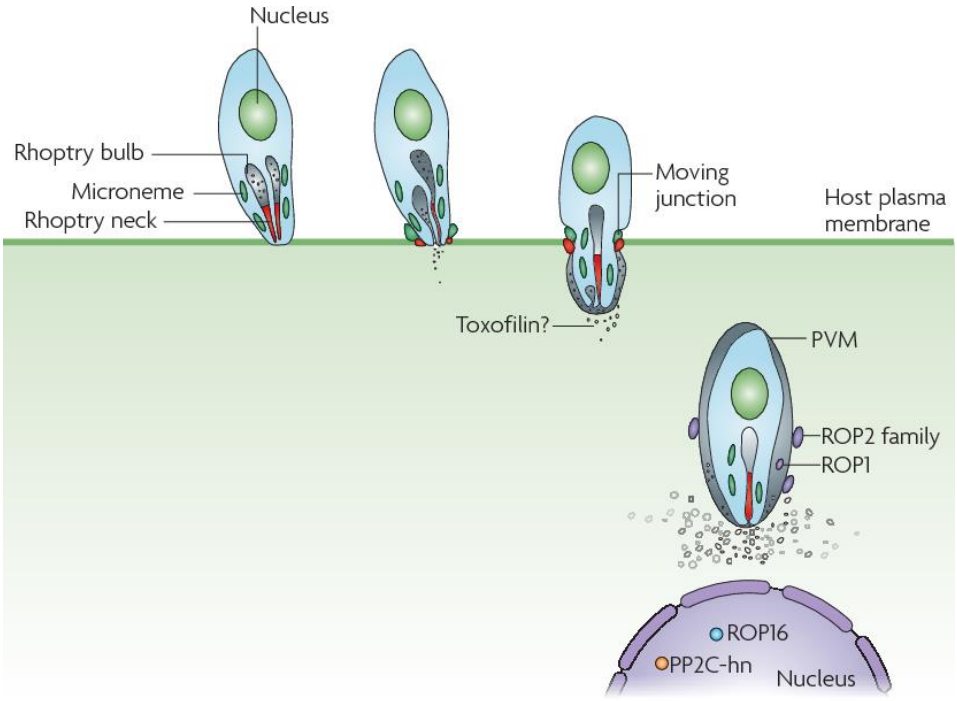



**Figure 13: Schematic representation of rhoptry proteins role during the invasion and PV formation.** Adapted from (Boothroyd and Dubremetz, 2008). The RON proteins (in red) are associated with *Tg*AMA1 protein to form the moving junction (MJ) allowing the parasite's entry into the host cell. During the invasion, the parasite surrounds itself with a protective structure, the parasitophorous vacuole (PV), whose membrane derives from the host cell. The ROP proteins (in gray) secreted during the invasion participate in the establishment and maintenance of this structure. The proteins *Tg*ROP16 and *Tg*PP2C-hn translocate to the nucleus of the host cell.

The majority of ROP proteins are kinases or pseudo-kinases that target host cell proteins to block the destruction of the PV and to ensure an adequate cellular environment to the parasite replication (Boothroyd and Dubremetz, 2008; Bradley and Sibley, 2007; Hakimi et al., 2017). Some ROPs are homologous to phosphatases such as protein phosphatase 2C (PP2C) (Gilbert et al., 2007), or proteases such as Toxopain-1 that plays a role in the final maturation of ROP proteins after the cleavage of the N-terminal located pro-domain (Que et al., 2002). Currently, the best-characterized ROP proteins belong to the ROP2 family which includes around fifty proteins (Boothroyd and Dubremetz, 2008; El Hajj et al., 2006; Peixoto et al., 2010). The proteins of the ROP2 family have a kinase-like domain in their C-terminal region and are found, for the most, at the level of the PVM (Hajj et al., 2007). Moreover, many ROPs are targeted to the host cytoplasm and nucleus and are involved in subversion of host signaling pathways or gene expression, respectively (Boothroyd and Dubremetz, 2008) and are therefore major virulent factors that promote parasite dissemination in the intermediate

host (Behnke et al., 2015, 2012). *T. gondii* type I strains are the most virulent and express ROP that are absent from the Type II strain genome or that display mutations that render them inactive (Yang et al., 2013). The best described example holds for the *Tg*ROP5/*Tg*ROP17/*Tg*ROP18 complex, located at the PVM, which induces the phosphorylation of several threonine residues within the GTPase domain of Immune-Related GTPases (IRG) thus preventing their oligomerization and accumulation at the PV and therefore the PV lysis (Etheridge et al., 2014; Fleckenstein et al., 2012). The inhibition of the activity of IRGs is specific to virulent type I strains. In fact, type II strains have a polymorphism within the gene encoding *Tg*ROP5, making this factor inactive and therefore altering the elimination of parasites by IRGs (Behnke et al., 2011).TgROP18 also phosphorylates the transcription factor ATF6β and induces its degradation by the proteasome causing a higher susceptibility to infection (Yamamoto et al., 2011) (Figure 14). Unlike the ROP proteins mentioned above, the kinase *Tg*ROP16 and the phosphatase *Tg*PP2C translocate to the host cell nucleus. The role of *Tg*PP2C has not been elucidated, but in its absence, a decrease in parasite growth has been observed in vitro (Gilbert et al., 2007). Conversely, *Tg*ROP16 is well characterized. *Tg*ROP16, a serine/threonine kinase, phosphorylates the transcription factors STAT3 and STAT6 (Signal Transducer and Activator of Transcription) leading to a strong decrease in the production of pro-inflammatory cytokines IL-12 and TNF-α and consequently of the establishment of the Th1 response in infected mice (Butcher et al., 2011; Ong et al., 2010) (Figure 14). Finally, rhoptries also contain a lipid fraction composed mainly of cholesterol, phospholipids (sphingomyelin and phosphatidylcholine), and saturated fatty acids (Foussard et al., 1991).

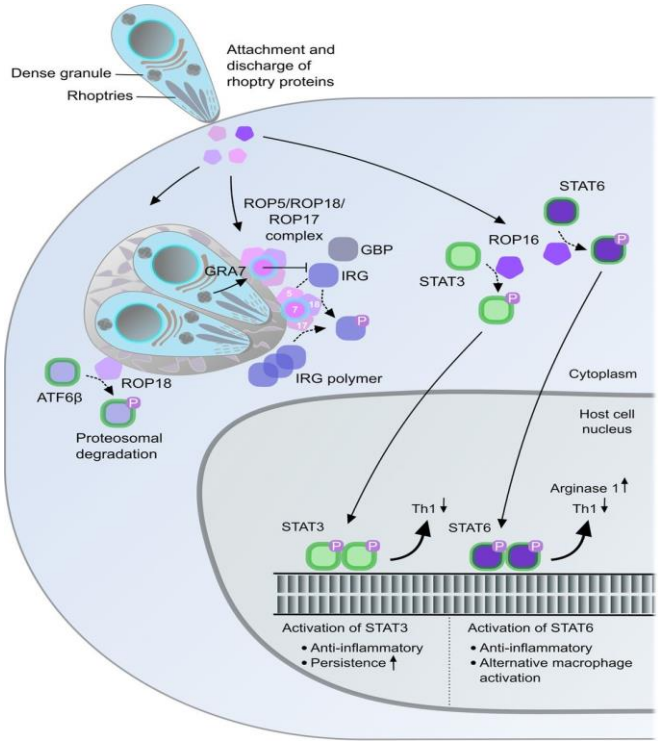



**Figure 14: Role of ROP proteins in modulating the activity of the host cell.** Adapted from (Hakimi et al., 2017). Following attachment of the parasite to the host cell, the ROP proteins are secreted into the host cytoplasm where they associate with the PV or are translocated to the host nucleus. The *Tg*ROP5/*Tg*ROP17/*Tg*ROP18 complex phosphorylates the IRGs preventing their accumulation at the PVM. *Tg*GRA7 alone or associated with the *Tg*ROP5/*Tg*ROP17/*Tg*ROP18 complex can also inhibit the IRGs. *Tg*ROP18 phosphorylates the transcription factor ATF6β inducing its degradation via the proteasome. *Tg*ROP16 phosphorylates STAT3 and STAT6 inducing anti-inflammatory responses.

### 4.3.4 Dense granules

Dense granules (DGs), so-called due to their high electron density observed by Transmission Electronic Microscopy (TEM) (Dubremetz and Dissous, 1980), have a dense microsphere structure with an approximate diameter of 200nm (Dubey et al., 1998; Mercier and Cesbron-Delauw, 2015). Unlike the rhoptries and micronemes located exclusively at the apical pole, dense granules are distributed through the parasite's cytoplasm and are variable in number according to the parasitic stage. There are approximatively 15 of them in the tachyzoite and sporozoite stages, and between 8-10 and 3-6 in the bradyzoite and merozoite stages respectively (Kim and Weiss, 2004). One of their particularities is that DGs are only observed in a subset of apicomplexan parasites and seems to be restricted to those forming tissue cysts such as *Toxoplasma*, *Neospora*, *Sarcocystis*, *Besnoita*, *Hammodia,* and *Frankelia* (Mercier and Cesbron-Delauw, 2015). DGs contain proteins named GRAs (dense GRAnules proteins)

secreted immediately after parasite invasion at the onset of PV formation (Carruthers and Sibley, 1997). The mechanisms by which GRA proteins are released outside of the parasite remain largely unexplored (Souza, 2006). However, it has been proposed that DGs navigate through small gaps separating the IMC plates rather than fusing directly with the membrane of the IMC (Dubremetz et al., 1993). Although the difficulty of DG purification makes it difficult to publish a complete proteome, around twenty GRA proteins have been reported so far (Mercier and Cesbron-Delauw, 2015) (Figure 15).

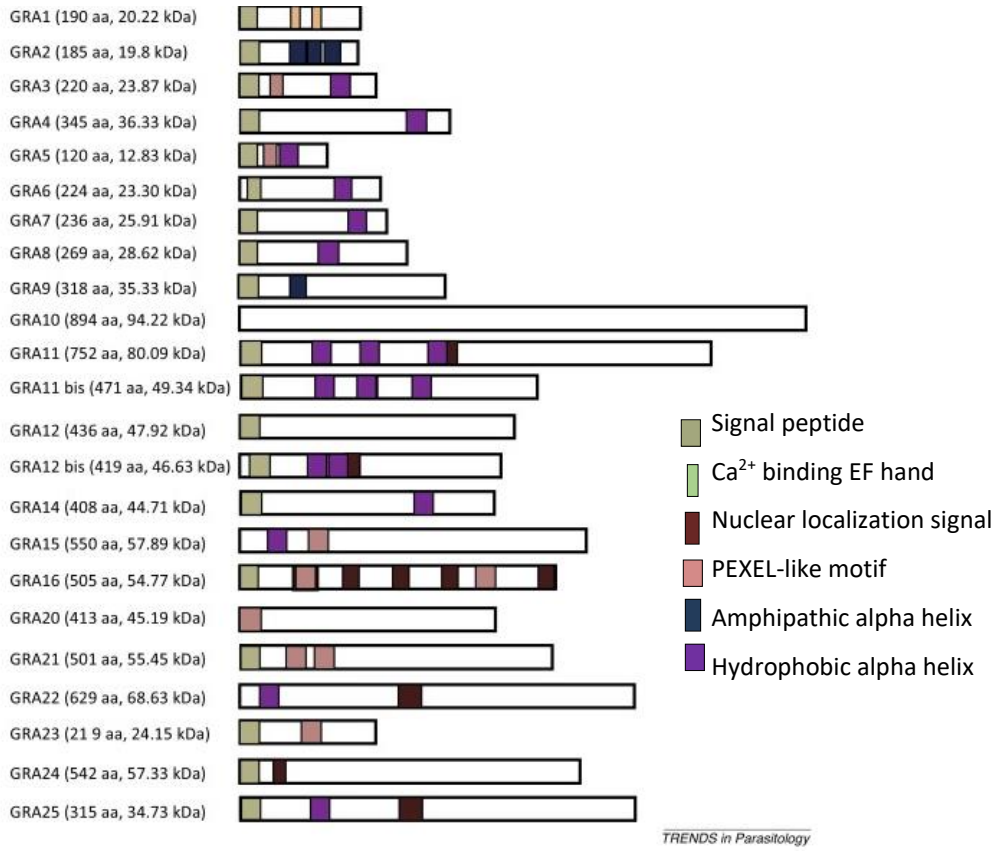



**Figure 15: Schematic representation of dense granule proteins (GRAs).** Adapted from (Mercier and Cesbron-Delauw, 2015). List of identified GRA proteins and associated motifs identified in the type II ME49 strain.

The majority of DG proteins (except for *Tg*GRA20) have an N-terminal signal peptide cleaved very early during transport through the ER and show very little or no homology to each other or to known proteins. The newly synthesized GRAs traffic from the Golgi apparatus to the parasite periphery via a mechanism dependent on *Tg*MyoF motor, a vesicular cargo transporter moving along the actin filaments (Heaslip et al., 2016). GRA proteins display

different important roles for the parasite, including maintaining the structure and integrity of the PV (Masatani et al., 2013) in particular by forming the intravacuolar network (IVN) (Mercier et al., 2002), importing nutrients from the host cell (Gold et al., 2015), and modulating the host immune response (Bougdour et al., 2013; Braun et al., 2013; Gay et al., 2016; Rosowski et al., 2011). The functions of the various characterized GRA proteins are detailed below:

*Tg*GRA2 and *Tg*GRA6 are essential for the formation of tubular membranes constituting the IVN and maintaining its structure to ensure notably the synchronicity of the parasite successive divisions (Mercier et al., 2002; Travier et al., 2008). The IVN also facilitates nutrient exchange with the host cell (Caffaro and Boothroyd, 2011). *Tg*GRA7 induces the formation of H.O.S.T (Host Organelle Sequestring Tubulostructures) structures which stimulate the internalization of host cell endolysosomes into the PV to promote the import of cholesterol, a lipid essential for parasite growth (Coppens et al., 2006). In addition, *Tg*GRA7 recruits and associates with *Tg*ROP5/*Tg*ROP18 complex to promote the phosphorylation of the IRG protein, Irga-6 (Hermanns et al., 2016). It was also suggested that the interaction of *Tg*GRA3 and *Tg*GRA5, inserted into the PVM, with CAMLG (CAlcium Modulating LiGand) could stimulate host RE recruitment to the PV, although this hypothesis has not been yet demonstrated (Ahn et al., 2006; Kim et al., 2008). Besides, *Tg*GRA3 interacts with the host Golgi apparatus and induces the formation of tubules promoting the internalization of host Golgi fragments into the PV, thereby dysregulating anterograde transport of the host cell (Deffieu et al., 2019). Likewise, *Tg*MAF1 (Mitochondrial Associating Factor 1) stimulates the recruitment of host mitochondria to the PV, indirectly modulating the cytokinic profile of the infected cell (Pernas et al., 2014).

Some GRAs can modulate positively or negatively key host signaling pathways involved in parasite persistence. This is the case for the proteins *Tg*GRA6 and *Tg*GRA15. In the type I strain, *Tg*GRA6 can interact with CAMLG and activate the transcription factor NFAT4 (Nuclear Factor of Activated T cell 4) which induces, among other things, the synthesis of the chemokines Cxcl2 and Ccl2 (Ma et al., 2014) (Figure 16). These two molecules activates the recruitment of immune cells which will then be diverted by the parasite for its dissemination (Coombes et al., 2013; Courret et al., 2006). In the type II strain, *Tg*GRA15, present at the PVM, is capable of interacting with TRAF factors (TNF Receptor-Associated Factors), such as

TRAF6, to induce the activation and nuclear translocation of the transcription factor NF-κB (Nuclear Factor Kappa B), thereby activating the production of IL-12 by macrophages (Jensen et al., 2011; Rosowski et al., 2011; Sangaré et al., 2019) (Figure 16).

On the other hand, *Tg*GRA16, *Tg*GRA24, and *Tg*IST (Inhibitor of STAT1 transcriptional activity) are exported to the host nucleus. *Tg*GRA16 forms a complex with host enzymes PP2A-B55 (Protein Phosphatase 2A) and HAUSP (Herpes Virus-Associated Ubiquitin Specific Protease) to modulate the expression of genes associated with the p53 tumor suppressor pathway and therefore cell cycle-associated genes (Bougdour et al., 2013; Hakimi and Bougdour, 2015) (Figure 16). On the other hand, *Tg*GRA24 promotes the autophosphorylation of the p38α MAPK (Mitogen-Activated Protein Kinases) protein, which activates a pro-inflammatory response mediated by IL-12 (Braun et al., 2013) (Figure 16). Finally, *Tg*IST negatively regulates the transcription of STAT1 dependent genes thereby protecting the tachyzoites from clearance by IRGs during the infection of a naïve cell (Gay et al., 2016) (Figure 16).

Moreover, the injection of tachyzoites deleted for different GRA proteins, such as *Tg*GRA2, *Tg*GRA3, *Tg*GRA4, *Tg*GRA6, *Tg*GRA7, *Tg*GRA8, *Tg*GRA9, *Tg*GRA12 or *Tg*GRA14, strongly inhibits the formation of cysts in infected mice, indicating a role of GRA proteins in cysts formation and the establishment of chronic infection (Fox et al., 2019, 2011).

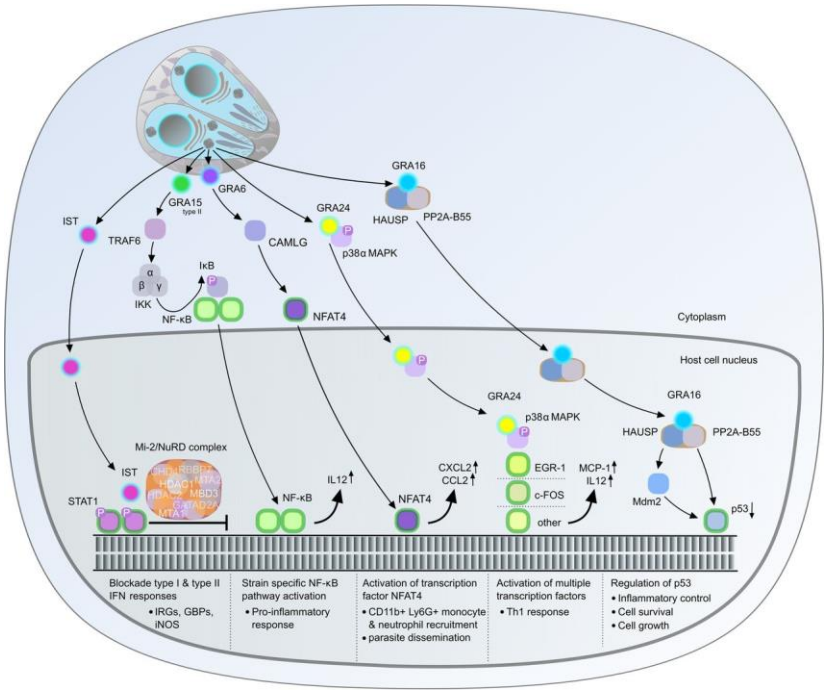



**Figure 16: GRA proteins role in the modulation of host cell activity.** Reprinted from (Hakimi et al., 2017). The GRA proteins are either localized at the PVM (*Tg*GRA15) or reside at the PV while

interacting with the cytosol of the host cell (*Tg*GRA6), or else are translocated in the host cell nucleus (*Tg*IST, *Tg*GRA24, and *Tg*GRA16). *Tg*IST inhibits the IFN responses by recruiting the Mi-2/NuRD complex repressing the binding of STAT1 to the associated promoters. *Tg*GRA24 forms a complex with p38α MAPK activating transcription factors, such as EGR-1 and c-FOS, to induce a Th1 response. *Tg*GRA16 forms a complex with HAUSP and PP2A-B55 to downregulate p53. In type II parasitic strains, *Tg*GRA15 activates TRAF6, which induces the activation of the NF-κB pathway responsible for the induction of a pro-inflammatory response. In type I parasitic strains, *Tg*GRA6 interacts with CAMLG to stimulate the transcription factor NFAT4, which induces the secretion of chemokines, such as CXCL2 and CCL2, stimulating the recruitment of immune cells at the site of the infection.

GRA protein must translocate through the PVM before being transported to the cytoplasm of the host cell. Recently, some of the processes allowing the export of these proteins and the exchange of nutrients have been elucidated (Figure 17):

- *Tg*ASP5, an Aspartyl protease located in the Golgi apparatus of the parasite, cleaves proteins containing an HT/TEXEL motif (Toxoplasma EXport Element), such as *Tg*GRA6, *Tg*GRA7, *Tg*GRA15, *Tg*GRA16, and *Tg*IST, inducing their maturation and export (Coffey et al., 2015; Curt-Varesano et al., 2016; Hsiao et al., 2013).
- The protein MYR1 (MYc Regulation 1), located at the PVM, is a translocon ensuring the export of a large part of GRA proteins (*Tg*GRA16, *Tg*GRA24, *Tg*IST, and *Tg*HCE1 (Host Cyclin E1)), thus modulating the host cell activity during the infection (Franco et al., 2016; Naor et al., 2018; Panas et al., 2019). The proteins MYR2 and MYR3 are secreted into the PV space and colocalize with MYR1 at the PVM. They are also essential for *Tg*GRA16 and *Tg*GRA24 translocation (Marino et al., 2018).
- The complex *Tg*GRA17/*Tg*GRA23, also located at the PVM, allows the passive transport of small molecules, such as nutrients, between the host cell and the PV (Gold et al., 2015).

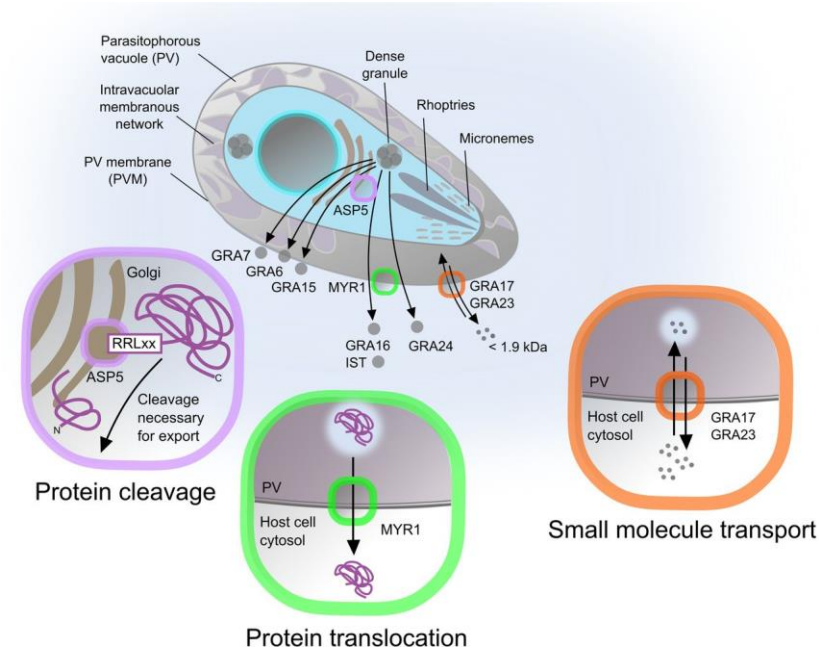



**Figure 17: Schematic representation of the export and the trafficking mechanisms of GRA proteins through the PVM.** Adapted from (Hakimi et al., 2017). The aspartyl protease *Tg*ASP5 cleaves the proteins *Tg*GRA6, *Tg*GRA7, *Tg*GRA15, *Tg*GRA16, and *Tg*IST, in the Golgi apparatus, to allow their export. *Tg*MYR1, located at PVM, allows the export of *Tg*GRA16, *Tg*GRA24, and *Tg*IST through the PVM to the host cytosol. *Tg*GRA17 and *Tg*GRA23, located at the PVM, are responsible for the transport of small molecules between the PV and the host cytosol.

# 5 *Toxoplasma gondii* lytic cycle:

The lytic cycle of the parasite is a stepwise process where the tachyzoites use their gliding machinery to locate a suitable host cell. The parasites then attach, re-orientate, and invade the host cell. Once inside, the parasites reside and replicate by endodyogeny within the PV. Finally, after lysing respectively the vacuole and the host cell, the parasites egress out to re-infect a neighboring host cell. The parasite is apt to accomplish these activities using a multitude of parasite effectors (Figure 18).

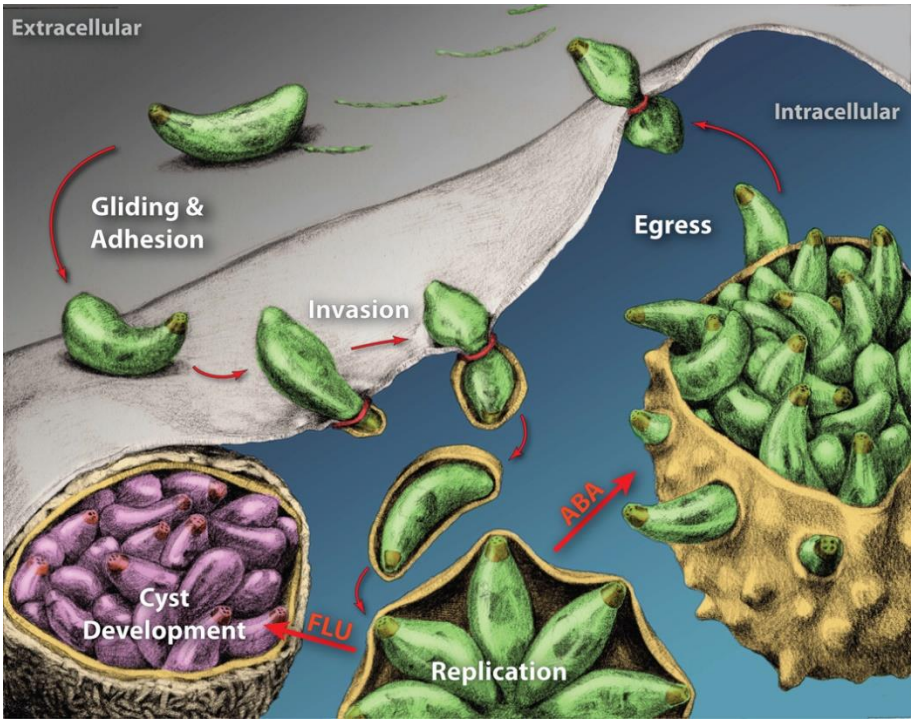



**Figure 18: *Toxoplasma gondii* lytic cycle.** Adapted from (Billker et al., 2009)**.** The extracellular tachyzoite uses gliding motility to move on the cell surface and adhere to the membrane of the host cell after MIC protein secretion. The invasion stage is mediated by the formation of the moving junction (MJ) (red ring) inducing the entry of the parasite into the host cell. This step is accompanied by the formation of a protective structure, the PV, in which the parasite is able to replicate. This replication leads to the accumulation of abscissic acid (ABA) triggering the exit of the parasites and the lysis of the infected cell. On the other hand, blocking the production of ABA by fluridone (FLu) prevents the egress of the parasites and induces cyst formation.

## 5.1 Gliding motility and adhesion

Gliding motility is a critical process for the parasite dissemination and invasion of the host cell. This process can be divided into several steps including the secretion of MIC adhesins and their insertion into the parasite membrane, the anchoring of these adhesins to actin filaments allowing their translocation via the actomyosin motors, and the disassembly of MICs/cellular receptor complex at the basal pole (Blader et al., 2015).

### 5.1.1 Microneme secretion and parasite adhesion

The first contact between the host cell and the parasite is mediated by adhesins belonging to the family of SAGs (Surface Antigen Glycoproteins) attached to the parasite surface. SAG proteins recognize and bind to cellular receptors of the sulfated proteoglycan type, thus allowing parasite attachment to the host cell (Carruthers and Boothroyd, 2007; He et al., 2002). Following this contact, the parasite secretes the MIC proteins, which once inserted into

the plasma membrane; recognize carbohydrates on the host cell surface. The first burst of microneme protein secretion is mediated by the increase of intracellular calcium levels (Lovett et al., 2002) in intracellular parasites. Indeed, a peak of intracellular $Ca^{2+}$ level was observed during parasite invasion (Arrizabalaga and Boothroyd, 2004) and before egress (Moudy et al., 2001; Withers-Martinez et al., 2014). This increase in the level of intracellular calcium is induced by several stimuli (Wetzel et al., 2004). Actually, $Ca^{2+}$ level increases during intracellular replication and contributes to the egress of the parasite. It involves the sensing of an increase in phosphatidic acid (PA) generated by a diacylglycerol (DAG) kinase 2 (TgDGK2) secreted into the parasitophorous vacuole (Bisio et al., 2019). Soon after parasite initial contact with the host cell, the level of extracellular potassium increases, and phosphatidylinositol-4,5-biphosphate (PI-(4,5)-P2), which is a substrate of phosphoinositide phospholipase C (PI-PLC), is formed. PI-PLC generates the secondary messengers, DAG and inositol triphosphate (IP3), which all trigger MIC protein secretion (Bullen et al., 2016). More specifically, DAG is converted into PA, which is known to be involved in various cellular processes such as signal transduction or exocytosis in mammals (Chasserot-Golaz et al., 2010). In fact, the study of Bullen et al (Bullen et al., 2016) showed a predominant role of *Tg*DGK1 (diacylglycerol kinase-1) in MIC secretion via the presence of PA. Moreover, IP3 formation stimulates the release of calcium leading to the activation of calcium-dependent protein kinases (CDPKs) which trigger the fusion of microneme organelles with the parasite plasma membrane and thereby the secretion of MIC proteins. This membrane fusion would be initiated by the protein *Tg*APH (acetylated-pleckstrin-homology domain-containing protein) which can bind the PA present at the parasite membrane. Furthermore, the major roles of *Tg*APH and *Tg*CDPK1 proteins in MIC secretion have been identified by knock-down (KD) and knock-out (KO) strategies respectively (Bullen et al., 2016; Lourido et al., 2010).

After secretion, MIC proteins are found at the apical pole after proteolytic cleavage by ROM proteins to allow, with the glideosome, the reorientation of the parasite necessary for the invasion (Frenal and Soldati-Favre, 2013; Rugarabamu et al., 2015). Among the characterized *Tg*ROMs, *Tg*ROM4, localized at the plasma membrane, is required for the cleavage of TgMIC2 (Shen et al., 2014b). The absence of *Tg*ROM4 induces an accumulation of *Tg*MIC2 over the entire surface of the parasite impairing the establishment of a MIC protein gradient at the apical pole necessary for the reorientation of the parasite and therefore host invasion (Shen

et al., 2014b). Several phenotypical characterization experiments have been carried out on different MIC protein depleted strains to decipher their functions. The majority of the MIC examined (*Tg*MIC1, *Tg*MIC2, *Tg*M2AP, *Tg*MIC3, *Tg*MIC4, *Tg*MIC5, *Tg*MIC6, and *Tg*SUB1) are not essential for parasite survival in-vitro. However, the absence of the proteins *Tg*MIC1, *Tg*MIC2 or *Tg*M2AP negatively impact parasite adhesion to host cells (Cérède et al., 2005; Gras et al., 2017; Harper et al., 2006). In vivo virulence experiments in mice carried out for the proteins *Tg*MIC1 and *Tg*MIC3 showed that the absence of *Tg*MIC1 or *Tg*MIC3 mildly affects parasite survival. However, a double KO mutant for both proteins strongly attenuates the virulence of the parasite, revealing a synergistic effect (Cérède et al., 2005).

### 5.1.2 The glideosome

The gliding motility mechanism is well preserved in Apicomplexa and involves a multiprotein complex called glideosome, dependent on the actomyosin motor located between the IMC and the parasite's membrane.

The actomyosin complex allows the parasite to move across a 2D substrate (Håkansson et al., 1999) and through 3D matrices (Leung et al., 2014). When parasites move over 2D substrates, they display three distinct forms of displacement: circular, helical, and twirling. Circular gliding is when parasites move across the substrate in a circular motion at average speeds of 1.5µm/s. They may also exhibit a helical motion, during which they project forward about one body length over the substratum in a biphasic flip along the longitudinal axis of the parasites. Finally, the parasites can move using a twirling motion, where they appear to spin clockwise while balancing on their basal end (Håkansson et al., 1999). During these three types of motions, the parasites shed their surface membrane, leaving behind a trail of surface antigens (SAG1) along with a variety of other proteins. However, in an extracellular 3D gel matrix, the parasites move exclusively in a spiral corkscrew-like manner (Leung et al., 2014).

The glideosome allows linking the parasite cytoskeleton via anchoring the GAPs to the IMC, and MIC adhesins to the parasite surface. This structure is composed of the protein Myosin A (*Tg*MyoA), the main component of the actomyosin motor, of the proteins *Tg*MLC1 (Myosin Light Chain 1), *Tg*ELC1 and *Tg*ELC2 (Essential Light Chain 1 and 2) regulating the motor activity (Herm-Götz et al., 2002), and the proteins *Tg*GAP40, *Tg*GAP45, and *Tg*GAP50 (Frénal et al., 2010; Gaskins et al., 2004). The motility of the parasite results from the displacement of *Tg*MyoA on the polymerized actin filaments. *Tg*MLC1 allows the anchoring of *Tg*MyoA at the

IMC level through its interaction with *Tg*GAP45 which acts as a junction between the IMC and the parasite membrane (Frénal et al., 2010). In *T. gondii*, there are two functional protein homologs of the protein *Tg*GAP45. At the apical pole, *Tg*GAP70 also interacts with *Tg*MyoA and *Tg*GAP80 present at the basal pole recruits the myosin *Tg*MyoC (Frénal et al., 2014) (Figure 19). Previously, the connection between *Tg*MIC2 and the actomyosin complex was attributed to the aldolase enzyme (*Tg*ALD) due to its ability to bind both the cytoplasmic tail of adhesins (here *Tg*MIC2) and actin. However, a recent study has shown that in the absence of *Tg*ALD, the parasite retains its capacity for motility and invasion (Shen and Sibley, 2014). The role of connector would rather be attributed to the protein *Tg*GAC (Glideosome Associated Connector) capable of binding actin, *Tg*MIC2, and PA (Jacot et al., 2016) (Figure 19).

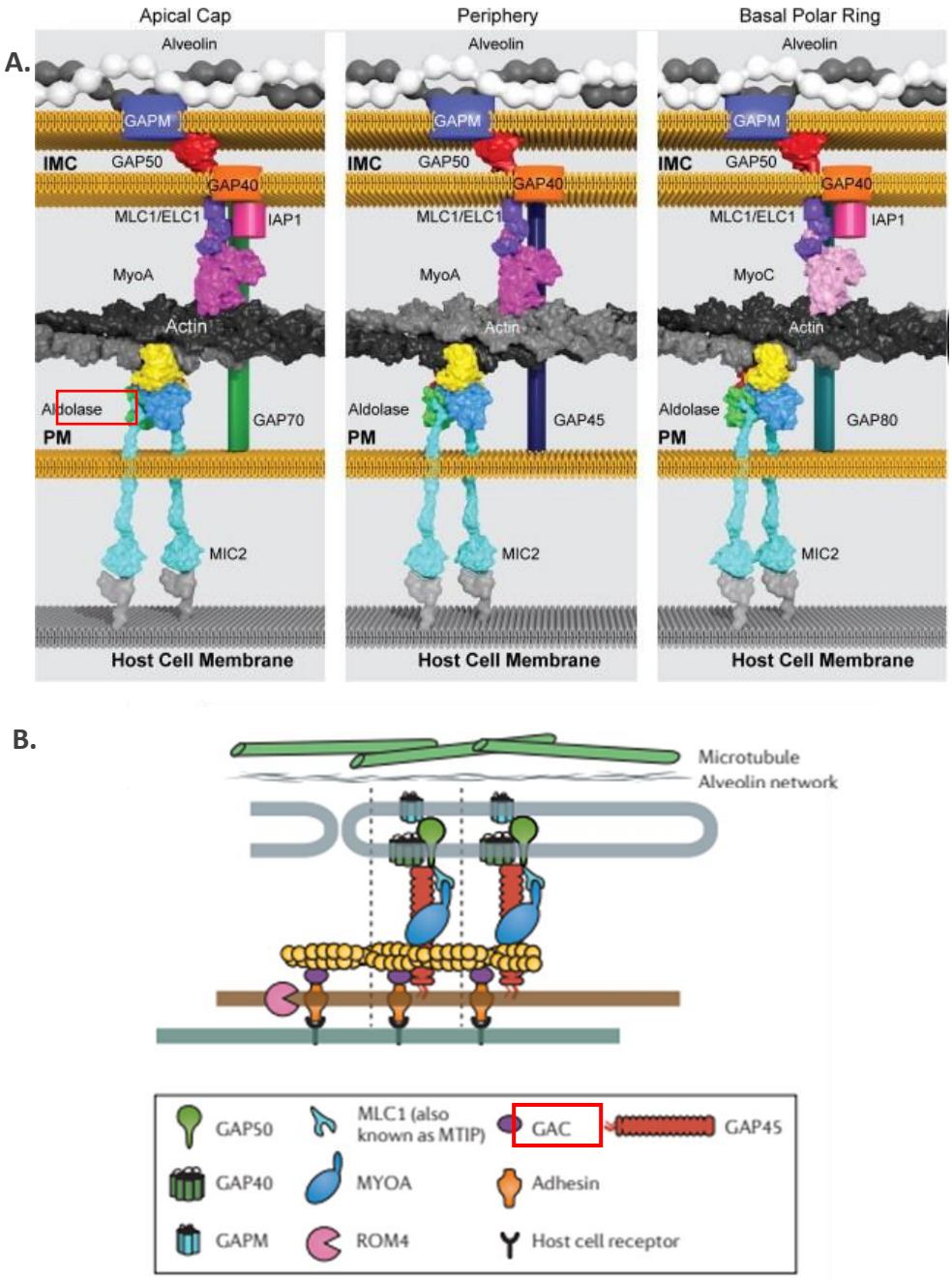



**Figure 19: Schematic representation of the glideosome. A.** Illustration of the different components of the glideosome at the apical pole (left), at the periphery (middle), and at the basal pole (right), Adapted from (Boucher and Bosch, 2015). **B.** New representation of the glideosome replacing the *Tg*ALD protein by *Tg*GAC protein as an actin connector with the adhesion *Tg*MIC2. Reprinted from *(Frénal et al., 2017a)*.

### 5.1.3 Actin dynamics

Unlike *Plasmodium spp*. which encode two isoforms of actin (ACTI and ACTII), *T. gondii* encodes a single actin gene, termed ACT1 (Janice M. Dobrowolski et al., 1997). In comparison to actin in other eukaryotic cells, *Toxoplasma* actin was found predominantly in the

monomeric globular state (~97 %), with almost no F-actin detected (Janice M. Dobrowolski et al., 1997). Actin is predominantly distributed across the conoid, and as short dynamic filaments in the glideosome anchored at the IMC (Yasuda et al., 1988). F-actin was predominantly seen in regions where the parasite is in contact with the plasma membrane (Skillman et al., 2013). To date, actin filaments in *T. gondii* have only been detected by electron microscopy (Janice M. Dobrowolski et al., 1997; Schatten et al., 2003; Shaw and Tilney, 1999). Reasons for this could be, that they form very short, unstable filaments of approximately 100nm in length, and their cytoplasmic concentration is very low, within the range of 8-10 µM (Sahoo et al., 2006), suggesting an evolutionary adaptation to control processes such as gliding motility. Recent studies revealed new biological roles for *Tg*Act1. For instance, it has been demonstrated that the directed DG transport is dependent on actin and *Tg*MyoF (Heaslip et al., 2016). More recently, using a new approach for F-actin imaging in parasites based on the expression of actin chromobodies (Cb), Periz el al have uncovered a role of a dynamic F-actin network in the recycling of MIC proteins from the mother to the forming daughter parasites, with the residual body acting as a storage and sorting hub for recycling and material exchange between cells (Periz et al., 2019).

No homologs of F-actin nucleating proteins, such as Arp2/3 complex that regulates actin polymerization in eukaryotic cells, have been identified within apicomplexan genome (Gordon and Sibley, 2005). However, as the parasite is thought to maintain predominantly a Globular-actin (G-actin) state, there must be many actin-binding proteins regulating its dynamics to sequester the monomers so they cannot form filaments. Indeed, *T. gondii* contains a strong actin depolymerization factor (ADF) and profilin (PRF), which may act to sequester the actin monomers (Mehta and Sibley, 2010; SKILLMAN et al., 2012).

Moreover, treatment of parasites with factors that alter actin dynamics affects many processes throughout the lifecycle, especially gliding motility and invasion. In particular, depolymerization of F-actin, through the use of Cytochalasin D or latrunculin B, affects both gliding motility and invasion (Dobrowolski and Sibley, 1996; Wetzel et al., 2003). Artificially polymerizing actin with jasplakinolide interferes with proper parasite motility and invasion (Poupel and Tardieux, 1999). This leads to the assumption that controlled polymerization of F-actin is essential for efficient motility and invasion. However, the characterization of a conditional ACT1 KO indicated that ACT1 is important but not essential for motility or

invasion. The ACT1 KO also highlighted a role for actin in tachyzoite morphology, apicoplast division, and egress (Egarter et al., 2014).

## 5.2 Invasion

Motility and invasion are both tightly controlled and require the sequential secretion of proteins contained in micronemes and rhoptries (Carruthers and Boothroyd, 2007). The invasion is a rapid process occurring in a few seconds (around 30 seconds), highly conserved among apicomplexan parasites. It involves finding and invading a suitable host cell that is thought to be driven actively by the parasite gliding machinery (Dobrowolski and Sibley, 1996). The parasites discharge their MIC proteins from their apical end to attach firmly to host cell receptors once the host cell is located (Carruthers and Tomley, 2008; Dowse and Soldati, 2004). Following this firm apical attachment, the parasites reorientate their apical pole and discharge, in a regulated manner, their second set of specialized secretory organelles, known as rhoptries into the host cytosol. Firstly, the RONs are discharged from the neck region into the host cytosol and return to the surface to form the MJ, an adhesive structure linking the parasite and the host cell membrane (Bichet et al., 2014; Lamarque et al., 2011). After MJ formation, the parasites sequentially secrete the ROP proteins to begin the formation of the PV made from the invagination of the host plasma membrane (Suss-Toby et al., 1996). The third set of secretory proteins, called dense granules, are constitutively secreted and play a role in the formation and continual modulation of the PV structure as well as the modulation of the host cell responses during parasite replication. At the end of the invasion, the tachyzoite resides within the PV and the PVM is closed (Mercier et al., 2005). (Figure 20)

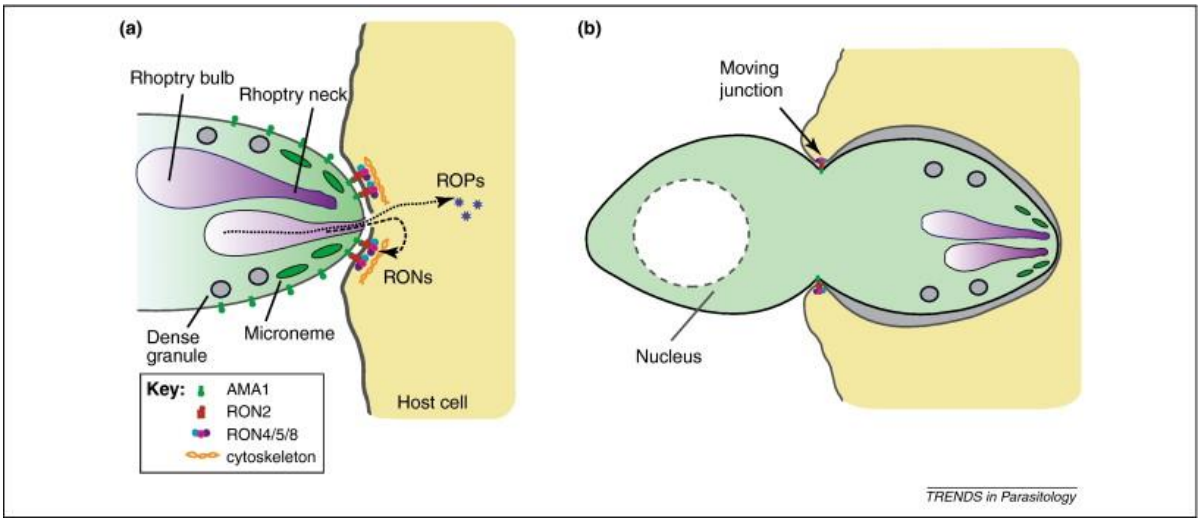



**Figure 20: Schematic representation of *Toxoplasma gondii* invasion process.** Adapted from (Tyler et al., 2011). After its reorientation, the parasite interacts via the protein *Tg*AMA1, anchored at its plasma membrane, with the RONs complex (*Tg*RON2/*Tg*RON4/*Tg*RON5/*Tg*RON5), secreted in the cytoplasm of the host cell, thus forming the MJ. The driving force provided by the glideosome allows the entry of the parasite which is accompanied by the formation of the PV, a protective structure necessary for the parasite's survival in the host cell.

### 5.2.1 *Tg*AMA1 and *Tg*RON complex to form the MJ

As mentioned earlier, the multistep invasion process involves the formation of a transient structure, the moving junction (MJ). The MJ results from the interaction of a RON protein complex with the microneme protein TgAMA1.

More precisely, *Tg*AMA1, which plays a central role in the invasion (Mital et al., 2005), is anchored at the parasite plasma membrane (Donahue et al., 2000; Hehl et al., 2000) and interacts with the RON proteins complex via *Tg*RON2, inserted into the membrane of the host cell. *Tg*AMA1 has long been considered essential for parasite survival since no inducible mutant could be obtained (Mital et al., 2005). However, the development of genetic tools in *T. gondii* made it possible to generate a KO strain for the *Tg*AMA1 gene (Bargieri et al., 2013). Using this mutant, it has been demonstrated that the RON proteins always localize at the MJ, even in the absence of *Tg*AMA1. This result would, therefore, indicate that *Tg*AMA1 has a role in the attachment stage preceding the invasion and not a role in the formation of the MJ which must require the intervention of another protein. Subsequently, Lamarque et al (Lamarque et al., 2014) showed that in the absence of *Tg*AMA1, the parasite can adapt by overexpressing proteins homologous to *Tg*AMA1 and *Tg*RON2. Thus, the parasite expresses *Tg*AMA2 protein capable of interacting with *Tg*RON2 to allow parasite's invasion. The double

KO of both *Tg*AMA2 and *Tg*AMA1 genes led to a further reduction in the invasion, which however remained partly effective. This result, therefore, suggests the existence of another alternative route to the *Tg*AMA2/*Tg*RON2 pair and led to the finding of another *Tg*AMA1 homolog, *Tg*AMA4. *Tg*AMA4 can bind to the protein *Tg*RON2L1, a homolog of *Tg*RON2 expressed in the sporozoite stage, also expressed in the *Tg*AMA1 KO. Another *Tg*AMA/*Tg*RON complex exists (*Tg*AMA3/*Tg*RON2L1), but is specific for the sporozoite stage. In summary, all these compensation mechanisms highlight the key role of *Tg*AMA/*Tg*RON complexes in the entry process, but also the adaptive capacity of the parasite to ensure its entry into host cells and perpetuate its lytic cycle (Figure 21).

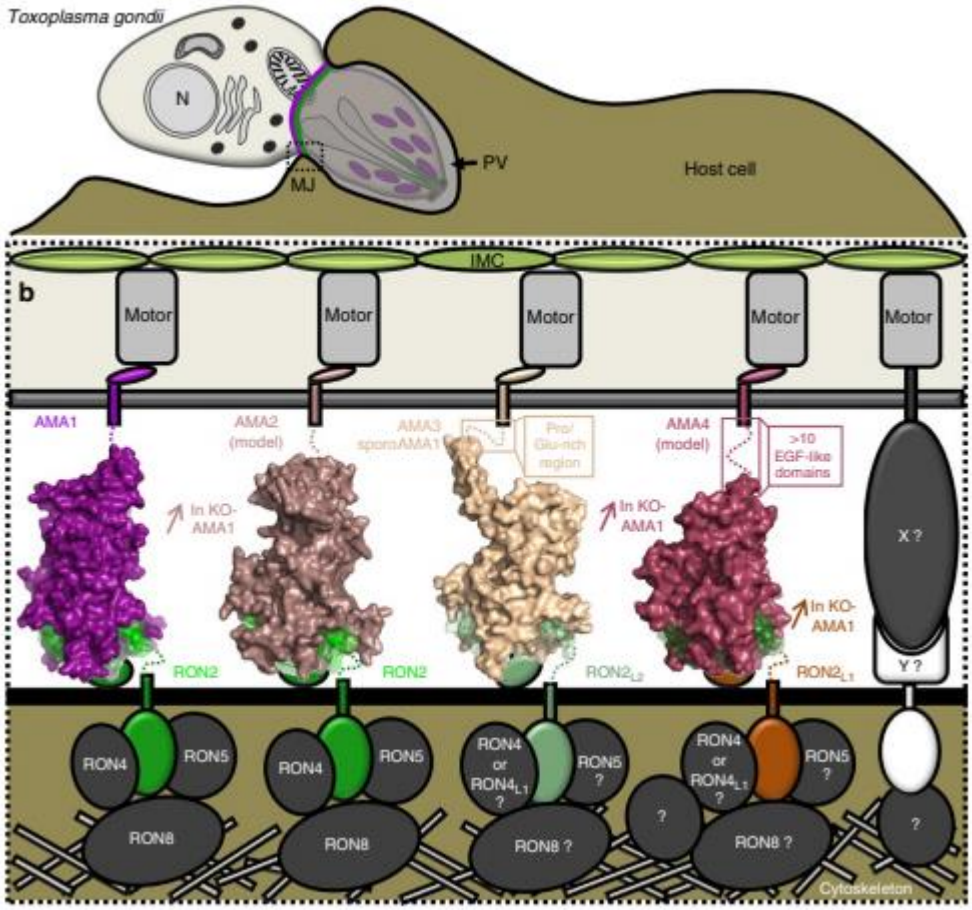



**Figure 21: Schematic representation of the different *Tg*AMA/*Tg*RON complexes forming the moving junction (MJ).** Adapted from (Lamarque et al., 2014). This figure illustrates (**a**) a tachyzoite partially invading the host cell and (**b**) the different proposed models of *T. gondii* MJ. Four different AMA/RON interactions, binding the parasite to the host interface, are illustrated here, with the cytoplasmic tail of AMA connecting the MJ to the gliding motor localized in the IMC. The RON complex, formed of RON4/RON5/RON8 tethered to RON2, localizes beneath the host plasma membrane where it may interact with the host cytoskeleton. The main invasion pathway used by *Toxoplasma* tachyzoites and *Plasmodium* merozoites is mediated by the AMA1/RON2 complex. Three additional pairs, homologs

to AMA1 and RON2, have been proposed. AMA3/RON2$_{L2}$ and AMA4/RON2$_{L2}$ are highly expressed during the sporozoite stage. AMA2/RON2 and AMA4/RON2$_{L1}$ demonstrate the molecular plasticity of the MJ by compensating the loss of AMA1. X and Y represent, yet unidentified, divergent components that might add to the architecture of the MJ.

Besides *Tg*RON2, the RON complex is composed of three soluble proteins secreted in the cytoplasm of the host cell (Alexander et al., 2005; Besteiro et al., 2009): *Tg*RON4 and *Tg*RON5 conserved in Apicomplexa (Shen and Sibley, 2012) and *Tg*RON8 found only in coccidia (Straub et al., 2009). A hypothesis put forward the role of *Tg*RON2 as a receptor, which could facilitate the active entry of the parasite into the host cell through the glideosome (Besteiro et al., 2009). Moreover, a recent study showed that the RONs complex diverts proteins from the host cell creating a physical link between the parasitic proteins and the host actin cytoskeleton (Guérin et al., 2017). This interaction would stabilize the MJ and create a cellular anchor and signaling platform promoting parasite invasion.

The role of *Tg*RON2 and *Tg*RON5 proteins in regulating invasion has been studied using an ATc-inducible strategy (Anhydro TetraCycline). In the case of the *Tg*RON2 mutant, the authors observed that its absence affects the expression and localization of *Tg*RON4 and *Tg*RON5 proteins (Lamarque et al., 2014). The study of the *Tg*RON5 mutant revealed its implication in the stabilization of *Tg*RON2 and the targeting of *Tg*RON4 from the bulb to the neck of the rhoptries (Beck et al., 2014). However, the absence of *Tg*RON2 and *Tg*RON5 does not seem to disturb the expression and the localization of *Tg*RON8. The presence of *Tg*RON8 at the MJ suggests that other proteins may be associated with this protein to form the MJ. In addition, *Tg*RON8 seems to be nonessential since a KO strain of the protein was obtained. However, it seems that the MJ is less stable in its absence although the other RONs forming the complex are properly addressed (Straub et al., 2011).

Recently, the laboratory of Maryse Lebrun has described a small family of rhoptry apical surface proteins (RASPs) that cap the extremity of rhoptries and play a role in their discharge. Indeed, depletion of RASP2, in both *T. gondii and P. falciparum*, ablates rhoptry secretion leading to a severe block in invasion and therefore intracellular proliferation of the parasite. Interestingly, RASP2 contains a C2 lipid binding domain and a PH-like domain that bind to phosphatidic acid (PA) and phosphatidyl-inositol 4,5 biphosphate (PIP2) upon MIC exocytosis. Their interaction mediate the attachment between the rhoptry and the PM allowing the

recruitment of hypothetical membrane fusion machinery such as SNARE proteins (Suarez et al., 2019).

### 5.2.2 ROP and GRA proteins implication in PV formation

The PV is a protective structure in which the parasite develops inside the host cell while escaping the immune system. The formation of the PV results from the invagination of the host cell membrane shortly after the MJ formation (Suss-Toby et al., 1996). Once formed, the PV membrane (PVM) undergoes several modifications which allow the exclusion of host transmembrane and lipid raft proteins except for GPi anchor proteins (Charron and Sibley, 2004; Mordue et al., 1999). All these changes allow the PVM to escape the endosomal pathway and thus protect it from lysosomal degradation providing a safe environment for the parasite to proliferate (Mordue et al., 1999).

The PV also consists of ROP proteins previously secreted in small vesicles called e-vacuoles in the cytoplasm of the host cell (Håkansson et al., 2001). Among the ROPs secreted, some of them have proven to be crucial for the in-vivo virulence in mice by modulating the host cell immune responses. GRAs secretion is carried out in two stages, firstly, a discharge just after the invasion in the lumen of the nascent vacuole, then a continuous secretion during the development of the parasite within the PV (Carruthers and Sibley, 1997). As mentioned before, these GRA proteins are found associated with several structures such as the PVM, the IVN (intravacuolar network) allowing the connection of the parasite to the PVM (Masatani et al., 2013), and an organelle sequestration structure “HOST” (such as GRA7) involved in the delivery of endolysosomes from the host cell to the PV (Coppens et al., 2006). Moreover, a sub-membrane network is set up thanks to GRA proteins (Lemgruber et al., 2008; Magno et al., 2005a). Thus, the PV will be able to recruit certain host cell organelles such as the ER (Magno et al., 2005b) and the mitochondria (Magno et al., 2005a; Pernas et al., 2014) and constitutes a physical barrier for the passage of host components to the parasite (Gold et al., 2015).

## 5.3 Cell cycle and intracellular replication

Once inside the host cell, the parasite resides within the PV, and then initiates its replication using a unique mechanism called endodyogeny. This mechanism is based on the formation of

two daughter cells within a mother cell, which is degraded at the end of the division process (Hu et al., 2002a) (Figure 22A).

### 5.3.1 Atypical cell cycle

*T. gondii*, similar to other Apicomplexa, has a modified cell cycle compared to higher eukaryotes. This atypical cell cycle consists of phases G1, S, and M, but lacks a G2 phase (Striepen et al., 2007). The parasite has a haploid genome (1N) which is replicated during the S phase of the cycle. During this phase of replication, the parasite pauses when its DNA content reaches around 1.8N marking the beginning of mitotic spindles formation and cytokinesis (Blader et al., 2015), although replication is not fully complete. Akin to mammals, cell division seems to be coordinated by the cyclins/cyclin-dependent kinases (CDKs) control system (Gubbels et al., 2008; Kvaal et al., 2002). However, no information on which cyclin/CDK complex governs a certain phase of the cycle was known. Recently, after the functional characterization of CrKs (cyclin related kinases), the cyclin/CrK complexes were associated with a precise phase of the cycle. Indeed, among the seven atypical P, H, L, and Y-type cyclins, as well as ten CrKs, five of the latter have been shown to play a role in the process of cell division (Alvarez and Suvorova, 2017). Thus, *Tg*CrK1/*Tg*CycL complex are essential for daughter cell formation. *Tg*CrK2 interacting with *Tg*PHO80 (P-type cyclin) would avoid cycle arrest in the G1 phase, *Tg*CrK5 would regulate an S phase checkpoint, while *Tg*CrK4 and *Tg*CrK6 non-interacting with cyclins, would be necessary for the duplication of the centrosome and the function of mitotic spindles respectively (Alvarez and Suvorova, 2017) (Figure 22B). Moreover, a cascade of coordinated mRNA expression progressing through the cell cycle have been revealed, and the mRNA expressed in tachyzoite stages are mostly cell cycle-regulated (Behnke et al., 2010). Tachyzoites mRNA transcripts appear just before the requirement of the encoding proteins, following a sequential expression. There are two major groups of cell cycle-regulated subtranscriptomes, one in G1 and a second in S/M. The G1 subtranscriptome contains genes regulating biosynthetic and metabolic functions, whereas the S/M transcriptome contains genes unique to the biological adaptations of Apicomplexa, such as parasite maturation, specialized organelles development, and daughter cells egress (Behnke et al., 2010).

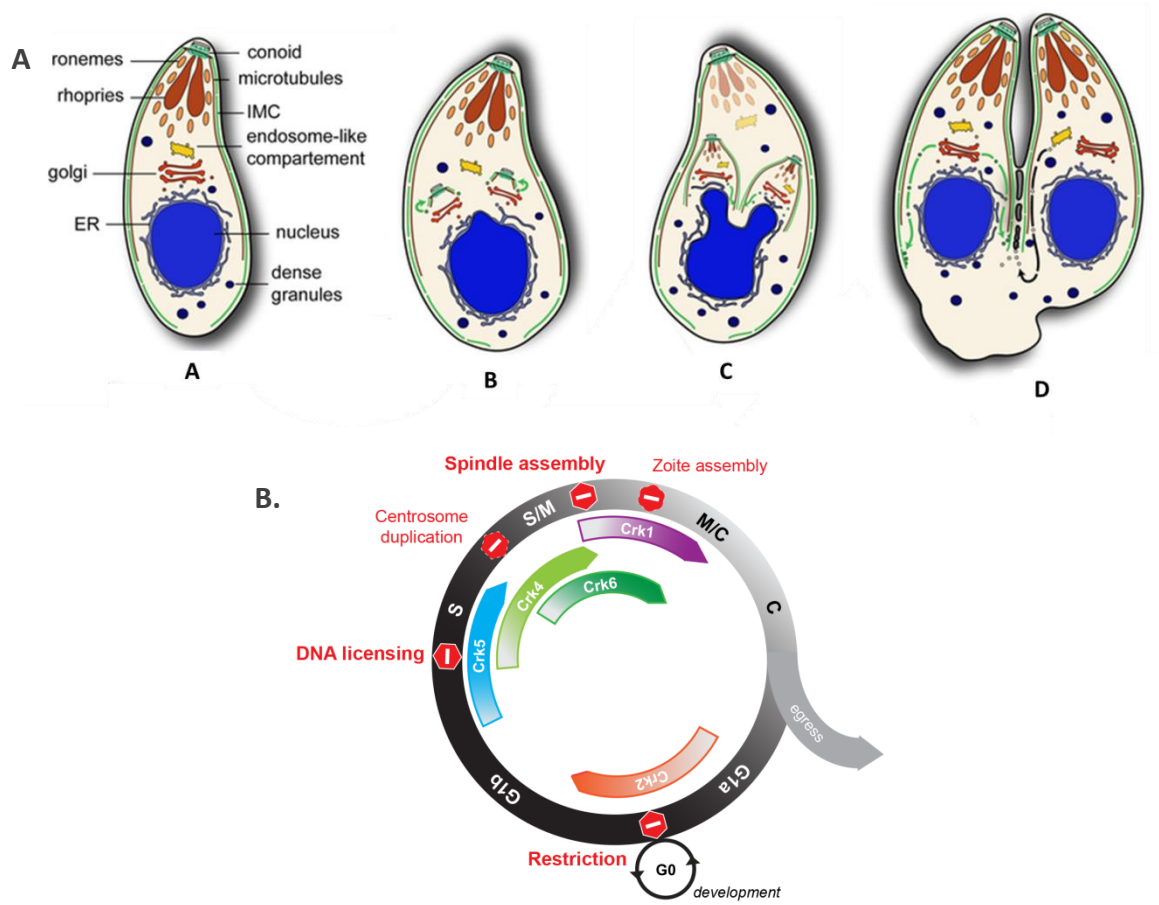



**Figure 22: *Toxoplasma gondii* parasite replication and cell cycle. A.** Schematic representation of the parasite's replication by endodyogeny, adapted from (Agop-Nersesian et al., 2010). **B**. Representation of checkpoints during the cell cycle in *T. gondii*, adapted from (Alvarez and Suvorova, 2017). The checkpoint at G1 is regulated by the protein *Tg*Crk2 which forms a complex with *Tg*PHO80 (orange arrow). The transition from the G1 phase to the S phase is under the control of the protein *Tg*Crk5 (blue arrow) to allow the initiation of the replication, while *Tg*Crk6 is involved in regulating the formation of mitotic spindles during metaphase (dark green arrow). Two other checkpoints would be specific to Apicomplexa with the proteins *Tg*Crk4 (light green arrow) necessary for the maintenance of the stoichiometry of the centrosome and *Tg*Crk1 (purple arrow) controlling the formation of daughter cells.

### 5.3.2 Cellular division and daughter cell formation

During *T. gondii* cellular division, cytokinesis and daughter cell formation start before mitosis is completed (Francia and Striepen, 2014). The apical pole of the parasite is defined by the apical polar ring (APR), which acts as a microtubule organization center (MTOC). Besides, *T. gondii* has a centriole-based MTOC, called the centrosome. Several studies have agreed on the major role played by the centrosome during mitosis, karyokinesis, and cytokinesis. During the initial stages of daughter cell formation, the centrosome containing 2 centrioles is located

near the APR. All the other times, the centrosome is associated with the spindle pole plaques, embedded in the nuclear envelope. During the G1 phase, the centrosome becomes associated with the Golgi apparatus which divides by lateral extension (Hartmann et al., 2006; Morrissette and Sibley, 2002b; Nishi et al., 2008). Once the division of the Golgi apparatus is completed, the centrosome migrates to the basal end of the nucleus, where it divides (Hartmann et al., 2006). The scission and the functional role of the centrosome are coordinated by several kinases including *Tg*Nek1 (NIMA related kinase) (Chen and Gubbels, 2013), *Tg*Ark 1/2/3 (Aurora-related kinase) (Berry et al., 2016; Suvorova et al., 2015), *Tg*CDPK7 ($Ca^{2+}$ dependent kinase 7) (Morlon-Guyot et al., 2014), and *Tg*MAPK-L1 (Mitogen-activated protein kinase L1) (Suvorova et al., 2015). The centrosome possesses two regions with separate functions: the proximal region or inner core, which organizes karyokinesis, and the distal region or outer core, which helps to regulate the initiation and the assembly of daughter cells. The two regions remain spatially close throughout the cell cycle and duplicate independently: the duplication of the inner core follows that of the outer core (Suvorova et al., 2015) (Figure 23).

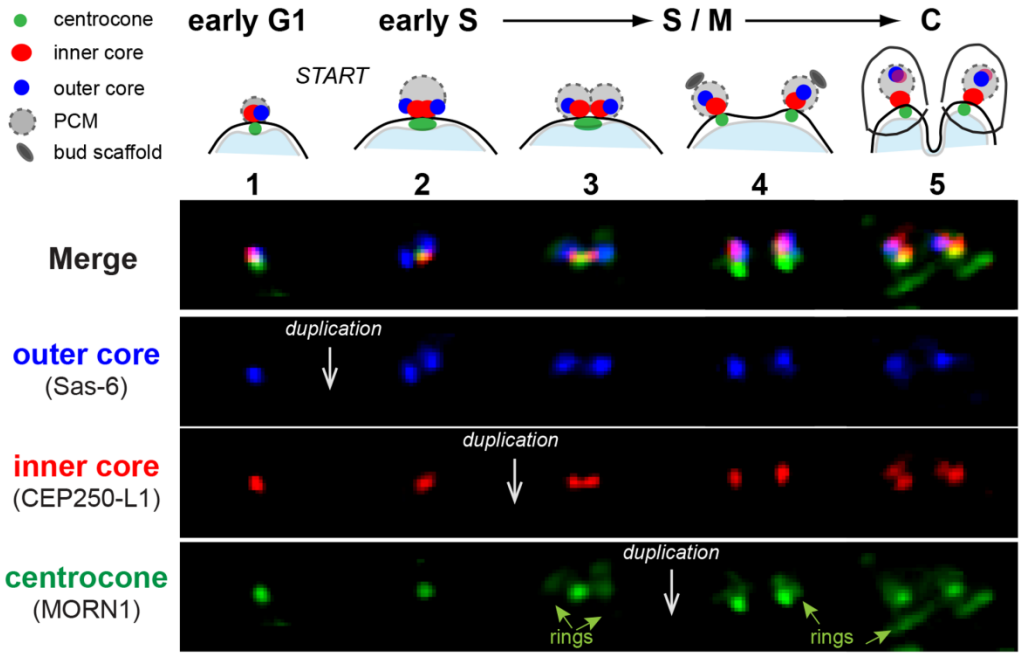



**Figure 23: Morphogenesis of the centrosome during the tachyzoite cell cycle.** Adapted from (Suvorova et al., 2015). Five morphological transitions are observed during the cell cycle between phases G1 and C. The duplication of the centrosome begins at the beginning of phase S by the duplication of the outer core (represented in blue using a *Tg*Sas-6 labeling) then the inner core shortly after (represented in red using a *Tg*CEP250-L1 labeling) to finish with the centrocone (MORN1 labeling in green).

Once divided, the centrosome returns to the apical pole marking the start of cytokinesis and the S phase (Hartmann et al., 2006). The assembly of daughter cells is initiated quickly after duplication of the centrosome, before the onset of mitosis, reflecting a particular mechanism of Apicomplexa. The phase S is marked by chromosomes duplication and segregation near the centrosome. The chromosomes are attached to the microtubule spindles via the kinetochore complex, assembled at the centromeres which are anchored to the centrosome by passing through the centrocone, a subcellular structure specific to Apicomplexa (Farrell and Gubbels, 2014) (Figure 24). Furthermore, the attachment of the chromosomes to the centrocone throughout the cell cycle would allow genome integrity to be maintained (Brooks et al., 2011).

The centrosome at the base of the conoid is physically linked to the budding cytoskeleton of the daughter cell by the SFA (Striated Fiber Assemblin) (Francia et al., 2012). The budding of daughter parasites is based on the formation of the cortical cytoskeleton and the intervention of the proteins *Tg*MORN1 and *Tg*ISPs. Indeed, *Tg*MORN1 forms a ring at the level of the apex and the basal pole and associates with the centrosome at the beginning of the division. As the division progresses, the rings, helped by the microtubule's polymerization, move towards the basal pole. Also, *Tg*MORN1 co-locates with the myosin proteins B and C and *Tg*Centrin2, the latter being able to play a role in the constriction process allowing the scission of daughter cells (Gubbels et al., 2006; Hu, 2008). The ISP proteins, in particular *Tg*ISP2, are essential to the development of the IMC derived from the Golgi apparatus of the mother cell (Beck et al., 2010). The IMC is assembled de novo during daughter IMC elongation within the mother cell, then followed by maternal IMC membranes recycling after the budding of daughters from the mother cell (Ouologuem and Roos, 2014). The machinery involved in IMC formation and recycling remains to be studied in detail; however, some key molecules are identified, for instance, two Rab11 isoforms: Rab11A and Rab11B. Rab11A associates with MLC1, a member of the glideosome, to regulate IMC formation and the delivery of a new PM to daughter cells in order to complete the cell division (Agop-Nersesian et al., 2009); while Rab11B is involved in the specific transport of vesicles derived from the Golgi to the immature IMC of growing daughter parasites (Agop-Nersesian et al., 2010).

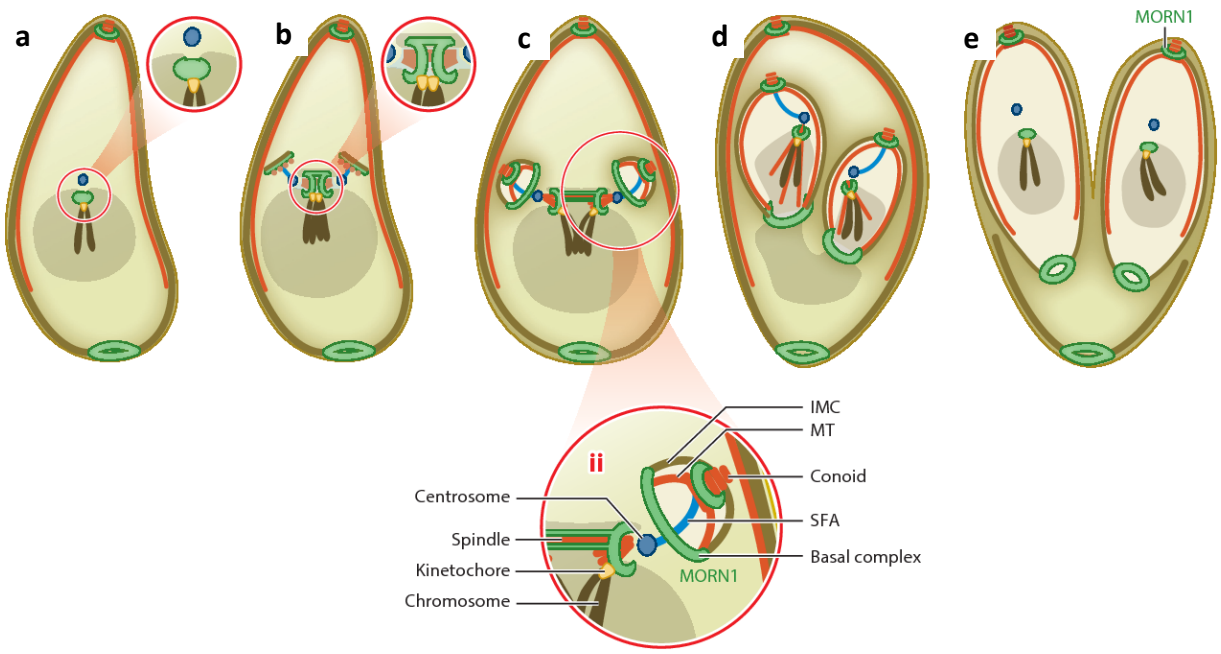



**Figure 24: Cell division of *T. gondii*.** Adapted from (Blader et al., 2015). (a and b) The chromosomes are grouped at the level of their centromeres and anchored to the centrocone via the kinetochore. (c) The assembly of daughter cells is driven by the cortical cytoskeleton from the apical pole to the basal pole. (d) The parasitic nucleus is anchored to the daughter cell in formation by the centrosome, itself attached to the conoid by the SFA fiber. (e) The constriction of the basal complex at the end of the cycle allows the separation of the daughter cells.

The different organelles are duplicated and assembled in the budding daughter cells in a precise order and the IMC formation is closed to complete division. First, the centrosome and the Golgi apparatus as mentioned above, followed by the apicoplast, the nucleus, the ER, and then the mitochondria are segregated to the daughter cells (Gubbels et al., 2008; Nishi et al., 2008). The secretory organelles (rhoptries and micronemes) are synthesized *de novo* in each daughter cell, not inherited from the mother cell (Nishi et al., 2008). Once formed, these specialized organelles are transported to the apical pole of the daughter cells, a process that is thought to be mediated by cytoskeletal components (Francia and Striepen, 2014). Then, the daughter cells inherit the maternal PM. Degraded maternal secretory organelles and parts of the mitochondrion, known as remnants, are packaged in the residual body (RB) at the end of the division process (Attias et al., 2019; Muñiz-Hernández et al., 2011; Nishi et al., 2008). When cytokinesis is completed (average of 6 hours to complete a cycle of division), the daughter cells are separated and able to start a new round of division (Gubbels et al., 2008).

## 5.4 Egress

After several replication cycles, the parasites exit the host cell by rupturing the PV and the host cell membrane to disseminate. Although the precise mechanisms are still unknown, several cellular and parasitic signals can modulate the egress of the parasite.

Early studies showed that the stimulation of several processes producing physiological changes in intracellular parasites that result in egress is triggered by a calcium signaling cascade (Endo et al., 1982). Indeed, it has been demonstrated that during its replication, the parasite continuously synthesizes abscisic acid (ABA) which when reaching a threshold level, triggers the egress (Nagamune et al., 2008). The ABA frees the intracellular calcium storage, triggering the secretion of micronemes and the parasite's exit. In addition, the replication of the parasite causes an acidification of the PV inducing the insertion of the microneme protein *Tg*PLP1 (Perforin Like Protein 1), which forms pores in the PVM and in the host cell membrane to facilitate the exit of the parasite (Kafsack et al., 2009; Roiko et al., 2014). Egress can also be mediated by NTPases (Nucleotide Triphosphate-degrading enzymes) that are secreted in the PV. These enzymes deplete the host cell in ATP by blocking the $Na^+/K^+$ dependent pumps, which results in a decrease in intracellular potassium and therefore parasite egress (Stommel et al., 1997). Moreover, it has been demonstrated that calcium-dependent kinases, in particular, CDPK1 and CDPK3, have been implicated in egress through their phosphorylating proteins of the motor complex and triggering microneme secretion (Gaji et al., 2015; Lourido et al., 2012, 2010; McCoy et al., 2012). Similarly to gliding motility and invasion, it has been shown that actin is also essential for egress. Treating vacuoles with high concentrations of CD blocks egress even after artificial induction with a calcium ionophore (Egarter et al., 2014; Moudy et al., 2001) similar to observations made in a conditional ACT1 KO parasite strain. In addition, the depletion of AKMT (apical complex lysine methyltransferase) compromises the parasite's invasion and egress, and thus severely impairs the lytic cycle. In this study, the authors showed that the parasites depleted for AKMT failed to disperse from the PV and egress from the permeabilized host cells (Heaslip et al., 2011).

Egress can also be stimulated by an immune response. In fact, CD8+ T lymphocytes, through perforins and the Fas/FasL cell death receptor, cause damage to the membrane of the host cell. These lesions cause a decrease in the potassium concentration and as mentioned above, the egress of the parasite (Moudy et al., 2001; Persson et al., 2007).

# 6 Regulation of protein trafficking:

Eukaryotic cells are partitioned into smaller sub-compartments which are termed organelles. Each organelle is surrounded by a phospholipid bilayer membrane and contains a unique set of proteins which enable them to carry out distinct functions, such as cargos sorting, membrane deformation, budding, translocation across the cytoplasm, and membrane fusion. Importantly, cargos (such as proteins and lipids) are transported by vesicles moving along cytoskeleton tracks and fusing with the target organelles, thereby regulating their intracellular trafficking. The best-explored intracellular trafficking pathways are the secretory (anterograde) and recycling or endocytic (retrograde) pathways.

Within the secretory pathway, proteins are synthesized in the endoplasmic reticulum (ER), and then transported to the Golgi apparatus, where a whole series of post-translational modifications take place. Finally, the proteins reach the trans-Golgi network, where they are sorted and oriented towards their subsequent destination, which could be the plasma membrane, the secretory granules, the sorting endosomal compartment or the late endosomes. On the other hand, in the endocytic pathway, the internalized molecules first appear in the early endosomes located in the vicinity of the plasma membrane. From there, these molecules are either rapidly recycled to the cell surface, as in the case of several receptors, or transported to late endosomes, located at the perinuclear region near the Golgi. Certain molecules, in particular receptors for lysosomal hydrolases, are then recycled to the trans-Golgi network, while molecules which will be degraded are packaged in the lysosomes (Figure25).

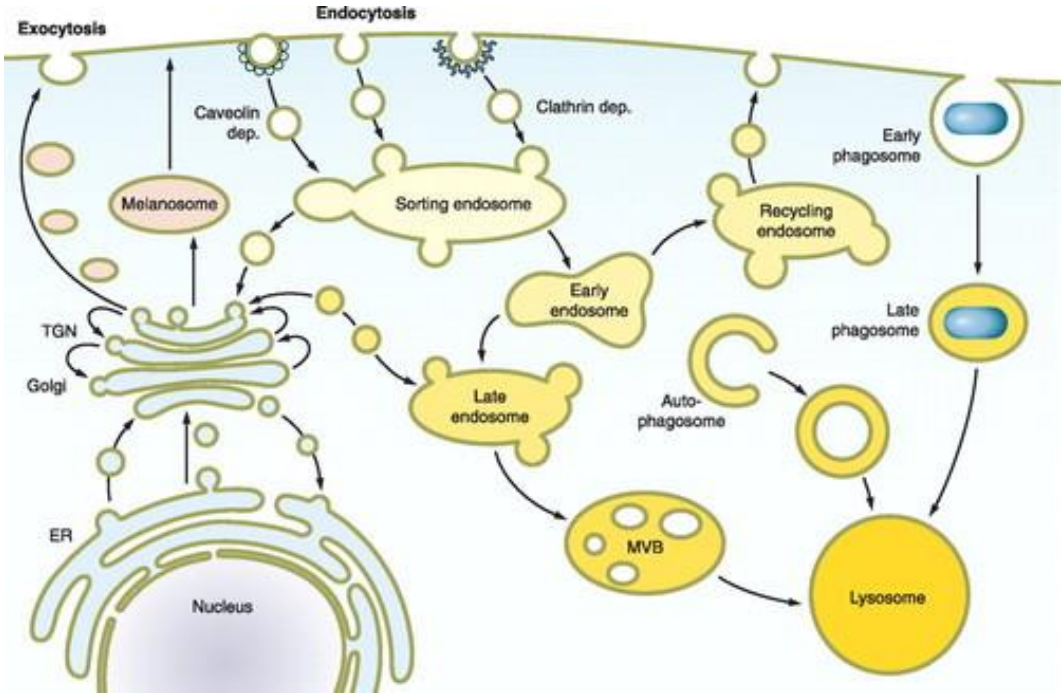



**Figure 25: The anterograde and retrograde pathways for protein trafficking.** Adapted from (Ligeti et al., 2012). A schematic representation of the two major protein trafficking routes. In the anterograde pathway (exocytosis), newly synthesized proteins translocate from the ER and enter the Golgi where they move from the cis Golgi complex towards the TGN (Trans-Golgi network), before being delivered to the plasma membrane. On the other hand, during endocytosis, cargoes are internalized from the plasma membrane to early endosomes where their fate is determined. Internalized proteins are either routed to the TGN for retrieval, or recycled to the plasma membrane, or destined to the lysosomes for degradation. The transition from late endosomes to lysosomes is mediated by multivesicular bodies (MVB). Phagocytosis, as well as autophagocytosis, target internalized particles directly to the lysosomes for degradation.

## 6.1 The Anterograde/Secretory pathway

In eukaryotic cells, each organelle has a specific function. Cells become organized and functional when the high amount of proteins produced is sorted, transported and localized to the correct cellular membrane or organelle. To achieve this, eukaryotic cells use the secretory pathway to deliver proteins, lipids and certain membrane-bound organelles via transporting vesicles to an acceptor membrane, and/or by releasing material outside the cell (Schekman and Orci, 1996). The mechanisms and the molecules implicated in the secretory pathway are well conserved across different species from the simplest organisms such as yeast to the most complex organisms such as mammalian cells (Bennett and Scheller, 1993; Gadila and Kim, 2016). Eukaryotic cells possess two different secretory pathways: the regulated pathway and the constitutive pathway (Moore and Kelly, 1985; Moore, 1987).

Proteins destined for the regulated pathway, such as hormones, are packaged into vesicles that are stored in the cytoplasm of the cell until their release is triggered by a specific signal. However, proteins exported by the constitutive pathway, such as plasma membrane proteins, are concentrated into clear and small vesicles that are secreted continuously and fuse directly with the plasma membrane (Figure 26).

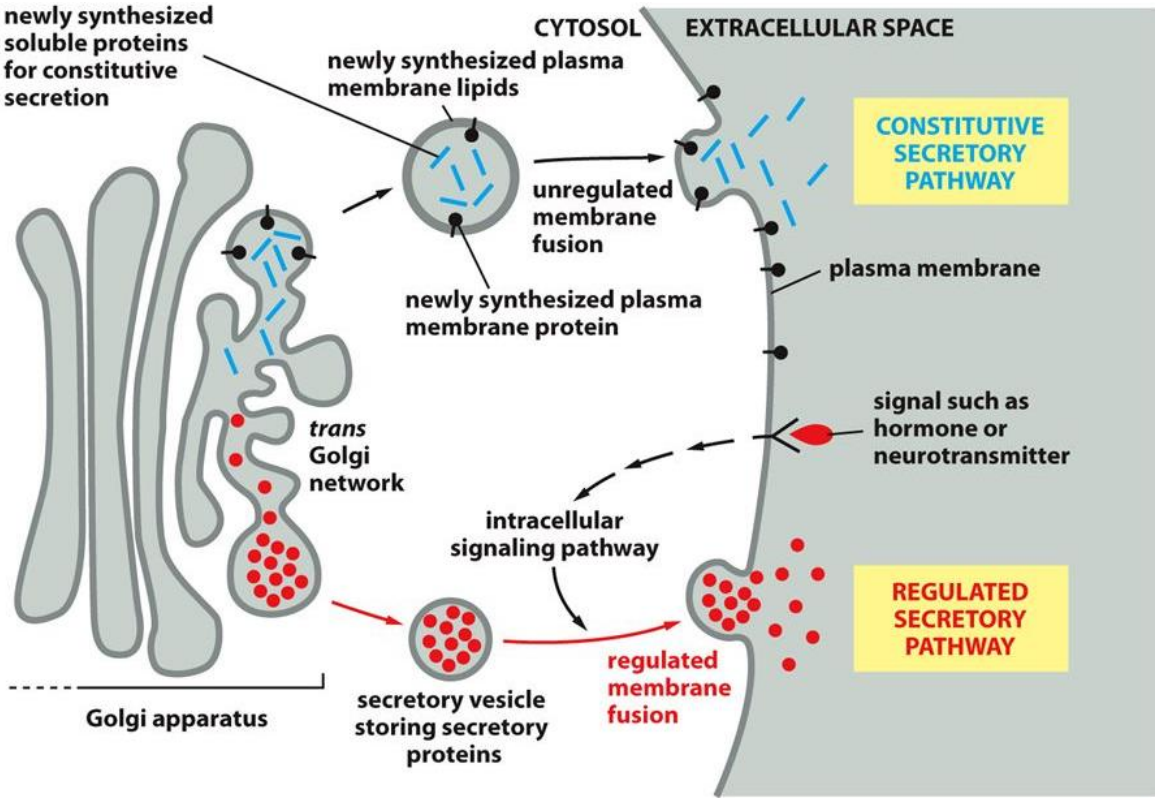



**Figure 26**: **The constitutive and regulated secretory pathways.** Adapted from (Shaib, 2016). Two types of exocytosis have been described. The constitutive secretion transfers proteins from the Golgi network towards the plasma membrane and is carried out by all cells. On the other hand, the regulated secretion takes place only in specialized cells and occurs in response to specific conditions, signals or biochemical triggers. Of note, membrane-bound and soluble molecules can both be directed down either pathway.

Proteins destined for the secretory pathway contain an N-terminal sequence recognized by the signal recognition particle (SRP) (Blobel and Dobberstein, 1975). Properly folded and assembled, proteins are generally packaged into coat protein complex II (COPII)-coated vesicles and transported from the ER to the Golgi complex (Barlowe et al., 1994; Rexach et al., 1994). These vesicles detach from the donor membrane (ER) via a process called budding to primarily mediate the movement of cargos between different compartments. Unlike membrane cargos that are sorted into the vesicles by a direct interaction between the

cytosolic domain of the cargo protein and the coat components, soluble cargos require the involvement of a transmembrane receptor to mediate the interaction (Wieland and Hartert, 1999). When the vesicles are completely formed, they lose their coat and fuse with each other to form vesicular tubular clusters or ER-Golgi intermediate compartment (ERGIC) (Aridor et al., 1995; Balch et al., 1984), before being transported by dynein along microtubule tracks, and finally tethered and fused to the *cis* side of the Golgi complex (Presley et al., 1997). Misfolded or unassembled subunits are degraded in the cytosol by the ubiquitin-mediated proteolytic pathway (Sommer and Wolf, 1998). Wrongly transported proteins and components needed for another round of transport are returned by retrograde transport to the ER (Pelham, 1988).

Newly produced proteins that arrive from the ER, enter the Golgi complex at the *cis*-side, where they are modified, processed and sorted, and then they exit at the *trans*-side. The transport of cargo through the Golgi stack is mediated by cisternal maturation and/or vesicular transport (Balch et al., 1984; Grasse, 1957). In the vesicular transport model, secretory cargo proteins, excluding resident Golgi proteins, move forward through the stack via COPI vesicles that follows the anterograde traffic (from earlier to later cisternae) (Elsner et al., 2003). While the cisternal maturation system suggests that secretory cargos move forward within the cisternal compartments from the *cis* Golgi towards the *trans*-side. The entire cisternae function as the transporting entity that, once reached the *trans*-face of the Golgi stack, would release its content for further transport to the cell surface. However, resident Golgi proteins, are recycled from older to younger cisternae along retrograde COPI vesicles (Elsner et al., 2003). Once pinched off from the TGN, vesicles are then actively transported along microtubules or actin filaments to the target compartment such as the endosomes or lysosomes. A classic example of cargo transport via the anterograde pathway (not destined to extracellular secretion) is proteins destined to lysosomes. Those proteins carry a special targeting signal, mannose-6-phosphate (M6P), and are sequestered by M6P receptors into vesicles bound to late endosomes. Here, the lysosomal proteins are separated from their receptors: vesicles containing the proteins are sent to lysosomes (Gadila and Kim, 2016), while the receptors are recycled back to the Golgi (Cecilia N. Arighi, 2004; Seaman et al., 1997). Another example is the transport of cargo molecules such as granzyme A, granzyme B, perforin and prosaposin to the late endosomes and to the lysosomes by a lysosomal sorting

receptor (called sortilin) localized at the TGN (Braulke and Bonifacino, 2008; Herda et al., 2012).

Following their transport to target organelles, vesicles are brought into proximity to the membrane for fusion to occur by tethering factors and finally the actual fusion is mediated by SNAREs (Soluble NSF Attachment protein Receptor) (Bonifacino and Glick, 2004). Rab GTPases together with the tethering factors are implicated in determining the specificity of vesicle targeting (Cai et al., 2007). Tethering factors mainly belong to either coiled-coil tethers or multisubunit tethering complexes (Sztul and Lupashin, 2006). The long coiled-coil tethers called Golgins consist usually of dimers that resemble long rod-like molecules. The most studied members of this class of tethers are p115 and EEA1 that are implicated in COPII vesicle fusion and homotypic fusion process between early endosomes respectively (Allan et al., 2000; Mills et al., 1998; Nelson et al., 1998). However, multisubunit tethering complexes consist of several subunits, including both quatrefoil tethers such as the oligomeric Golgi complex (COG) and non-quatrefoil tethering complexes, such as the transport protein particle (TRAPP) and the exocyst. COG associates with COPI subunits both in mammalian and yeast and functions as a tethers between *cis*-Golgi and COPI (Suvorova et al., 2002; Zolov and Lupashin, 2005). TRAPP1 complex is anchored to the Golgi and functions as a tether for incoming COPII vesicles (Barrowman et al., 2000; Sacher et al., 2001). TRAPP2, located in the *cis*-Golgi, is functionally linked to ARF1 and coatomer and could be involved in intra-Golgi traffic (Sacher et al., 2001, 1998). The exocyst complex, located at the plasma membrane, is formed of eight subunits and is believed to be involved in vesicles tethering (TerBush et al., 1996). Moreover, tethering factors also interact with coat components and SNAREs (Cai et al., 2007).SNAREs mediate the final step of vesicle docking and fusion with the target membrane. SNAREs are short membrane associated proteins containing a characteristic SNARE motif (Jahn and Scheller, 2006; Weimbs et al., 1997) (Figure 27). They are present on both membranes of the two fusion partners. Soon after vesicle tethering to the target membrane, v-(vesicular) and t- (target organelles) SNAREs form a four-helix bundle called trans-SNARE or SNAREpins to brings the membranes close to each other and thereby ‘zipping’ them together, thus mediating their fusion (Hanson et al., 1997; Monck and Fernandez, 1994; Weber et al., 1998). Following membrane fusion, the SNARE complex disassemble and trans-SNARE convert to a cis-SNARE. α-SNAP and NSF (N-ethylmaleimide sensitive factor) are recruited to

disassemble the complex, a process consuming a considerable amount of energy (Söllner et al., 1993).

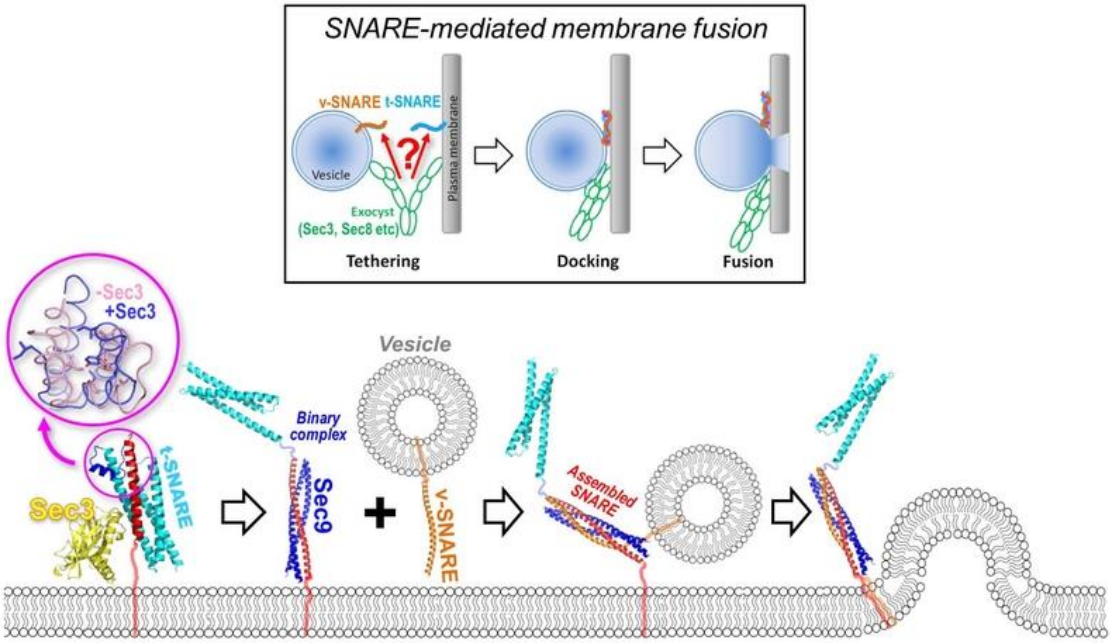



**Figure 27: Tethering, docking, and fusion of vesicles.** Adapted from (Gang Dong, 2017). The complex mechanism of vesicle fusion is mediated by SNARE proteins. The process of vesicle tethering and doking to the plasma membrane is carried out by the exocyst complex that interacts directly with the t-SNARE protein, enabling the next step of vesicle fusion.

## 6.2 The Retrograde/Recycling pathway

Endocytosis is a vesicle-mediated process used by the cell to internalize cargo molecules from the surface (such as extracellular macromolecules, plasma membrane lipids, transmembrane proteins and their ligands) into the intracellular region of the cell. This pathway regulates many physiological roles including nutrient uptake, cell signalling, cell adhesion, and developmental processes in response to morphogens.

Several endocytic pathways have been described; however they can all be pooled into two distinct pathways: clathrin-mediated endocytosis (CME) and clathrin-independent endocytosis (CIE) (Grant and Donaldson, 2009) (Figure 28). The best-studied endocytic pathway is the CME defined by a requirement for the protein clathrin, the major component of the endocytic vesicle coat. While the CIE pathways (also known as caveolar endocytosis) generally depend on cholesterol-rich domains, such as rafts. The caveolar endocytosis will not

be further discussed, since *T. gondii* does not express caveolin/cavin proteins required for this process (Ford et al., 2002). The cytoplasmic domain of cargoes that undergo CME typically possess linear sequence motifs, or covalent modifications such as ubiquitylation and phosphorylation, that recruit adaptor proteins from the cytosol in a highly ordered manner, leading to their packaging into clathrin-coated vesicles transported inside the cell. In mammalian cells, CME facilitates the internalization of transmembrane receptors (and their associated ligands) via the association with clathrin adaptor proteins such as the four-subunit complex AP2 (Boucrot et al., 2012; van Dooren et al., 2009). Clathrin adaptors in turn bind to the clathrin lattice that encases the forming vesicle which drives and/or stabilizes membrane curvature (Boucrot et al., 2012; van Dooren et al., 2009). The large GTPase dynamin forms a coil around the neck of budding vesicles, and mediates the vesicle scission (Boucrot et al., 2012; Ezougou et al., 2014; Scheuring et al., 2011). These vesicles are then uncoated by the heat shock cognate protein HSC70 and its cofactor auxillin/GAK (Boucrot et al., 2012; van Dooren et al., 2009). Soon after, the small GTPase Rab5 mediates the fusion of uncoated endocytic vesicles with one another and with early endosomes (EE). The EE is mildly acidic, which facilitates dissociation of some ligands from their receptors. The majority of internalized ligands does not recycle, but instead is transported from the lumen of the EE to late endosomes, and eventually degraded in the lysosomes. However, the receptors are either returned to the plasma membrane directly or indirectly via recycling endosomes, transported to the TGN, degraded in lysosomes, or transcytosed to the opposite membrane of polarized cells (Elkin et al., 2016). Finally, “hybrid” organelles, which mature back into lysosomes through sorting and fission, are formed through the fusion of late endosomes with the pre-lysosomes.

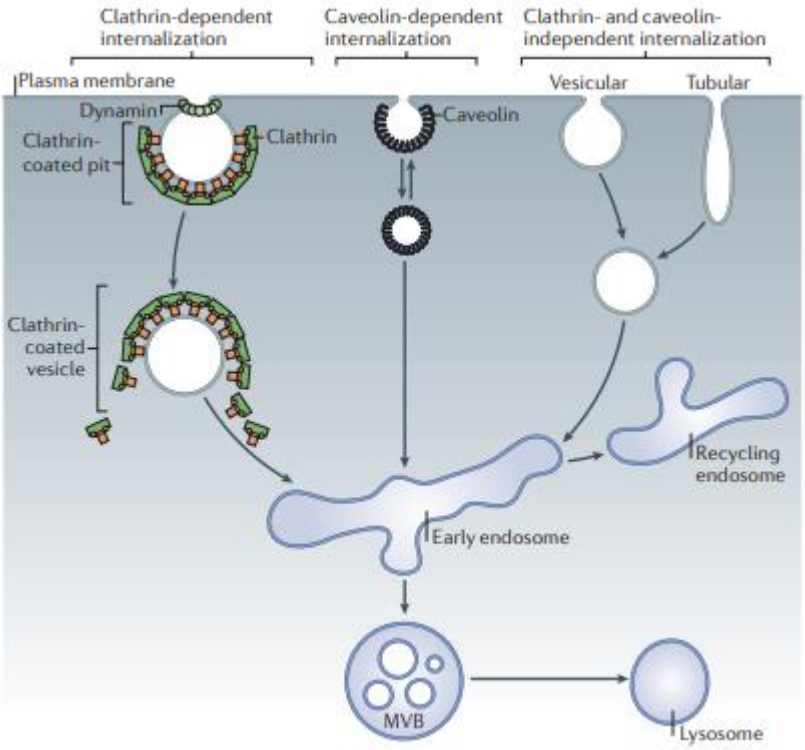



**Figure 28: Clathrin-dependent and –independent endocytosis.** Adapted from (McMahon and Boucrot, 2011). Two types of endocytosis have been identified. In the clathrin-mediated endocytosis, cargoes are internalized by a process that requires the involvement of clathrin proteins. The clathrin-independent endocytosis does not use a clathrin coat, but rather depends on cholesterol-rich domains. Internalized proteins are trafficked into endosomes where they are sorted either into multivesicular bodies (MVB) or lysosomes for degradation or recycled back to the plasma membrane.

The endosomal recycling pathway is a dynamic process that controls and balances the composition of the plasma membrane, by sorting and re-exporting internalized cargoes (Grant and Donaldson, 2009). The disruption of this balance leads to a variety of diseases such as cancer and neurodegenerative diseases (Mellman and Yarden, 2013; Schreij et al., 2016). The internalized membrane components, after being sorted in the EE, can either return to the plasma membrane or progress along the degradative pathway (Figure 29). While the process of cargo internalization from the plasma membrane and the mechanisms mediating their transport along the degradative pathway are well understood, the machinery regulating the sorting and recycling of cargo is not fully characterized. There are two recycling pathways back to the plasma membrane: the fast recycling pathway where recycling occurs directly from the EE, and the slow recycling pathway where recycling occurs indirectly via a distinct subpopulation of recycling endosomes (REs), often referred to as the endosomal recycling compartment (ERC). A wide variety of cell surface receptors internalized into EEs undergo

endosomal recycling, including receptor tyrosine kinases (such as ErbB family members, IGF1R, FGFRs, c-Met), G protein-coupled receptors (such as Par1, chemokine receptors, beta-adrenergic receptors), cell adhesion molecules (integrins and cadherins), and carrier proteins such as the transferrin receptor (involved in iron uptake), low-density lipoprotein receptor (involved in cholesterol uptake) and the glucose transporter Glut4 (Taguchi, 2013). Like any other dynamic process, the endosomal recycling pathway is tightly regulated to ensure the delivery of the right cargo to the right place. Members of the Rab GTPase family play a central role in the regulation of this process.

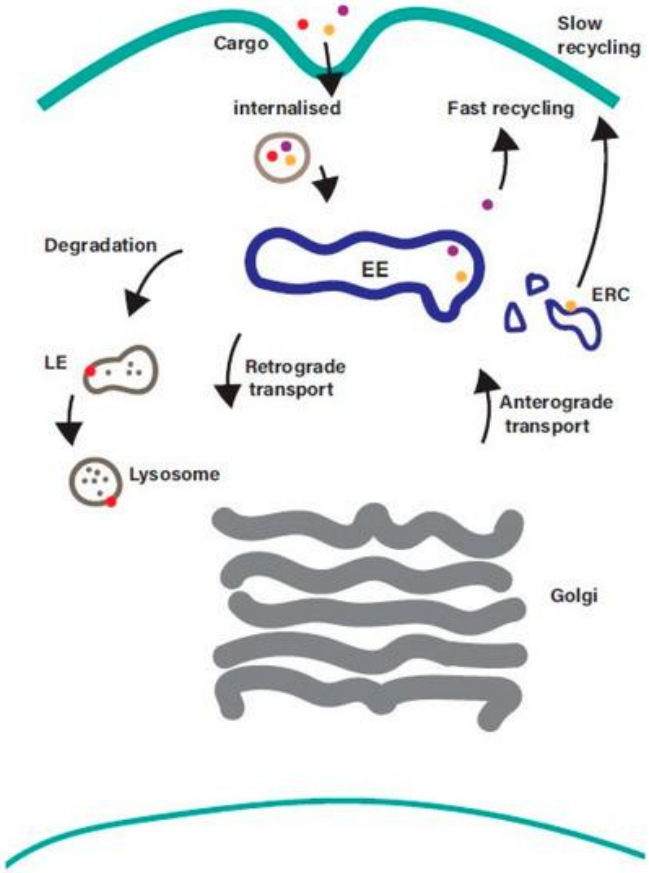



**Figure 29: The endosomal recycling pathway.** Adapted from (O'Sullivan and Lindsay, 2020). Cargoes internalized from the cell surface reach the early endosome (EE), where they are sorted to the retrograde trafficking pathways. Cargoes are returned to the plasma membrane either directly from the EE (fast recycling pathway) or indirectly from the endosomal recycling compartment (ERC) (slow recycling pathway).

## 6.3 Rab GTPases

Ras superfamily contains five major kinds of small GTPases including Ras, Rho, Ran, Rab and Arf (Colicelli, 2004). The GTPase proteins of each subfamily have similar structures, sequences and functions. However, different family proteins play multiple and divergent roles. Therefore, Ras superfamily proteins are versatile and are key regulators of virtually all fundamental cellular processes. Rab GTPase family is the biggest member of the Ras superfamily and key proteins to control vesicle trafficking. These so-called small "G" proteins are monomeric GTPases of small sizes around 25 KDa. They act as molecular switches inside cells. Their activities are regulated by factors that promote binding and hydrolysis of guanosine triphosphate (GTP) to guanosine diphosphate (GDP). They alternate between GTP-bound "active" and GDP-bound "inactive" forms to carry out their functions. While the exchange of GDP to GTP is catalyzed by guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs) stimulate a Rab's intrinsic rate of GTP hydrolysis, thus inactivating the Rabs by converting bound GTP to GDP. GDP dissociation inhibitors (GDIs) maintain Rabs in their "inactive" state by extracting GDP-Rabs from membranes and forming soluble complexes (Bos et al., 2007; Stenmark, 2009). Indeed, to help the extraction of Rabs from the high affinity Rab-GDI complex, membrane-localized GDI displacement factor (GDF) functions to disrupt the high affinity Rab–GDP–GDI complexes and to promote the release of Rabs (Sivars et al., 2003) (Figure 30A).

Rab proteins are key regulators of vesicular trafficking via interacting with various effector proteins in respective pathways. They are involved in all stages of intracellular trafficking according to their subcellular distribution, and play roles in all steps of membrane trafficking including vesicle budding, transport, tethering, docking and fusion (Segev, 2001; Stenmark, 2009; Zerial and McBride, 2001). During each step, a unique set of Rab interacting proteins/effectors are required. The small GTPases Arf and Sar regulate the retrograde and anterograde pathways respectively, by participating in coat protein complex-I (COPI) and coat protein complex-II (COPII) formation, respectively (Barlowe et al., 1994; Memon, 2004). In addition, several Rabs are also involved in the coat budding process. For example, GTP-bound Rab9 recruits its effector TIP47 and directs the vesicle transport of the mannose-6- phosphate receptors (M6PRs) from the late endosomes to trans-Golgi network (TGN) (Aivazian et al., 2006; Carroll et al., 2001; Díaz and Pfeffer, 1998). Other Rabs have been involved in vesicle

budding and fission such as Rab35 (Kobayashi and Fukuda, 2013; Kouranti et al., 2006). Moreover, it is crucial to release the coat from the vesicle, a process that termed uncoating, to allow the fusion of the vesicle with acceptor membranes. Therefore, Rabs also play a role in uncoating. For example, Rab5 regulates the early endocytic pathway and is found on clathrin-coated vesicles (CCVs). It is implicated in the AP-2 uncoating process. With the action of its GAP, GAPVD1 (GTPase activating protein and VPS9 domain—containg protein 1), Rab5 releases μ2 kinase from the clathrin adaptor AP-2 preventing it from phosphorylating its μ2. Rab5 can also modulate PtdIns(4,5)P2 levels through recruitment of effectors such as PtdIns(3)P kinases or PtdIns phosphatases (Christoforidis et al., 1999; Semerdjieva et al., 2008; Shin et al., 2005). Furthermore, Rab proteins often use motor proteins (kinesins/dyneins and myosins) along actin- or microtubule-based cytoskeletal structures to promote vesicle movement. Rab27A interacts with its effector melanophilin/Slac2-a that binds to the actin motor myosin Va (MyoVa) in melanocytes to regulate the transport of melanin-containing melanosomes to the plasma membrane (Bahadoran et al., 2001; Hume et al., 2001; Matesic et al., 2001; Strom et al., 2002; Wu et al., 2002; X Wu, 2001). The recycling of plasma membrane is regulated by Rab11, which interacts with myosin Vb (MyoVb) through its effector, Rab11 family interacting protein 2 (Rab11-FIP2) (Hales et al., 2003). Another major membrane trafficking pathway relies on microtubules in animal cells, aside from the above vesicle transport processes, which are driven by actin. Actin filaments usually facilitate slower and short-range local transport events, while microtubules provide high-speed, long-range transport (Jordens et al., 2006). It has been proposed that Rabs interact with microtubule-based motors to regulate these pathways, either via kinesins (plus-end directed motors) or the dynein (minus-end directed motors) family. A well-studied case is Rab7, which interacts with its effector Rab-interacting lysosomal protein (RILP) to recruit the dynein-dynactin motor complex to transport cargos along microtubule, coordinating the trafficking of late endosomes and lysosomes or the centrosome (Johansson et al., 2007; Jordens et al., 2001). Finally, Rabs regulate tethering and fusion processes, by interacting directly with SNARE proteins or SNARE related proteins. Rab5, which is found on early endosomes, plays a critical role in endocytic pathway through the function of its numerous effectors. Rab5 effectors, EEA1 and rabenosyn-5, interact with the SNARE proteins, Syntaxin13, Syntaxin6 and

the SM protein VPS45, respectively (Nielsen et al., 2000; Simonsen et al., 1999), promoting thus homotypic early endosome fusion (McBride et al., 1999) (Figure 30B-C).

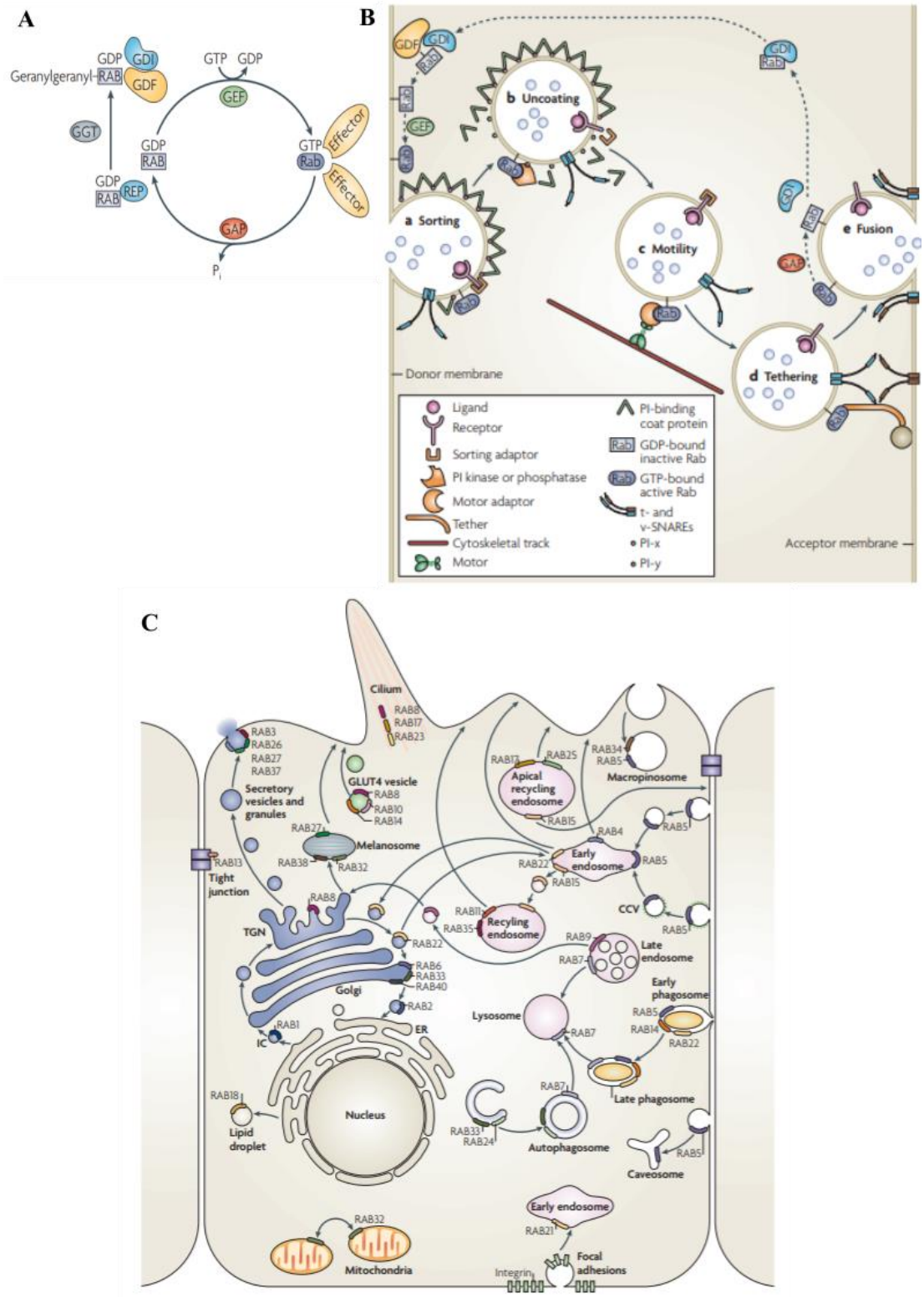



**Figure 30: Rab GTPases.** Adapted from (Stenmark, 2009). (A) Rab GTPases activating cycle. Rab GTPases switch between two conformations: a GTP-bound active form and a GDP-bound inactive

form. The activation of Rab GTPase is catalyzed by a guanine nucleotide exchange factor (GEF) that exchange GDP for GTP. Once activated, Rab binds to multiple effector proteins to promote vesicle trafficking. The hydrolysis of GTP back to GDP is mediated by GTPase activating proteins (GAP), a process leading to Rab inactivation, thus effectors dissociation from the Rab protein. (B) Role of Rab GTPases in vesicle trafficking. The Rab GTPase and its effectors control different steps of membrane trafficking such as cargo sorting, vesicle uncoating, vesicle motility, tethering, and fusion. (C) Localization and presumed functions of Rab GTPases. Over 70 Rab proteins have been identified. They localize to virtually every organelles of the secretory system and additionally to other specific sites or organelles within a cell. They are involved in the formation of transport intermediates, the active transport of such intermediates along microtubules and actin filaments, as well as in tethering and fusion of transport intermediates to the target compartment.

## 6.4 Rab11

The Rab11 protein family consists of three members: Rab11A, Rab11B, and Rab25 that share a high sequence homology (Welz et al., 2014). Rab11A is ubiquitously expressed and predominantly localized to the pericentriolar ERC/RE (endosomal recycling compartment/recycling endosome) (Ullrich et al., 1996). Rab11B was also shown to localize to the ERC (Lai et al., 1994; Schlierf et al., 2000), but its expression is restricted to the brain, testes and heart (Lai et al., 1994). Rab25 is expressed specifically in epithelial cells of the lung, kidney and gastric tract (Goldenring et al., 1993). In non-polarized and in polarized cells Rab11 localizes to perinuclear recycling endosomes (Casanova et al., 1999; Green et al., 1997; Ullrich et al., 1996). Furthermore, Rab11 has been reported to localize to the TGN, to post-Golgi secretory vesicles and recycling endosomes (Chen et al., 1998; Urbé et al., 1993). Rab11 is known to be involved in numerous functional roles such as secretion of growth factors and cytokines at the plasma membrane, delivery of proteins at cell-cell junctions, and recycling of transmembrane proteins, such as the transferrin receptor and integrins during cell motility (Guichard et al., 2014). Indeed, Rab11 regulates the transport of many receptors and adhesion molecules, such as α-amino-3-hydroxy-5-methyl-4- isoxazolepropionic acid(AMPA) receptor, rhodopsin, epidermal growth factor (EGF) receptor, Toll like receptor 4 (TLR4), E-cadherin and N-cadherin (Kelly et al., 2012). Thereby, Rab11 regulates numerous cellular activities, such as ciliogenesis, cytokinesis, cell migration and adhesion, as well as cell polarity establishment in epithelial cells (Hobdy-Henderson et al., 2003; Knödler et al., 2010). Studies performed in Drosophila and mammalian cells proved a role of Rab11A in cytokinesis. As a matter of fact, Rab11 and Arf6 together with family Rab11 interacting proteins, FIP3 and FIP4, have been implicated in targeting recycling endosomes to the cleavage furrow/midbody for

the completion of abscission, the final step of cytokinesis (Horgan and Mccaffrey, 2012; Wilson et al., 2005). The depletion of Exo70p, a component of the exocyst complex, which localizes at the cleavage furrow and interacts with Arf6 led to the cytokinesis failure (Fielding et al., 2005). Rab11 is known in mammalian cells to regulate exocytic events by stimulating the fusion of vesicles to the plasma membrane via its binding to the exocyst complex subunit Exo70 (Takahashi et al., 2012). Also Rab11 interacts with its effector Sec15 to anchor the exocyst at the vesicular side (Wu et al., 2005) (Figure 31).

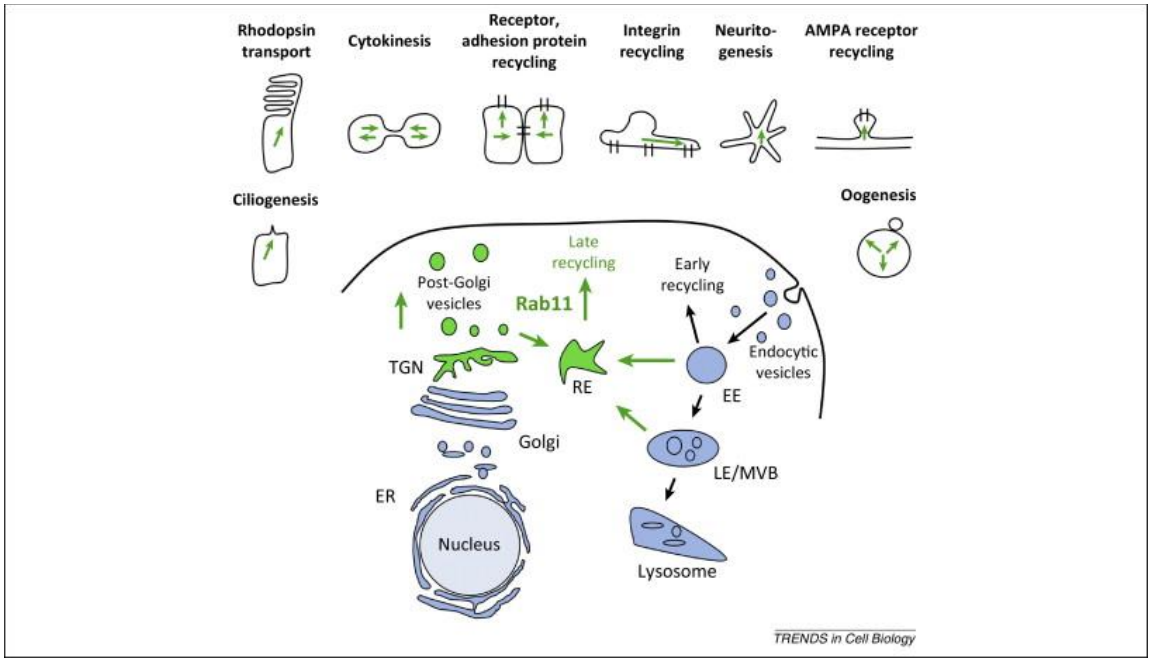



**Figure 31: Rab11 localization and functions.** Adapted from (Welz et al., 2014). Rab11 is localized at the TGN, in post-Golgi secretory vesicles, and in recycling endosomes (in green). Rab11 has diverse cellular functions. It is implicated in the late or slow recycling process of proteins at the plasma membrane, but not in the early or fast recycling process. Additional functions for Rab11 are depicted above with green arrows.

### 6.4.1 Rab11 and motor proteins

Rab11 recruits distinct motor adaptors to execute its functions. Rab11 forms complexes with kinesins and dynein for bidirectional movement along microtubule tracks or with myosins for actin filament-dependent transport. The Rab11 subfamily was shown to interact with the C-terminus of myosin Vb (Myo5B) tail by yeast two-hybrid screening (Lapierre et al., 2001). Then, an evolutionarily conserved family of Rab11-effectors which have been termed the Rab11 family-interacting protein (RAB11FIP) was identified by proteomic screenings (Kelly et

al., 2012). The best-studied Rab11-FIPs-motor protein complex is the Rab11-FIP2-MyoVb complex which has been implicated in a wide range of endosomal recycling processes. For instance, this complex mediates transport of AMPA receptors from recycling endosomes at the base of the spine to postsynaptic membranes (Wang et al., 2008). Another example is the role of Rab11-FIP3 complex in vesicle transport to the cleavage furrow, where they are tethered prior to fusion via interactions with Arf6 and the exocyst, in a dynein dependent manner (Fielding et al., 2005; Horgan, 2004). Rab11 moves from recycling endosomes to autophagosomes upon autophagy induction, and interacts with HOOK, which acts as a negative regulator of endosome maturation. HOOK is a motor adaptor that anchors endosomes to microtubules. Rab11 removes HOOK from mature late endosomes and inhibits its homodimerization to facilitate the fusion of endosomes with autophagosomes (Szatmari et al., 2013) (Figure 32).

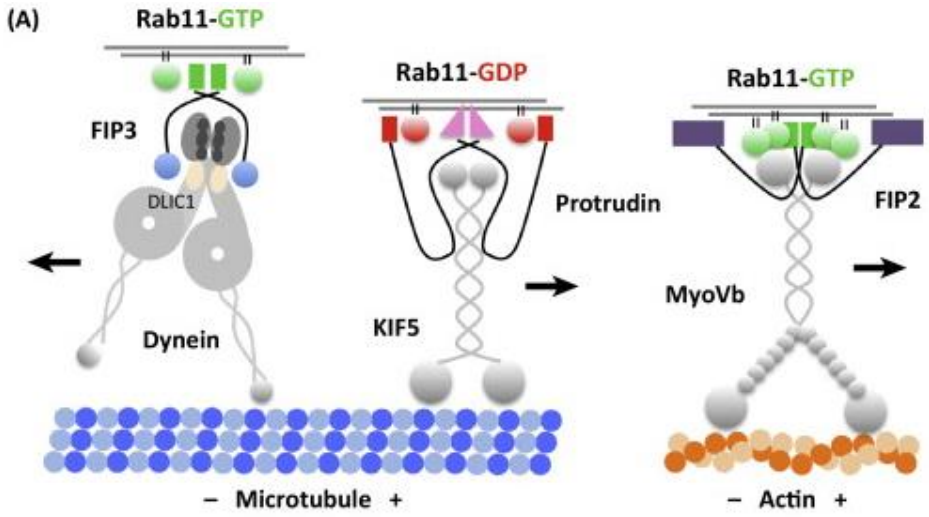



**Figure 32: Rab11 and motor protein complexes.** Adapted from (Welz et al., 2014). Rab11-GTP (represented by green sphere) and its effector FIP3 interact with dynein light intermediate chain 1 (DLIC1) bound to the microtubule "- "end to mediate endosomal transport. However, protrudin mediates the interaction of KIF5 with Rab11-GDP (represented by red sphere) to form a complex directed to the microtubule "+" end. The binding of Rab11-GTP (green sphere) to FIP2 recruits the actin motor protein myosin Vb (MyoVb). The formed complex slides toward the "+" end of actin filaments.

### 6.4.2 Rab11 regulators

Like any other small Rab GTPases, the function of Rab11 is dependent on its structural conformation changing via its interaction with GEF, GAP, GDI and other effectors. In general, the discharge of GDP from GTPases is very slow but can be accelerated by GEFs to yield

effective activation in cells. Rab GTPase GEFs can be subdivided into at least four types based on their functional domains (Cherfils and Zeghouf, 2013; Hutagalung and Novick, 2011). DENN (Rab35 GEF) and Vps9 (Rab21/22 GEF) motifs with the surrounding of other domains constitute the conserved catalytic domains of the GEF subfamilies (Delprato et al., 2004; wu et al., 2011). However, Sec2 (Sec4 GEF) and the TRAPP (Ypt1/Rab1 GEF) complex that work as dimeric and pseudo-dimeric complexes respectively, are the unique GEF subfamily (Burton et al., 1993; Cai et al., 2008). A calmodulin-binding protein related to Rab3 GDP/GTP exchange protein (Crag) was identified in Drosophila as the only GEF for Rab11 (Xiong et al., 2012). On the other hand, GAPs accelerate the slow intrinsic GTPase activity to exchange GTP-bound form to GDP-bound form. The GTPase GAPs are subfamilies specific, similarly to the GEFs (Calmels et al., 1998). To date, most GAPs share a common conserved TBC (Tre-2/Cdc16/Bub2) domain (Albert et al., 1999; Albert and Gallwitz, 1999; Seals et al., 2000; Strom et al., 1993). TBC1D11, TBC1D15 and Evi5 (the ectopic viral integration site 5 protein homolog), three GAPs that have been found so far to activate Rab11 (Fuchs et al., 2007). However, GEFs and GAPs functional mechanisms have not been yet fully understood.

### 6.4.3 Rab11 in diseases

Given its importance in many fundamental intracellular trafficking processes, the Rab11 subfamily is implicated in numerous physiological disorders. It has been demonstrated that many Rabs including Rab11 promote tumor cell migration and invasion, and consequently exhibit their effects on tumorigenesis and metastasis by interruption of intracellular signal transduction (Yoon et al., 2005). Rab11 mediates α6β4 integrin trafficking, thereby enhancing cancer cell invasion in breast cancer (Yoon et al., 2005). Moreover, the overexpression of Rab11c (Rab25) oncogene is associated with poor prognosis in breast and ovarian cancer patients, and leads thereby to an increase in the aggressiveness of cancer cells (Cheng et al., 2004). However, Rab11c (Rab25) acts as a tumor suppressor gene in colon cancer. Some Rab proteins including Rab11 are related to several prevalent neurological diseases: it is considered as a causative factor in the neurodegenerative disorder Huntington's disease and Alzheimer disease (Greenfield et al., 2002; Li et al., 2009). In type 2 diabetes, Rab11 plays a role in glucose transporter GLUT4 trafficking from storage vesicles to the endocytic recycling pathway (Kaddai et al., 2008). The majority of intracellular pathogens hijack Rabs involved in endocytic trafficking. Rab11a associates with chlamydial inclusions (a non-acidified vacuole

where the pathogen replicates), increasing thus the number of infectious particles generated (Lipinski et al., 2009; Rzomp et al., 2003). Similarly, Rab11a increases the release of influenza virus particles, therefore affecting the lytic cycle of influenza A virus (Bruce et al., 2010).

## 6.5 FTS/HOOK/FHIP complex

The endogenous cytoplasmic HOOK/FTS/HIP complex, termed FHF, comprises three proteins: "fused toes" (FTS), Hook, and "FTS and Hook-interacting protein" (FHIP) (Mattera et al., 2020; Xu et al., 2008; Yao et al., 2014). The FHF complex was first identified by a proteomic analysis of FTS interacting proteins in human embryonic kidney 293T cells (Xu et al., 2008). In this chapter, we will describe the function of the three components of this complex.

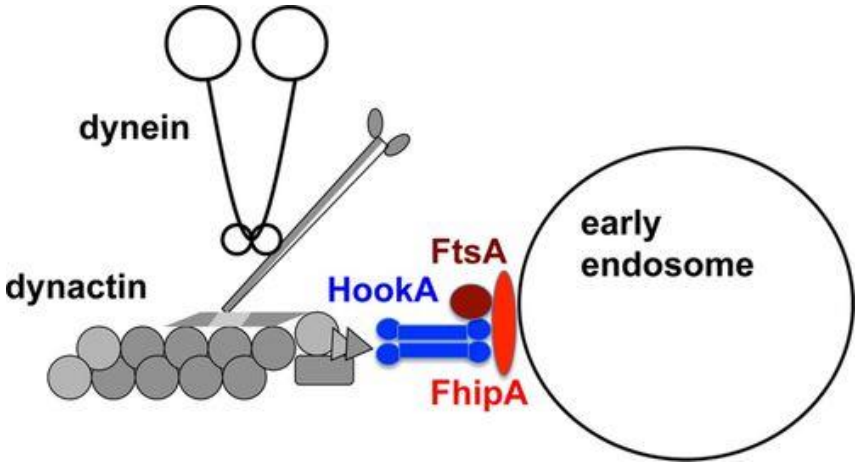



**Figure 33: Schematic representation of the FHF complex in Aspergillus.** Adapted from (Yao et al., 2014). FtsA/HookA/FhipA interact together to form the FHF complex. The C-terminus of HookA (depicted as a dimer in blue) interacts directly with FtsA (brown), while its N-terminus interacts with dynein/dynactin complex. Most likely, FhipA interacts directly with FtsA and with the early endosome. FhipA-HookA interaction involves the C-terminus of HookA, mediating thus HookA-endosome interaction.

### 6.5.1 HOOK

Amongst the eukaryotic endosomal trafficking proteins, the HOOK protein family consists of broadly conserved proteins that contribute to endosomal trafficking. HOOK proteins possess a highly conserved globular N-terminal putative microtubule binding domain, a central coiled-coil motif that mediates homodimerization, and a divergent C-terminal domain thought to mediate cargo binding (Walenta et al., 2001). They are cytoplasmic, and in some cases, they

display an enriched localization with cellular organelles (Walenta et al., 2001). Hook proteins (HookA or Hok1), were first identified as potential dynein adaptors in filamentous fungi (Bielska et al., 2014; Zhang et al., 2014). In the fungus *Ustilago maydis*, HOOK1 contributes to early endosome motility by coordinating dynein and kinesin-3 motors, while in *Aspergillus nidulans*, it was described as an adaptor regulating dynein-mediated early endosome transport (Bielska et al., 2014; Zhang et al., 2014). In both fungal species, HOOK proteins interact with dynein via their N-terminus, and bind to cargo via their C-terminus (Bielska et al., 2014; Yao et al., 2014; Zhang et al., 2014). However, in *Drosophila melanogaster,* HOOK was shown to be important for late endosome formation (Krämer and Phistry, 1999, 1996).

Most eukaryotes encode a single HOOK isoform; however, mammals have three paralogues, which appear to have specific functions and cell tropisms (Dwivedi et al., 2019; Olenick et al., 2018). HOOK1 is implicated in spermiogenesis (Mendoza-Lujambio et al., 2002). HOOK2 protein associates with the centrosome and contributes to the establishment and maintenance of centrosome structure, function, and homeostasis (Guthrie et al., 2009; Moynihan et al., 2009; Szebenyi et al., 2007). HOOK3 localizes to the Golgi and was originally described as a Golgi-associated protein (Walenta et al., 2001). Further, mammalian HOOK1 and HOOK3 have been implicated in a variety of endosomal trafficking pathways (Luiro et al., 2004; Maldonado-Báez et al., 2013; Xu et al., 2008). Indeed, by immunoprecipitation studies, hook1 was shown to interact with Rab7, Rab9 and Rab11; however immunofluorescence assays performed in cells showed a colocalization only between HOOK1 and Rab7 (Luiro et al., 2004). Its interaction with Rab7 and Rab9 suggested a regulatory function of HOOK1 in late endocytic organelle compartments (Luiro et al., 2004). Moreover, it has been demonstrated that HOOK1 interacts with the homotypic vacuolar protein sorting (HOPS) complex to probably promote homotypic fusion and clustering of both early and late endosomes/lysosomes in mammalian cells (Luiro et al., 2004; Richardson et al., 2004). In a distinct study, mammalian HOOK1 was shown to be also implicated in the recycling of specific clathrin independent endocytic (CIE) cargos via endosomes decorated with Rab11 and Rab22 (Maldonado-Báez and Donaldson, 2013) (Figure 34). Recently, a study performed in hippocampal neurons suggested that Hook1 and Hook3 are involved in Rab5 retrograde motility in axons by binding to cargo through C-terminal interactions with FTS and FHIP proteins, similar to fungus (Guo et al., 2016; Xu et al., 2008; Yao et al., 2014). Mammalian

HOOK proteins were also shown to act as adaptor molecules for the molecular motor dynein and play a role in vesicular trafficking by anchoring vesicles to microtubule tracks via their interaction with Rab proteins (Krämer and Phistry, 1996; Maldonado-Báez et al., 2013; Maldonado-Báez and Donaldson, 2013). Thus, HOOK proteins are coupled to the endosomal trafficking pathway, but their precise biological functions are in fact poorly understood.

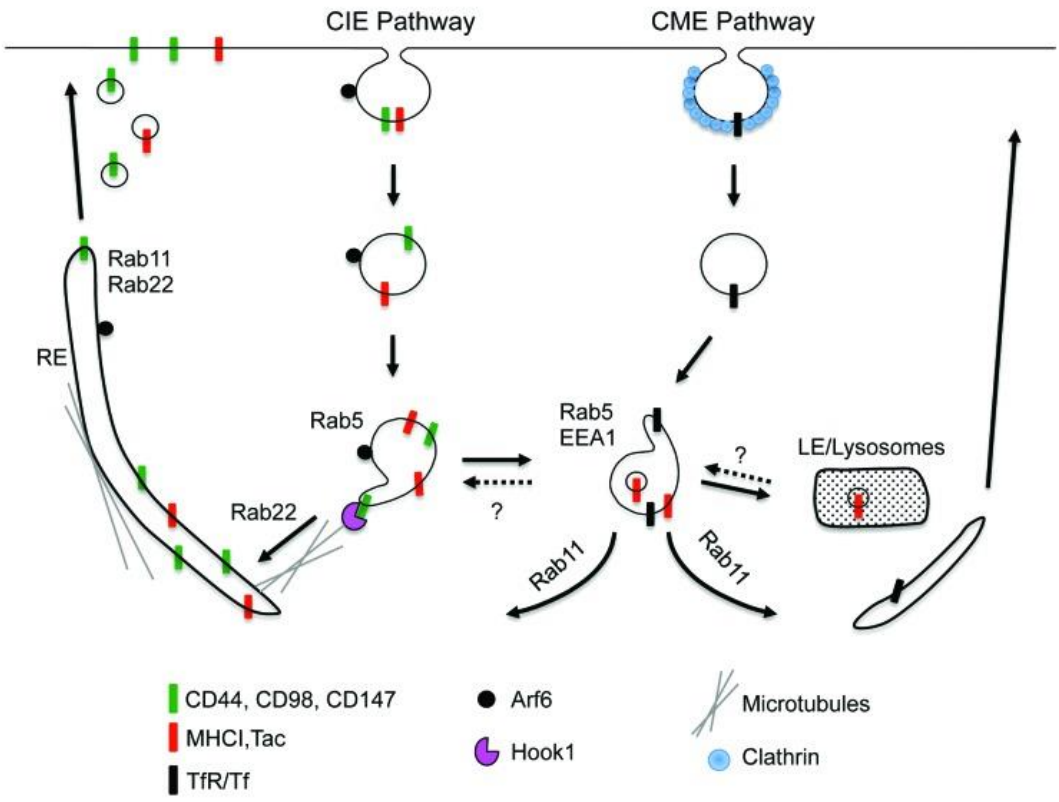



**Figure 34: Role of HOOK1 and Rab11 in clathrin independent endocytosis (CIE).** Adapted from (Maldonado-Báez and Donaldson, 2013). Prototypical CIE cargo proteins (represented by red bars) are internalized in an Arf-6 dependent manner, without the involvement of clathrin. Once internalized, the vesicles mature or fuse to Rab5-EEA1-transferrin positive early endosomes. Cargo proteins are then either recycled back to the plasma membrane in a Rab11/Rab22 dependent manner or directed to late endosomes (LE)/lysosomes for degradation. CIE cargo proteins carrying cytoplasmic sorting motifs (represented by green bars) traffic directly to recycling endosomes (RE), avoiding thus endosomes associated with EEA1 and transferrin. HOOK1 mediates the recycling of CIE cargo proteins, such as CD98 and CD147, via its interaction with microtubules at the level of the RE.

### 6.5.2 FTS and FHIP

The FTS gene was initially identified as one of six genes deleted in a mouse mutant called *Fused Toes* (Lesche et al., 1997). Subsequently, FTS was identified in an interaction

screen with AKT1 and proposed to regulate Akt phosphorylation by PDK1 (Remy and Michnick, 2004). FTS is an inactive variant of an E2 ubiquitin-conjugating enzyme domain-containing protein, known to frequently form relatively tight complexes with other proteins. Xu et al used a proteomic approach and identify FTS-associated proteins in order to elucidate its cellular function (Xu et al., 2008). They demonstrated that the β-sheet surface of the ubiquitin-conjugating domain of FTS interacts with all three human Hook proteins (Hook1, Hook2, and Hook3) via a conserved helix in the C terminus of Hook proteins, to form a stable complex. They also identified an uncharacterized FTS-Hook Interacting Protein (FHIP) and demonstrated that FHIP interacts with the HOOK– FTS complex to form a tightly bound complex referred to as FHF complex (Xu et al., 2008).

It has been elucidated that the FHF complex promotes endosomal trafficking by coordinating vesicle movement, tethering, or both via the homotypic vesicular protein sorting complex (HOPS complex)(Xu et al., 2008). Another study demonstrated that Rab5A interacts directly with FHIP subunit that recruits the FHF complex to endosomes. Rab5 and its effector FHF that interacts with dynein-dynactin, regulate neuronal polarity by promoting retrieval of somatodendritic proteins from the axon (Guo et al., 2016). Moreover, a similar FHF complex was identified in *Aspergillus*. The interaction of all the three members of the complex seems to be critical for dynein-mediated early endosome movement (Yao et al., 2014) (Figure 33). Recently, Bonifacino et al co-purified the multimeric FHF complex and additional proteins with the ε subunit of the adaptor protein AP-4 in tandem affinity purification followed by mass spectrometry (TAP-MS). They uncovered a role of AP-4-FHF complex interaction in the cellular distribution of AP-4 and the transmembrane autophagy protein ATG9A (Mattera et al., 2020).

# 7 Protein trafficking in *T. gondii*

*T. gondii* possesses an endomembrane system composed of a single endoplasmic reticulum (ER) interconnected with the nucleus and a single Golgi stack. However, the existence of an endosomal/lysosomal sytem has not been fully established. It should be noted that in *T.gondii*, materials must be endocytosed across both the PVM and the parasite plasma membrane, unlike yeasts, mammals and plants, where the plasma membrane is the only barrier for endocytic cargoes. Most importantly, the last decade has clearly demonstrated

that *T. gondii* has functionally repurposed its endocytic machinery to the secretory pathway in order to ensure the biogenesis of the apical secretory organelles (Carruthers, 2013; Venugopal and Marion, 2018) (Figure 35). However, some of the mechanisms of protein trafficking described in mammals and yeasts are conserved in *T. gondii*.

## 7.1 *T. gondii* endo-secretory system

This section will describe the anterograde/secretory and the endocytic pathways in *T. gondii*.

### 7.1.1 The anterograde pathway in *T. gondii*

The anterograde route in higher eukaryotes is the route by which the neo-synthesized proteins are transported from the ER via the Golgi, to the plasma membrane. As in mammals, treatment of the parasite with brefeldin A (BFA) inhibits the activation of ribosylation factors (ARF1) (Chardin P and McCormick F, 1999) essential for the maintenance of the Golgi, nuclear envelope and the ER.

- **Trafficking from the ER to the Golgi:** Maintaining the membrane flow between the ER and the Golgi requires recycling of the membrane components. This process is provided by the COPI complex composed of an ARF-GTP protein for association with the Golgi membrane, and COPα; β; β ’; γ; δ; ε; ζ proteins, for the formation of the mantle on the vesicle. A study has shown that, as in mammals, the cytosolic units KKXX and DXE, essential for Golgi-ER transport by COPI and ER-Golgi by COPII, are present in *T. gondii*. However, the patterns allowing the maintenance of neo-synthesized proteins in the ER differ in the parasite (Hoppe and Joiner, 2000).
- **Trafficking from the Golgi to endosomes:** In *T. gondii*, it has been demonstrated that the type 1 transmembrane receptor, *Tg*SORTLR (*T. gondii* Sortilin-Like Receptor) and the clathrin adaptor AP-1 both located at the TGN are key factors regulating the sorting and exit of rhoptry and microneme proteins from the TGN towards the endosomal-like compartments (ELC) (Sloves et al., 2012; Venugopal et al., 2017). In contrast, no precise mechanism governing the sorting and release of GRA proteins from the Golgi has been described. The DrpB protein belonging to the family of dynamins is specific to Alveolates. It is essential for the cleavage of vesicle addressed to micronemes, rhoptries and dense granules (Breinich et al., 2009). Clathrin is a cytosolic protein complex forming a mantle around vesicles (Liu et al., 1995; Wang et

al., 2013). The functional unit is represented by the triskelion formed by three hexameric branches. A clathrin heavy chain 1 (CHC1) gene has been identified in *T. gondii*, and the protein is exclusively localized at the TGN (Pieperhoff et al., 2013). Unlike mammals, the absence of clathrin at the plasma membrane of the parasite raised the question of clathrin-dependent endocytosis in *T. gondii*. For targeting specificity, clathrin associates with the adapter complexes AP-1, AP-2, respectively at the TGN and at the plasma membrane in mammals (Hirst and Robinson, 1998). However, of these two complexes only AP-1 is well conserved and mediates the transport of cargos between the TGN and the endosomal-like compartment (ELC) in *T. gondii* (Carruthers, 2013; Nevin and Dacks, 2009; Venugopal et al., 2017). The AP-3 complex found in *Plasmodium* is exclusively localized at the TGN and is involved in biogenesis of apical organelles (Fomovska et al., 2012). AP-4 is well conserved in *Toxoplasma* and *Plasmodium*, however, AP-5 is only found in *Toxoplasma* (Nevin and Dacks, 2009). Proteins such as Endosomal Sorting Complexes Required for Transport (ESCRT) are also poorly conserved in the phylum, however, the Vps4 subunit of ESCRT III is found in *P. falciparum*, located in the MVB. And when the PfVps4 gene is introduced into *T. gondii*, the protein localizes in early endosomes (Yang et al., 2004).

- **Trafficking from endosomes to apical organelles:** In endosomal compartments, most microneme and rhoptry proteins undergo proteolytic maturation. *T. gondii* has an acidic compartment called VAC (standing for Vacuolar Compartment), which may be homologous to the lysosome in mammals. VAC contains cathepsin-like proteases involved in the maturation of ROP and MIC proteins. In addition, electron microscopy reveals the presence of another plant vacuole-like compartment (Miranda et al., 2010)(Francia et al., 2011). The PLV, “Plant Vacuole-like”, contains *Tg*VP1 pyrophosphatases, *Tg*CPL cathepsins, and *Tg*AQP1 aquaporins. The morphological and functional links between VAC and PLV have not yet been fully demonstrated. The set of specific VAC and PLV compartments and early and late endosomal compartments take the name of "Endosomal-Like Compartment or ELC" in *T. gondii*. The endosomal CORVET (class C core vacuole / endosome tethering) and vacuolar HOPS (homotypic fusion and vacuole protein sorting) complexes (Nickerson et al., 2009; Peplowska et al., 2007; Seals et al., 2000) are essential for endosome transition,

lysosome maturation and endo-lysosomal trafficking (Ostrowicz et al., 2010; Solinger and Spang, 2013). A recent study has shown that Vps11, a key protein in both complexes, is essential for the biogenesis of apical organelles (Morlon-Guyot J et al., 2015). In mammals, the CORVET and HOPS complexes interact respectively with Rab5, the marker of early endosomes and Rab7, the marker of late endosomes. In *T. gondii*, 15 genes encoding Rab proteins have been identified. Most of these proteins reside in the Golgi and ELC compartments. Overexpression of the Rab2, 4, 5A, and 5C proteins affects the growth of the parasite in vitro. The depletion of Rab5A and Rab5C located in the early endosomes, leads to a drastic defect on the localization of ROP proteins and on a sub-population of microneme proteins: MIC3, MIC8, MIC11 (Kremer et al., 2013), which demonstrates that segregation in the transport of apical proteins takes place at the level of the ELC, and is ensured by the function of the Rabs in *T. gondii*. Rhoptries, similarly to micronemes, are formed de novo late during daughter assembly (Nishi et al., 2008). However, in contrast to micronemes, rhoptries are formed first as immature organelles called “pre-rhoptries”, derived from the TGN/ELC that traffic along the classic secretory pathway (Dubremetz, 2007). Little is known about the maturation of pre-rhoptries into mature rhoptries. However, it has been demonstrated that the pre-organelle elongates the rhoptry neck towards the conoid and matures directly into rhoptries (Dubremetz, 2007).

- **Trafficking from TGN to the plasma membrane (PM):** *T. gondii* PM is mainly populated by glycosyl-phosphatidylinositol (GPI)-anchored proteins. Fusing a soluble protein to a signal peptide and either a transmembrane domain or a GPI-anchor signal leads to its targeting to the PM via a constitutive vesicular flow directly from the TGN (Karsten et al., 1998). Contrary, the protein SAG1 deleted from its GPI anchor is targeted to the vacuolar space via dense granule transport, leading to the hypothesis that DG represent the default constitutive secretory pathway in *T. gondii* (Striepen et al., 1998)*)*. The rhomboid protease, *Tg*ROM4, is a transmembrane protein inserted in *T. gondii* PM. It has been suggested that this protein could reach the surface by default, since domain-exchange and truncation attempts did not lead to a region involved in *Tg*ROM4 targeting to the PM (Sheiner et al., 2008). Furthermore, *Tg*ASP1 is a type II transmembrane aspartyl protease located to a novel post-Golgi vesicular

compartment. During replication, *Tg*ASP1 relocalizes to the IMC, suggesting that it might be implicated in the trafficking of proteins to the IMC (Shea et al., 2007).

### 7.1.2 The retrograde pathway in *T. gondii*:

In mammals and yeast, the retrograde pathway is the set of mechanisms allowing the recycling of molecules to the Golgi and the plasma membrane.

- **Endocytosis** is one of the basic mechanisms of vesicular trafficking, consisting of invagination of the plasma membrane resulting from the internalization of extracellular molecules (Trousdale and Kim, 2015). Usually this invagination is clathrin-dependent (AP2), however, there are also non-clathrin-dependent invagination mechanisms involving the protein Endophilin-A2 (EndoA2) in vesicle cleavage (Renard et al., 2015). There is also endocytosis with a calveola mantle composed of proteins called Cavin (Cav1) (Johannes et al., 2015). In the case of degradation of molecules, vesicles travel from the early Rab5 positive endosomal compartment via the positive Rab7 compartment and end up in mature lysosomes or the digestive vacuole, containing proteolytic enzymes. However, in *T. gondii* no classically clathrin-dependent endocytosis mechanism, similar to what is known in mammals, has been described. Recently, it has been shown that the parasite was able to ingest and digest soluble proteins in its cytosol initially present in the cytosol of the host cell (Dou et al., 2014). However, the mechanisms involved in the entry of cytosolic proteins from the host cell into parasites are not known yet.
- **The recycling pathway** allows the essential return of certain transmembrane receptors or transporters from early or late endosomes to the TGN or the plasma membrane. In mammals and yeast, recycling to the TGN is ensured by a protein complex called the Retromer complex, composed of Vps proteins (Vacuolar protein sorting) 26, 29 and 35 and Sorting Nexin (SNX) proteins (Seaman et al., 1998). The three Vps proteins are well preserved in *T. gondii*. *Tg*Vps 35 and *Tg*Vps26 were identified as binding partners of *Tg*SORTLR. In addition, by immunofluorescence, *Tg*Vps26 showed a strong colocalization with *Tg*SORTLR receptor at the level of the TGN (Sloves et al., 2012). Recently, it has been shown that the conditional KO of *Tg*Vps35 affects the biogenesis of all the apical

organelles (Morlon-Guyot J et al., 2015), suggesting that the function of the retromer complex is essential for the parasite. More recently, it has been demonstrated that the *T. gondii* Retromer complex harbors a trimer Vps35-Vps26-Vps29 core complex with the absence of SNX proteins (Sangaré et al., 2016). Moreover, Sangaré et al proved that the retromer complex is crucial for secretory organelle biogenesis and maintaining a proper parasite architecture and integrity. This complex plays a role in the recycling of *Tg*SORTLR, by binding to the C-terminal tail of the latter, between the ELC and the TGN, to ensure proper protein trafficking to secretory organelles rhoptries and micronemes (Sangaré et al., 2016). So far, Rab11A/B GTPases were not shown to be involved in cargo recycling in *T. gondii* however additional studies are required to further explore this aspect of vesicular trafficking in *T. gondii*.

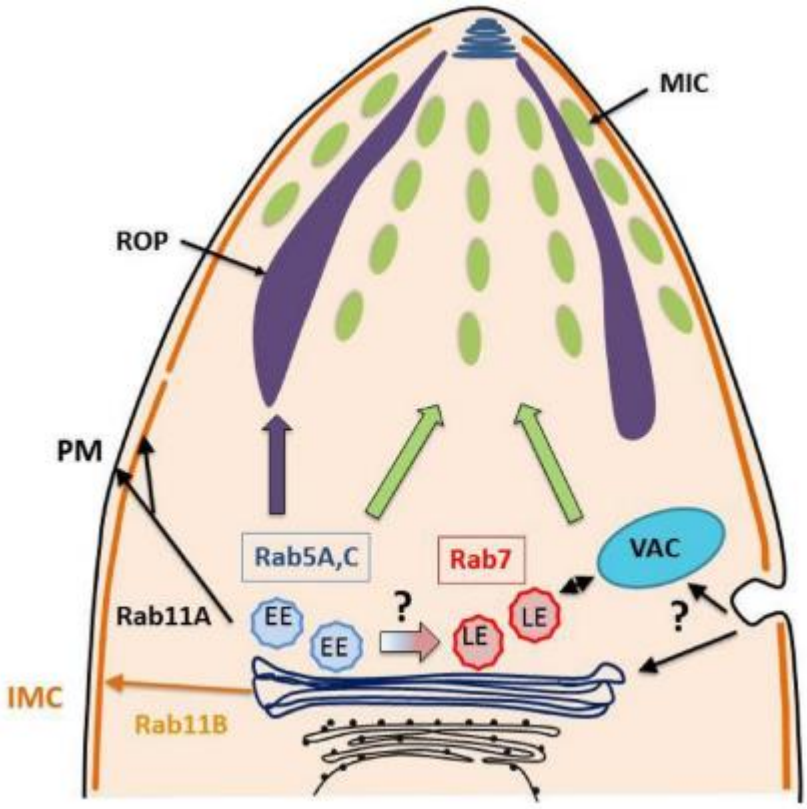



**Figure 35: *T. gondii* uses its endolysosomal system to transport proteins destined for the anterograde pathway.** Adapted from (Venugopal and Marion, 2018). In *T. gondii*, Rab5 and Rab7 localize at early (EE) and late (LE) endosomes respectively in close proximity to the Golgi apparatus, and are implicated in ROP and MIC biogenesis. Unlike in mammalian cells, the transition from EE to LE in *T. gondii* has never been investigated yet. Similarly, to other Eukaryotes, the recycling compartment, containing Rab11A or Rab11B, regulates the secretion of newly synthesized Inner

Membrane Complex (IMC) proteins from the endosomal compartments or from the TGN, to the forming buds of daughter cells and to the plasma membrane (PM) respectively. The digestive vacuole (VAC), localized in proximity to the LE, contains the cathepsin-like protease (CPL) and is implicated in MIC processing.

## 7.2 Dense granule biogenesis and secretion

As mentioned earlier, GRA proteins play a wide-range of functions. For instance, specific GRA proteins are inserted into the PV membrane (PVM) and trigger the recruitment of host cell organelles, such as the Golgi apparatus and the endoplasmic reticulum (ER) ensuring host lipid scavenging by the parasite (Laliberte and Carruthers, 2008; Romano et al., 2012). In addition, such as ROP proteins, GRA proteins can be secreted beyond the PVM to actively modulate host gene expression and immune responses triggered upon infection (Hakimi and Bougdour, 2015; Mercier and Cesbron-Delauw, 2015). Finally, some GRA proteins released into the vacuolar space play an essential role for the establishment of chronic toxoplasmosis by ensuring cyst formation into muscular and neuronal tissues (Mercier et al., 2002; Mercier and Cesbron-Delauw, 2015). While the molecular mechanisms regulating ROP and MIC protein release during parasite invasion have been well studied, the mechanisms regulating GRA proteins biogenesis and exocytosis at the parasite plasma membrane remain unexplored. By contrast to higher eukaryotic cells, immature DG similar to immature secretory granules have never been observed in *Toxoplasma*. Soluble proteins, if supplemented with a GPI signal anchor, are delivered to the plasma membrane via transport vesicles. However, if they were endowed with a signal peptide, soluble proteins are targeted to DG before being secreted into the PV (Karsten et al., 1998). Thus, DGs are considered to be the default constitutive secretory pathway for soluble proteins in *T. gondii*, based on the observation that the SAG1-GFP fusion protein (full product or truncated of its GPI anchor (SAG1ΔGPI)) is transported within DGs before being released into the vacuolar space (Striepen et al., 1998).

It was hypothesized that the mechanism sustaining DG biogenesis is based on the retention and condensation of GRA proteins, due to the similarity of DG with the mammalian version of dense core granules. The existing model of sorting-by-retention observed in higher organisms relies on the selective aggregation of regulated secretory proteins which limits their capacity to escape from maturing granules during constitutive vesicle budding (Arvan

and Castle, 1998). Subtle changes such as mild acidification or an increase in calcium concentration leads to this aggregation of the regulated secretory proteins (Chanat, E. & Huttner, W. B., 1991). The majority of DG proteins are considered as transmembrane proteins, not soluble, upon their secretion into the PV (Lecordier et al., 1999). While the transmembrane domain carrying GRA proteins are trafficked as soluble cargoes to the DG (Labruyere et al., 1999; Lecordier et al., 1999; Sibley et al., 1995), transmembrane surface proteins are delivered to the parasite plasma membrane (Gendrin et al., 2008). It was shown that some GRA proteins interact together to form these aggregates using their N-terminal hydrophilic domain, which is the case for GRA5 and GRA6 (Braun et al., 2008; Gendrin et al., 2008). Moreover, protein aggregation is unlikely due to a decrease in the pH level or to signals motifs within the protein, since DGs were never described as an acidic compartment and the fact that *Tg*AP-1 (localized at the TGN) does not recognize the signature YXXϕ motif (localized in the cytoplasmic tails of GRA4 and GRA7) (Ngô et al., 2003). However, whether any additional interaction of other co-factors intervenes in this process or whether $Ca^{2+}$ plays a role in the regulating it needs to be further explored.

Secretion from DGs can be observed just after invasion is completed. It takes place at the level of the apical part of the parasite, and it was suggested that it could occur at the level of the suture between two plates of the IMC (Carruthers and Sibley, 1997; de Souza, 2005; Dubremetz et al., 1993). This secretion, which occurs simultaneously with the development of PV and its membranous systems, suggests that the particular mechanism which makes it possible to regulate the secretion of DGs is directly linked to the formation of PV. Unlike the secretory granules of eukaryotic cells, DGs seem to store proteins in both soluble and aggregated form (Adjogble et al., 2004; Braun et al., 2008; Labruyere et al., 1999; Sibley et al., 1995). This two-state storage could be the source of two types of secretion: the soluble non-aggregated fraction could be released constitutively; while the aggregated fraction would be released only in response to a signal.

- **The constitutive secretion:** The fusion of the DG with the plasma membrane is likely aided or enhanced by proteins involved in vesicular transport and fusion, namely small GTPases of the Rab family, soluble accessory factors of the NSF (Nethylmaleimide Soluble Factor) machinery, SNARE / SNAP (Soluble NSF Associated Protein) (Chaturvedi et al., 1999), and ARF-1 (ADP-Ribosylation Factor 1) (Liendo et al., 2001).

Furthermore, *Tg*Rab6 appears to indirectly regulate the trafficking of DG since its overexpression prevents the secretion of soluble DG proteins, which are then recycled to the Golgi apparatus and the ER (Stedman et al., 2003).

- **The regulated secretion:** Unlike in mammalian cells where the regulated event of granule fusion with the plasma membrane follows an elevation of intracellular calcium level, in *T. gondii*, calcium stimulation has no effect on the release of DG content (Chaturvedi et al., 1999), even though it initiates the secretion of MIC proteins (Carruthers et al., 1999). Recently, it has been reported that DG release is negatively regulated by cytosolic calcium ion ($Ca^{2+}$), in contrast to microneme exocytosis (Katris et al., 2019). The elevation of $Ca^{2+}$ levels, using a wide range of modulators of both $Ca^{2+}$ and cGMP, leads to a decrease in DG secretion. The use of mutant parasite lines depleted for *Tg*CDPK1 or *Tg*CDPK3, known for their defect in $Ca^{2+}$-dependent secretion, supported the role of cytosolic $Ca^{2+}$ levels in down-regulating DG secretion (Katris et al., 2019). However, incubation of parasites with heat-inactivated serum induces the secretion of DGs (Coppens et al., 1999; Darcy et al., 1988). However, the most common serum proteins such as bovine serum albumin, IgG, transferrin, and various cytokines do not induce DG secretion (Coppens et al., 1999).

In summary, the secretion of DG content could depend on two distinct mechanisms based on the level of the granular material compaction. It could lead to either a constitutive mechanism, dependent on ARF-1, during intracellular parasitic development (Liendo et al., 2001), or to a mechanism regulated by an unknown mechanism, totally independent of ARF-1, ensuring the rapid secretion of DG proteins when these proteins are required to constitute a functional PV, notably at the onset of parasite invasion and PV formation.

## 7.3 *T. gondii* Rab11

In contrast to humans, which express over 70 Rabs, *T. gondii* possesses a limited number of 13 Rabs that include two isoforms of Rab11: Rab11A and Rab11B (Kremer et al., 2013). As mentioned earlier, Rab11 is involved in trafficking of recycling endosomes in Eukaryotes. The function and localization of *Tg*Rab11A and *Tg*Rab11B were characterized in the *T. gondii*. Little is known about the role played by *Tg*Rab11A in host cell invasion and replication in apicomplexan parasites, and the molecular mechanisms regulating these processes remain

undefined. Although Rab11 was first found to be associated with parasite rhoptries by a proteomic analysis (Bradley et al., 2005), another study showed that this interaction is highly dynamic (Agop-Nersesian et al., 2009). In this latter study, a Rab11A strain tagged N-terminally with cMyc and mCherry was overexpressed under the control of the destabilizing domain ddFKBP (called DD) and upon the induction with shield-1 (Agop-Nersesian et al., 2009; Herm-Götz et al., 2008). This work showed that *Tg*Rab11A not only partially colocalizes with rhoptries, but also with the pro-peptide of MIC2 associated protein (M2AP), a marker of the endosome-like compartments. Furthermore, alteration of *Tg*Rab11A function, using an overexpressed mutated (GTPase domain – N126I) inactive form of the protein, does not affect the biogenesis and trafficking of apical secretory organelles (rhoptries and micronemes), nor the biogenesis of DG. The only defect observed was in the completion of IMC formation in daughter parasites leading to a block at a late stage of cell division. In addition, *Tg*Rab11A was found to be required for SAG1 delivery at the parasite plasma membrane (Agop-Nersesian et al., 2009), suggesting a role for *Tg*RAb11A in constitutive secretion.

*Tg*Rab11B location is highly dynamic and depends on the cell cycle of the parasite. It is accumulated at the Golgi close to the nucleus at the initial phase of cell division. While during cell division, *Tg*Rab11B accumulated at the growing IMC of the nascent daughter cells. *Tg*Rab11B function was determined using an overexpressing mutated inactive form of the protein. The ablation of *Tg*Rab11B impairs the IMC formation leading to an impairment in daughter cell budding (Agop-Nersesian et al., 2010). However, no defect in sub-pellicular microtubules or conoid formation was observed, indicating that both processes are not mechanistically linked. All these results together suggested a role of *Tg*Rab11B in the transport of Golgi derived vesicles to the nascent IMC of daughter cell (Agop-Nersesian et al., 2010).

# Objectives

The apicomplexan parasite, *Toxoplasma gondii,* is characterized by the presence of specific secretory organelles: the rhoptries, micronemes and dense granules that sequentially release their content into the host cell enabling parasite invasion and replication into a parasitophorous vacuole. Dense granule proteins, which are constitutively secreted during parasite replication, are key players ensuring parasite survival and dissemination by modulating host signaling pathways. However, the mechanisms regulating their biogenesis and exocytosis at the parasite plasma membrane remain unknown. In general, despite being crucial for the development of the infection, the mechanisms regulating exchanges between the parasite and its external environment (including endocytosis of host material) have been poorly investigated. In mammalian cells, Rab11 is located at the Trans-Golgi and in recycling endosomes and regulates distinct steps of vesicular trafficking by associating with many different effectors: vesicular budding from the donor compartment, vesicular transport by binding to molecular motors (such as myosin/dynein/kinesin), vesicle anchoring by binding to tethering complexes (such as exocyst complex components), and vesicle fusion by binding to SNAREs proteins. Rab11 has been shown to be involved in numerous biological processes such as cell division and cell migration, notably by regulating exocytosis of key regulators factors (Takahashi et al., 2012). In *T. gondii*, Rab11A was shown to also regulate cytokinesis as well

as the delivery of the GPI-anchored surface antigen SAG1 at the plasma membrane of the parasite, suggesting a role of Rab11A in the constitutive secretory pathway in *T. gondii*.

Thus, in a first part of my PhD project, we investigated a putative role of *Tg*Rab11A in dense granule secretion and plasma membrane protein delivery to the parasite surface. We also aimed to elucidate the mechanisms by which *Tg*Rab11A regulates those processes, notably whether *Tg*Rab11A modulates vesicle transport and/or vesicle docking/fusion with the parasite plasma membrane.

Moreover, at the molecular level, Rab11 is known in mammalian cells to regulate the exocytic process by stimulating the docking and subsequent fusion of vesicles to the plasma membrane via its binding to the exocyst complex subunit Exo70 (Takahashi et al., 2012). However, the exocyst complex components are not encoded in the genome of *T. gondii* and in Apicomplexa as a whole (Klinger et al., 2013). Hence, the question remains opened on how exocytosis is regulated in these parasites.

A previous study in the lab identified a novel *Tg*Rab11A binding partner, presenting a unique HOOK domain, that we called *Tg*HOOK. This protein was found to localize in vesicles spread in the parasite cytosol but enriched in the apical region with a strong accumulation at the parasite apical tip. The HOOK protein family consists of broadly conserved proteins that contribute to endosomal trafficking by acting as adaptors between vesicular cargos and the molecular motor dynein (Olenick et al., 2016). Interestingly, in *T. gondii*, the sub-pellicular microtubule network originates from the conoid placing the minus end of the microtubules at the apical tip of the parasite, where dynein accumulates. Thus, at the beginning of this project, we proposed a working model in which the *TgR*ab11A-*Tg*HOOK interaction would regulate apically polarized secretory events, occurring notably during parasite adhesion and invasion of host cells in a regulated manner. This second part of my thesis project was performed in close collaboration with the laboratory of Dominique Soldati-Favre (Geneva University), notably a PhD student from her lab, David Dubois.

# Materials and Methods

# Chapter II – Materials and Methods

## 1 Cell culture

### 1.1 Culture maintenance and growth of host cells and parasites:

Tachyzoites were maintained *in vitro* in monolayers of confluent human foreskin fibroblasts (HFF) cells cultured in complete Dulbeccos's Modified Eagles Medium (DMEM) GlutaMAX-1 (GibcoLife Technologies) supplemented with 10% Fetal Bovine Serum (FBS) (GibcoLife Technologies), and 1% Penicillin-Streptomycin (GibcoLife Technologies). *Toxoplasma gondii parasites* were grown in ventilated tissue culture flasks at 37°C in a 5% $CO_2$ incubator. HFF passaging is done by trypsinisation of the monolayer with 0.25% Trypsine-EDTA solution (Gibco).

Fully egressed parasites were then passed in a new T25 flask containing fully grown HFF. Since mechanical lysis is required for some experiments, infected host cells were scraped and lysed by sequential syringe passage with 17-gauge and 26-gauge needles and filtration through a 3-µm Whatman membrane filter to separate parasites from host cell debris. The filtered parasites are washed in a sterile 1X PBS solution then counted in a Malassez cell for the different applications. In most cases, parasites were centrifuged at 2200rpm for 10 minutes at room temperature or at 4°C if needed.

All the parasites used in this work are type I RH parasites in the tachyzoite stage. Three so-called "parental" parasitic strains were used to generate different mutants: the RHΔhxgprtΔku80 strain (a strain deleted for the ku80 gene promoting homologous recombination (Huynh and Carruthers, 2009)), the RHΔhxgprtΔku80 TATi strain (combining a high rate of homologous recombination and an tetracyclin (ATc) inducible system (Sheiner et al., 2011)), and the RHΔhxgprt Δku80 Tir1 strain (combining a high rate of homologous recombination and an Auxin-inducible degradation (AID) system (Brown et al., 2018)). The different strains used are listed in Table 3.

**Table 3: List of T. gondii strains used in the study**

| Strains | Genotype | Origin |
|---|---|---|
| mcherryRab11A-WT | Knock-In (KI) RHΔhxgprtΔku80; DDFKBP-cMyc-mcherry-Rab11A-WT; HXGPRT | Transfected In house |
| mcherryRab11A-DN | KI RHΔhxgprtΔku80; DDFKBP-cMyc-mcherry-Rab11A-DN; HXGPRT | Transfected In house |
| HOOK-KO | Direct Knock-Out (KO) RHΔhxgprtΔku80; gRNA for HOOK KO (pSAG1::CAS9-GFP-U6::sgHOOK1) | Soldati-Favre D |
| cMycHOOK-iKD | Inducible Knock-down (iKD) RHΔhxgprtΔku80 Tati; gRNA for iKO tet system (pSAG1::CAS9-GFP-U6::sgHOOK2); PCR 5'COR-pT8TATi1-HX-tetO7S1myc | Soldati-Favre D |
| cMycHOOK-iKD / FTS-HA | cMycHOOK-iKD strain (raw above) transfected with plicFTS-HA, DHFR | In house |
| FTS-mAID-HA | AID RHΔhxgprt Δku80 Tir1; gRNA for FTS-mAID-HA (pSAG1::CAS9-GFP-U6::sgFTS) ; PCR FTS-YFP-mAID-HA-HX | Soldati-Favre D |
| HIP-mAID-HA | AID RHΔhxgprt Δku80 Tir1; gRNA for HIP-mAID-HA (pSAG1::CAS9-GFP-U6::sgHIP) ; PCR HIP-YFP-mAID-HA-HX | Soldati-Favre D |
| HOOK-HA | KI RHΔhxgprtΔku80; plicHOOK-HA, DHFR | In house |
| HOOK-HA / APR1-2Ty | HOOK-HA strain (raw above) transfected with gRNA for APR1-2Ty (pSAG1::CAS9-GFP-U6::sgAPR1); PCR APR1-2Ty-HX | In house + Soldati-Favre D |
| FTS-cMyc | KI RHΔhxgprtΔku80; plicFTS-cMyc, HX | In house |
| FTS-cMyc / APR1-2Ty | FTS-cMyc strain (raw above) transfected with gRNA for APR1-2Ty (pSAG1::CAS9-GFP-U6::sgAPR1); PCR APR1-2Ty-DHFR | In house |
| FTS-HA | KI RHΔhxgprtΔku80; plicFTS-HA, DHFR | In house |
| cMycDLC8a-iKD | Inducible Knock-down (iKD) RHΔhxgprtΔku80 Tati; gRNA for iKO tet system (pSAG1::CAS9-GFP-U6::sgDLC8a); PCR 5'COR-pT8TATi1-HX-tetO7S1myc | Soldati-Favre D |

# 2 Molecular Biology:

## 2.1 Genomic parasite DNA extraction

Genomic DNA (gDNA) was isolated from type I RH*Δku80* parasite strain using Promega Wizard genomic DNA purification kit. Nuclei Lysis solution was added to the pellet of freshly egressed parasite. Once resuspended, RNase was added to it and incubated at 37°C for 15-30 minutes. Next, protein precipitation solution was added at room temperature, and the mix was vortexed briefly and incubated on ice for 5 minutes. Then, the mix was centrifuged at 14000rpm for 4 minutes at 4°C. The supernatant was transferred to a new eppendorf tube containing isopropanol to precipitate the DNA, then mix and centrifuge at 14000rpm for 30 minutes at 4°C. The supernatant was discarded, and the pellet was washed with 70% ethanol, and centrifuged at 14000rpm for 30 minutes at 4°C. The ethanol was then discarded, and the pellet was left to dry completely at room temperature. The genomic DNA was suspended in 40µl Milli Q water and allowed to dissolve before measuring the DNA concentration using nanodrop spectrophotometer (GE Healthcare) and stored at -20°C for future use.

## 2.2 Genetic engineering

### 2.2.1 List of plasmids used in our study

**Table 4**: **List of plasmids from external labs used for parasite transfection**

| **Plasmid** | **Laboratory** |
|---|---|
| DD-cMyc-mcherry-Rab11A-WT | Meissner M(Kessler et al., 2012) |
| DD-cMyc-mcherry-Rab11A-DN | Meissner M(Kessler et al., 2012) |
| IMC3-YFP | Gubbels MJ |
| SAG1ΔGPi-GFP | Heaslip A (Heaslip et al., 2016) |
| pNTP3-GT1-HA | Blume M (Blume et al., 2009) |
| pT8-ROM4-Ty | Soldati-Favre D |
| Rab5-HA | Carruthers V |
| Rab7-HA | Carruthers V |
| pLinker-APR1-2Ty | Soldati-Favre D |
| pSAG1::CAS9-GFP-U6 | Sibley LD (Shen et al., 2014a) |
| pGEX6P3-GST-Rab5 | In house (Sangaré et al., 2016) |
| pGEX6P3-GST-Rab7 | In house (Sangaré et al., 2016) |

| | |
|---|---|
| pGEX6P3-GST-Rab11B | In house (Sangaré et al., 2016) |

### 2.2.2 List of primers generated by our lab and used in our study

**Table 5: List of primers used in this study.**

| **Oligonucleotides** | |
|---|---|
| CGGggatccGAACAAAAACTCATCTCAGAAGAGGATCTGATGGCG GCTAAAGATGAATACTACG | Fw primer for GST-Rab11A |
| gcggccgcTCAGGCGGAACAGCAGCCAC | Rev primer for GST-Rab11A |
| GGGACCCCTCCGCCGTGGAGAGTTAAAAGCGCTAGCAAGGGCTC GGG | Fw primer for C-terminus KI of APR1-Ty in plicFTS-cMyc |
| CAAAAACTGATACCGAGTGTCGCACTGGCAATACGACTCACTATA GGG | Rev primer for C-terminus KI of APR1-Ty in plicFTS-cMyc |
| GAGGGCGAAAGCGGTTGCCTTCGGgttttagagctagaaatagc | gRNA for FTS iKD tet system pSAG1::CAS9-GFP-U6::sgFTS |
| CGCCACAGGAAAGGCAGGGTCTTGCCCGTCcatgtttgcggatccgggg | Fw primer PCR for FTS iKD Tet system (HXGPRT) |
| GCTACAGCTGAGAGCTCCGTGACTTCGATGCAGGTCCTCCTCGGA GATGA | Rev primer PCR for FTS iKD Tet system (HXGPRT) |
| tacttccaatccaatttagcGCAAAGATGACATGGCGAAGCAGATGATG | Fw primer for plicHOOK-HA (DHFR) |
| tcctccacttccaattttagcCGCCTCCCGAGGTGTGACAGAATC | Rev primer for plicHOOK-HA (DHFR) |
| TACTTCCAATCCAATTTAGCATTCCGTCATGGGACAATCTTCGAG | Fw primer for plicFTS-HA (DHFR) and plicFTS-cMyc (HXGPRT) |
| TCCTCCACTTCCAATTTTAGCTTCGGCGTTGAAGAGGTTGGCGCC | Rev primer for plicFTS-HA (DHFR) and plicFTS-cMyc (HXGPRT) |
| ATACCGTTTCGCATCCTAGCGTTTTAGAGCTAGAAATAGCAAGTT AAA | 5’ gRNA for FTS-KO pSAG1::CAS9-GFP-U6::sgFTS1 |

| | |
|---|---|
| GTTTGGGTCGGAAGGCAGTACGTTTTAGAGCTAGAAATAGCAAGTTAAA | 3' gRNA for FTS-KO pSAG1::CAS9-GFP-U6::sgFTS2 |
| GCCCTTTCGTCTTCCAAGAAAACTGGTTGGTCACGAGCCGGATCCATTATGCGTGA | Fw primer PCR for FTS KO (HR1-FTS-dhfr) |
| CCGAGAGACCTGCTTCGGATAATTATTCGCTGAGCTACTAGTGGATCGATCCCCCG | Rev primer PCR for FTS-KO (HR2-FTS-dhfr) |
| AACTTGACATCCCCATTTAC | Cas9 generic Rev |

## 2.3 Cloning methods

The gDNA mentioned above in section 2.1 is used for all the amplifications necessary for the construction of plasmids. All primers used for the PCRs are listed in Table 4. The PCRs were carried out using the enzyme Phusion® High-Fidelity DNA Polymerase (NEB) according to the manufacturer's recommendations and the following parameters:

| | | | |
|---|---|---|---|
| Initial Denaturation | 95°C | 5mins | |
| Denaturation | 95°C | 30sec | 25-35 cycles |
| Annealing | Primer dependent (≈ 50-65°C) | 30sec | |
| Elongation | 72°C | 30sec/Kb | |
| Final elongation | 72°C | 10mins | |
| Storage | 4°C | ∞ | |

Agarose gel electrophoresis separates DNA fragments based on fragment sizes. It was performed to verify PCR amplifications and all subsequent DNA analysis steps.

DNA fragments were purified using NucleoSpin Gel and PCR Cleanup kit (Machery Nagel) after PCR amplification or restriction digestion. Throughout this study, the restriction enzymes used, and their respective buffer were supplied by NEB®.

## 2.4 Schemes describing the different molecular cloning strategies used in our project

- **ddFKBP inducible over-expression system**

Rab11A regulated over-expression was achieved using the ddFKBP also called DD system (Herm-Götz et al., 2008). The plasmids coding for both Rab11AWT and Rab11ADN (containing the point mutation N126I in the GTPase domain) under the DD system are gifts from Dr Markus Meissner. Briefly the gene of interest (Rab11A) was placed under the influence of the alpha tubulin based p5RT70 promoter and the DD destabilization domain, which is followed by a cmyc epitope tag alone or together with a fluorescent mCherry tag. When transfected, the plasmid integrates randomly into the genome. Following MPA (mycophenolic acid) and Xanthine drug selection against the HXGPRT selection marker, parasites were cloned. In the absence of Shield-1, the recombinant protein is targeted to the proteolytic degradation pathway. When the synthetic ligand Shield-1 is added, the protein expression is stabilized and accumulated in the parasites over time and in a Shield-1 dose-dependent manner.

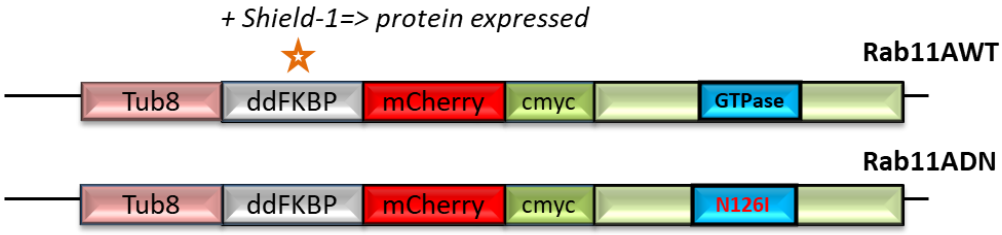



**Figure 36:** Schematic diagram of the plasmids used to generate the DD-Rab11AWT and DD-Rab11ADN strains.

- **Endogenous gene tagging using the pLIC system or "Knock-In" (KI)**

In the laboratory, KIs are performed using the LIC (Ligation Independent Cloning) strategy (Huynh and Carruthers, 2009). This consists of amplifying by PCR the 1-2 kb sequence upstream of the stop codon of the target gene containing a unique restriction site (not present in the pLIC vector) to allow its integration into the genome by simple homologous recombination.

The PCR product (gene of interest) amplified using the primers listed above, was ligated into the pLIC plasmid previously linearized with the PacI restriction enzyme. The unique linearization site in the middle of the gene of interest was then used to linearize the pLIC plasmid containing the sequence of interest. Upon DNA transfection, the integration into the genome is achieved by a single homologous recombination event in the RHΔKu80 parasite

strain. This strategy was used to generate the following parasite lines: HOOK-HA, FTS-cMyc, and FTS-HA.

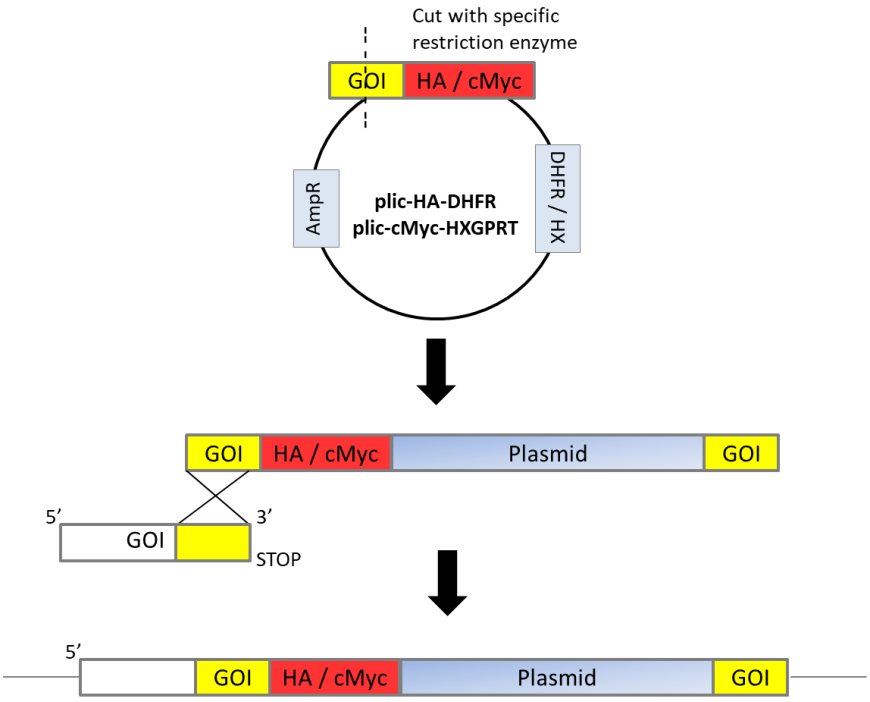



**Figure 37:** Schematic diagram of the strategy used to generate the HA or cMyc endogenously tagged KI parasitic lines.

- **Direct Knockout (KO) using the CRISPR/Cas9 system**

The CRISPR/Cas9 technology was used by the laboratory of Dominique Soldati-Favre to disrupt the *Tg*HOOK gene in RHΔhxgprt Δku80 strain via non-homologous DNA repair. For that, a guide RNA (gRNA) targeting the *Tg*HOOK gene was generated and inserted in the CRISPR/Cas9 plasmid following the method described by Sidik et al (Sidik et al., 2014). The successful transfection of the plasmid led to a frame shift mutation leading to a premature stop codon (Shen et al., 2014a).

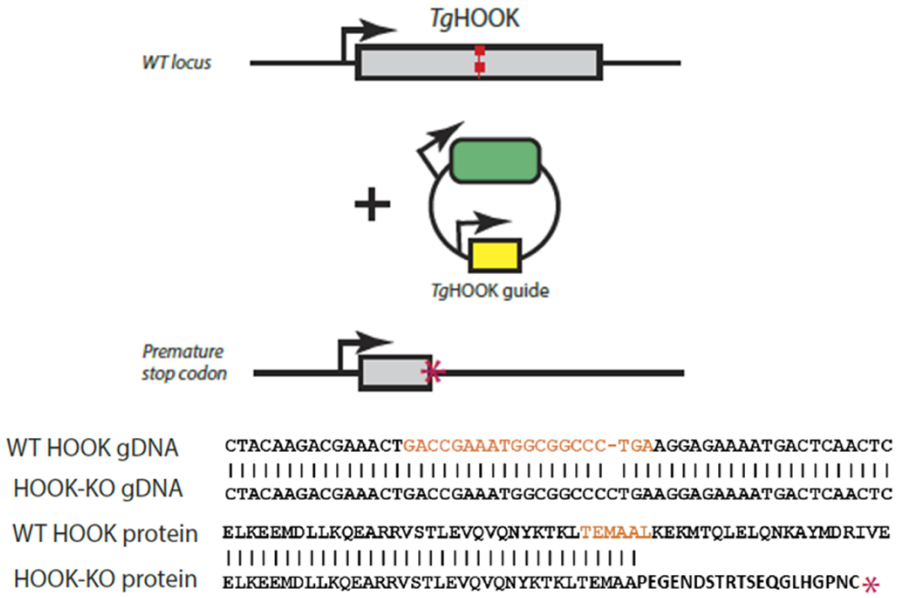



**Figure 38:** Schematic diagram of the strategy used to generate the HOOK-KO strain.

- **Gene KO using the double CRISPR/Cas9 gRNA system and cassette replacement**

This technique is used to generate clean KO using two gRNAs targeting the 5' and 3' ends of the coding sequence of the gene of interest (GOI) to facilitate the insertion of the cassette. Simultaneously, a PCR amplicon containing either the hypoxanthine-xanthine-guanine phosphoribosyl transferase (HXGPRT) or the dihydrofolate reductase (DHFR) selection cassette flanked by short homology regions localized at the 5' and 3' ends of the gene of interest is generated. Upon successful transfection of the plasmid and the PCR amplicon, the entire coding sequence is replaced by the selection cassette.

I tried this technique to generate a KO parasite strain for the *Tg*FTS gene using the gRNA and primers listed above in Table 5. The CRISPR/Cas9 plasmid containing the two gRNA and the PCR amplicon encoding for the DHFR cassette were successfully generated and transfected in the cMycHOOK iKD strain. I succeeded in obtaining a non-clonal population 48hrs after transfection, but we did not succeed in isolating positive clones over the selection period, likely reflecting the essentiality of the gene for the parasite lytic cycle. We also attempted to generate an inducible knock-down of *Tg*FTS by using the tetracycline repressor-based inducible knock-down system (see below). Meanwhile, the laboratory of Dominique Soldati-Favre (our collaborator) had generated the inducible mutant FTS-mAID-HA and HIP-mAID-HA strains using the AID system explained later on.

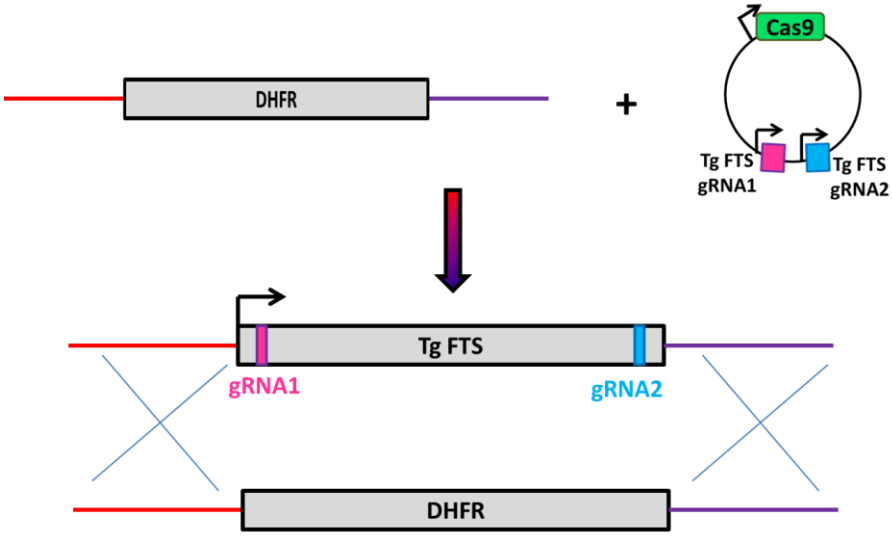



**Figure 39:** Schematic diagram of the strategy used to generate the FTS-KO strain.

- **Tetracycline Repressor-Based Inducible Knock-Down**

This approach (described in Jacot and Soldati-Favre, 2020) was applied by the laboratory of Dominique Soldati-Favre to generate the cMycHOOK-iKD strain. We also used it to generate the cMyc*Tg*FTS-iKD parasite strain. We generated the CRISPR/Cas9 plasmid containing the gRNA for FTS iKD Tet system (listed above) and the PCR fragment encoding a constitutively expressed TATi-1 cassette, an HXGPRT selection cassette, a tetO-inducible promoter and an N-terminal Myc epitope-tag. We failed in obtaining positively transfected parasites. However, meanwhile the lab of D. Soldati-Favre had generated the FTS-mAID-HA strain.

This technique consists on generating a gRNA targeting the 5'end of the GOI directly before the start codon (ATG) and inserting it in the CRISPR/Cas9 plasmid. Simultaneously, a PCR fragment encoding a constitutively expressed TATi-1 cassette, an HXGPRT selection cassette, a tetO-inducible promoter and an N-terminal Myc epitope-tag is generated. The double homologous recombination at the locus of interest is triggered following the transfection of both the CRISPR/Cas9 plasmid and the PCR amplicon in the RHΔhxgprt Δku80 strain. Upon successful recombination, the addition of anhydrotetracycline (ATc) induces a conformational change in TATi-1, resulting in its dissociation from tetO, thereby silencing the expression of the gene.

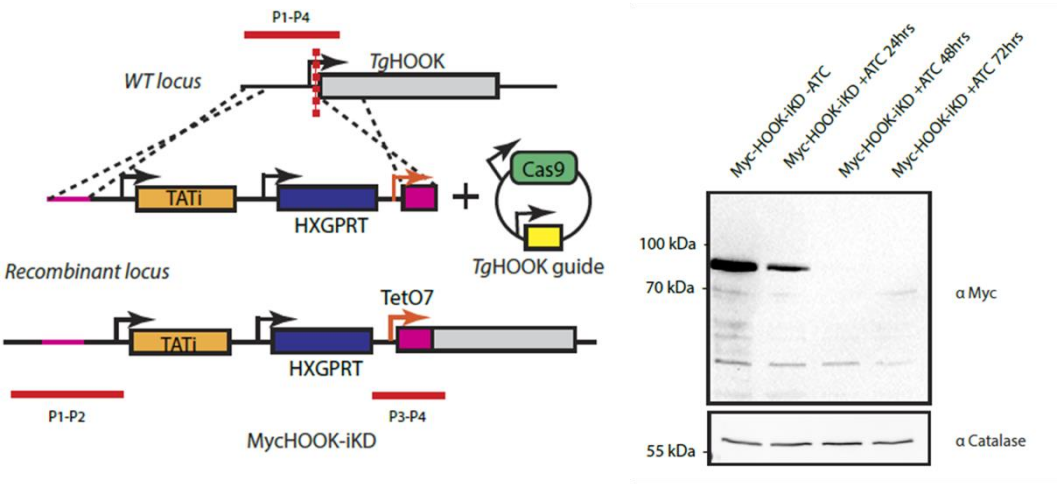



**Figure 40:** Schematic diagram of the strategy used to generate the cMycHOOK-iKD strain (left). The regulation of cMycHOOK-iKD was assessed by WB. After 48 hours ±ATc the protein was no longer detectable. Catalase was used as a loading control (right).

- **Auxin Induced Degron (AID) system**

The AID system was applied by the laboratory of Dominique Soldati-Favre to generate both FTS-mAID-HA and HIP-mAID-HA strains. This consists of generating a gRNA that cuts at the 3'end of the gene and insert it in the CRISPR/Cas9 plasmid. Following this step, a gene-specific miniAID (mAID) tagging cassette is amplified by PCR. The tagging amplicon contains a 5' homology flank-linker-(m)AID-3HA and Floxed HXGPRT-3' homology flank. Upon CRISPR/Cas9 plasmid (containing the specific gRNA sequence) and PCR transfection, a C-terminal mAID is added to the endogenous locus of *Tg*FTS and *Tg*HIP in a Transport Inhibitor Response 1 (Tir1) expressing strain to target the protein for proteosomal degradation upon the addition of auxin indole-3-acetic acid (IAA).

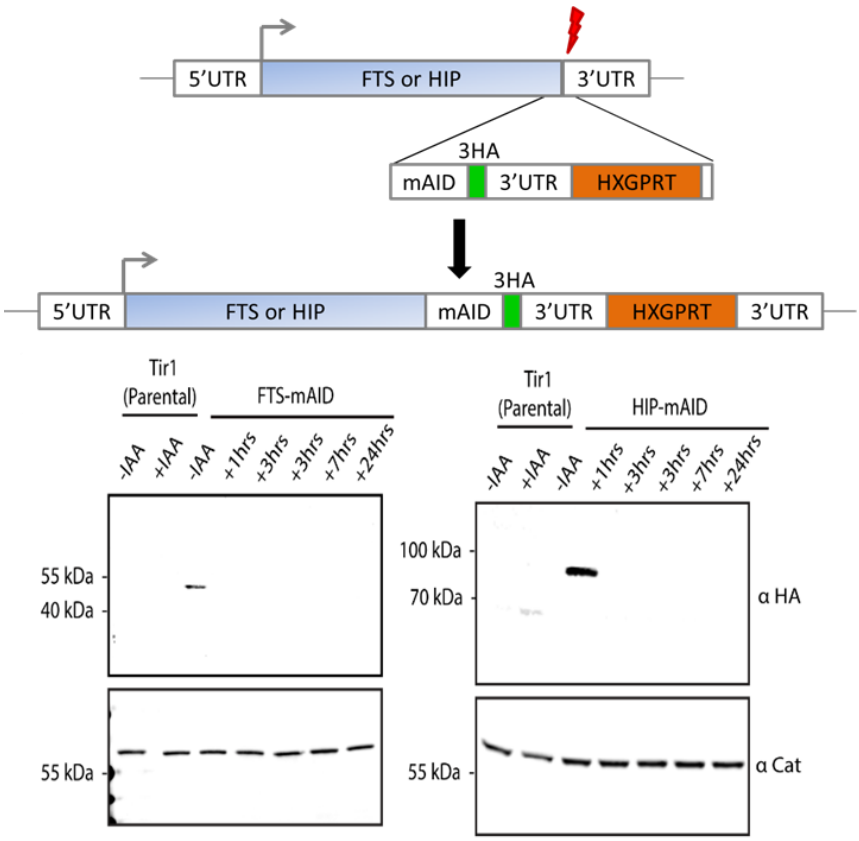



**Figure 41:** Schematic diagram of the strategy used to generate the FTS-mAID and HIP-mAID strains (up). The regulation of FTS-mAID and HIP-mAID was assessed by WB. After 1 hour ±IAA, the protein FTS was no longer detectable, while the protein HIP was no longer detectable after 3 hours ±IAA. Catalase was used as a loading control (down).

## 2.5 Parasite transfection

Parasite transfection is carried out by electroporation of $10^6$ freshly lysed and purified tachyzoites. The plasmids are precipitated with sodium acetate then taken up in a transfection solution called cytomix (120mM KCl; 10mM K2HPO4 / KH2PO4 pH7.4; 25mM HEPES pH7.6; 2mM EGTA pH7.6; 5mM MgCl2; 0.15mM CaCl2; at final pH 7.6). In parallel, the parasites are resuspended in cytomix supplemented with 5 mM of reduced glutathione (GSH) and 2 mM ATP. The plasmid is added to the parasitic suspension in a tank to allow electroporation using a BTX Electro Cell Manipulator 600 according to the following parameters: a voltage of 1.5kV.cm-1, a capacitance of 25µF and a 24Ω resistance.

Transient or stable transfections were performed in $10^6$ parasites with 50 µg of the plasmids indicated in table 1 and parasites were allowed to invade HFF cells for 24 h prior analysis.

## 2.6 Drug selection and cloning of transgenic parasites

The transfected parasites are cultured on HFFs and selected according to their resistance cassette. Drugs allowing the selection of mutant parasites are added to the culture medium 3 hours post-transfection as follows:

• DHFR (DiHydroFolate Reductase): selection of parasites for 3 days in the presence of 2µM of pyrimethamine (Sigma),

• HXGPRT (Hypohanthine Xanthine Guanine PhosphoRibosylTransferase): selection of parasites 5 days in the presence of 25µg/mL of mycophenolic acid (Eurogentech) and 50µg/mL of xanthine (Sigma),

• CAT (Chloramphenicol AcetylTransferase): selection of extracellular parasites 4 weeks in the presence of 34mg/ml of chloramphenicol (Sigma).

The efficiency of transfection in the non-clonal population is verified by immunofluorescence (IFA) using antibodies directed against the different tags added to the genes of interest when possible. The clonal lines are obtained by limiting dilution after depositing a parasite per well in a 96-well plate. The parasites are cultured for 7 days and then screened by IFA. The positive clones were passed into T25 flasks and continued in cultures for experimental analyses and frozen using freezing medium (10% DMSO in FBS) as stocks for future use.

# 3 Cell biology:

## 3.1 Immunofluorescence assays (IFA)

Infected confluent HFF monolayers where fixed with 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS) for 20 minutes, before being quenched with 50mM $NH_4Cl_2$ for 15 minutes, permeabilized and blocked with 0.1% Triton or 0.05% saponin dissolved in 5% BSA-PBS for 30 minutes. Samples were then incubated with primary antibodies in 0.1% triton or 0.05% saponin dissolved in 2% BSA-PBS for 1hour, followed by 3 washes with PBS. Goat anti-mouse, goat anti-rabbit or goat anti-rat immunoglobulin G secondary antibodies conjugated to Alexa Fluor488 or Alexa Fluor594 or Alexa Fluor647 (Molecular Probes, Invitrogen) were then added to the coverslips for 30 minutes. Images were acquired using using Zeiss LSM880 confocal microscopy equipped with an airyscan module at 63X magnification. All images were processed using Carl Zeiss ZEN software.

Antibodies used for IFA experiments are the following: rabbit anti-HA (Cell Signaling Technology), mouse anti-cMyc (Thermo Scientific), rat anti-cMyc (Abcam), mouse anti-SAG1 (in house), rabbit anti-GAP45 (D. Soldati-Favre), mouse anti-MIC2 (JF Dubremetz), rabbit anti-M2AP (V. Carruthers), mouse anti-ROP 2-3 (from J.F. Dubremetz), rat anti-SORTLR (in house), mouse anti-tubulin (Sigma Life Sciences), mouse anti-ProM2AP (V. Carruthers), mouse anti-Rab11A (In house), mouse anti- IMC3 (Gubbels, M.J.), mouse anti-GRA1 (Biotem), mouse anti-GRA2 (Biotem ), rabbit anti-GRA3 (JF Dubremetz), mouse anti-GRA5 (Biotem), rabbit anti-RON4 (M. Lebrun), rabbit anti-Eno2 (in house), mouse anti-Ty (D. Soldati-Favre).

## 3.2 Plaque Assay

HFF monolayers were infected with 100µL of serially diluted parasites (1/100, 1/1000 and 1/10000) and allowed to proliferate for 7 days ± IAA. Plaques were fixed in 4% paraformaldehyde, 0.05% glutaraldehyde (PAF-Glu), 10 minutes quenched in 0.1M glycine-PBS and subsequently stained with Crystal Violet (Sigma-Aldrich) for 10min. Data are representative of three independent biological experiments.

## 3.3 Parasite intracellular growth assay

DDFKBP-Myc-mCherryRab11A-RHΔku80 (from here on designated as Rab11A-WT) and DDFKBP-Myc-mCherryRab11A-DN-RHΔku80 (from here on designated as Rab11A-DN) were allowed to invade HFF monolayers for 1h then treated with or without 1µM of shield-1 for additional 20h, before fixation with 4% paraformaldehyde (PFA).

RHΔku80Tati and cMycHOOK- iKD strains were pre-treated or not for 48 hours with ATc (1mg/mL), then freshly egressed parasites were inoculated on HFF coated coverslips ($10^5$/well in a 24 well plate) in normal media or media with ATc and let grow overnight before in 4% PFA fixation.

*For the AID system,* $10^5$ freshly egressed parasites were inoculated onto HFF coated coverslips in presence or absence of IAA. 24hrs or 30hrs post-infection, the parasites (± IAA) are fixed with 4%PFA for 20min and quenched in 600µL 0.1M glycine-PBS.

Growth was assessed via immunofluorescence assay after staining of GAP45 (1/5000) for Rab11A—WT and Rab11A-DN parasites, of both cMyc (1/200) and GAP45 (1/5000) for cMycHOOK- iKD parasites, and of both HA (1/200) and GAP45 (1/5000) for FTS-mAID, HIP-

mAID and ΔHOOK parasites. The number of parasites per vacuole was counted for 100 vacuoles for each condition. Three independent experiments were performed, and collected data were presented as mean value ± SD of experiments.

## 3.4 Invasion assay

Freshly egressed extracellular parasites expressing Rab11A-WT and Rab11A-DN were harvested and subsequently treated for 2 h with 1µM of Shield-1 before the invasion assay is performed. By contrast, freshly egressed cMycHOOK-iKD parasites previously grown in presence or absence of ATc for 48hours and mAID-tagged parasites previously grown ±IAA for 24hours were used to perform the experiment. Parasites were seeded onto HFF monolayers in a 24-well plate at a concentration of $2*10^6$ parasites ($4*10^6$ parasites for Rab11A-DN) / 500µl complete medium containing Shield-1 or ATc or IAA when needed / coverslip. The plate was centrifuged for 2 min at 1000rpm at RT to trigger immediate adhesion and synchronized invasion events. Parasites were then shifted to 37°C for 1 h. The coverslips were washed with PBS – three times prior to fixation. Cells were fixed in 4% PFA for 10 min and subjected to a red/green invasion assay.

Briefly, adherent external parasites were labeled without permeabilization with mouse anti-TgSAG1 antibodies, followed by secondary anti-mouse antibodies coupled to Alexa488. After cell permeabilization with TritonX100 0.1%, invaded intracellular parasites were detected using rabbit anti-TgGAP45 antibodies followed with a secondary anti-rabbit antibody coupled to Alexa594. All parasites labeled green-red were considered as extracellular, while parasites exclusively red (positive for GAP45) were considered intracellular. At least 100 parasites were counted for each condition performed in triplicate. Data represent mean values ± SEM from three independent biological experiments.

## 3.5 Attachment assay:

This assay was performed using different parasite strains:

- Extracellular Rab11A-WT and Rab11A-DN parasites pre-treated or not for 2h with 1 µM of Shield-1.
- The RHΔhxgprtΔKu80 parasite strain was used as a control for the HOOK-KO parasites.

- The RHΔhxgprtΔKu80 Tati parental parasite strain and cMyc-HOOK ikD parasites pre-treated or not for 48h with ATc.
- The Tir1 parental strain, FTS-HA mAID and HIP-HA mAID parasite lines pre-treated or not overnight with IAA.

Freshly egressed extracellular parasites were counted and resuspended in Endo buffer (44.7mM K2SO4, 10mM Mg2SO4, 100mM sucrose, 5mM glucose, 20mM Tris, 0.35% wt/vol BSA—pH 8.2) containing 1 µM cytochalasin D. $1x10^6$ parasites were then seeded onto confluent HFF cells grown on glass coverslips, spun down for 2 min at 1000rpm and incubated for 15 min at 37°C in the presence of 1 µM cytochalasin D. The coverslips were washed with PBS before fixation with PFA 4% for 10 min. The Red/ Green assay was performed (see "Invasion assay"). Data are representative of three independent biological experiments.

## 3.6 Motility (Trail deposition) assay

This assay was performed using different parasite strains:

- Extracellular Rab11A-WT and Rab11A-DN parasites pre-treated for 2 h with 1 µM of Shield-1.
- RHΔhxgprtΔKu80 parasite strain was used as a control for HOOK-KO parasites.
- RHΔhxgprtΔKu80 Tati and cMycHOOK-kD parasites pre-treated or not for 48hours with ATc.
- Tir1, FTS mAID and HIP mAID parasites pre-treated or not overnight with IAA.

Glass slides were pre-coated with 100µg/ml BSA-PBS and incubated at 37°C for 45 minutes, then washed three times with PBS and allowed to dry. Freshly egressed extracellular parasites were harvested, counted, and suspended in HHE buffer (HBSS, 10mM HEPES, 1mM EGTA) containing either 1 µM of Shield-1 or ATc or IAA when needed. $1*10^6$ parasites were seeded per well and incubated for 15 min at 37°C. Parasites were then fixed with 4% PFA in PBS for 10 min at RT. A standard IFA protocol was followed wherein only SAG1 was used as the primary antibody diluted in blocking buffer (5% FBS-PBS), followed by goat anti-mouse immunoglobulin G secondary antibodies conjugated to Alexa Fluor 488. No permeabilization was performed. Coverslips were mounted on 5µl Mowiol placed on the slides and allowed to

dry at RT. At least 100 parasites per coverslip were counted for the presence or absence of a SAG1-positive trail. With internal triplicates, the experiment was performed 3 times. Mean values ± SEM was calculated. The slides were observed under the confocal microscope and the trails deposited by the parasites and highlighted by SAG1 staining were imaged.

## 3.7 Conoid extrusion assay

Freshly egressed extracellular Rab11A-WT and Rab11A-DN parasites were harvested and treated for 2h with 1µM of Shield-1. Parasites were then counted and resuspended in HS buffer (20mM HEPES, 138mM NaCl, 2.7mM KCl, 10% FBS—pH7.2). Conoid extrusion was induced with 2% ethanol for 30s and parasites were seeded on poly-L-lysine (Sigma) coverslips prior fixation with PFA 4% in PBS. At least 100 parasites were counted for each condition performed in triplicate. Data represent mean values ± SEM.

## 3.8 Conoid extraction assay

Freshly egressed extracellular HOOK-HA and FTS-cMyc parasites were harvested, counted, and suspended in HHE buffer. $2x10^6$ parasites were allowed to settle onto poly-L-lysine-coated coverslips for 15 min at room temperature (RT). Coverslips were then treated with 10mg/ml deoxycholate (DOC) in PBS for 10 min, prior fixation with PFA 4% in PBS for 10 min at RT or -20°C methanol for 3 min. Samples were stained with rabbit anti-HA, rat anti-cMyc, and mouse anti-tubulin antibodies (listed above).

## 3.9 Excreted secreted antigens assay

This assay was performed using Rab11-WT ±Shield and Rab11A-DN ±Shield parasites.

$10^6$ freshly egressed extracellular parasites were harvested. Parasites were mixed with an equal volume of pre-warmed intracellular (IC) buffer (5 mM NaCl, 142 mM KCl, 1 mM MgCl2, 2mM EGTA, 5.6 mM glucose and 25 mM HEPES, pH 7.2) and spun down at 1500rpm, RT for 10 min. The pellet was washed once in the IC buffer under similar conditions and then resuspended in Egress buffer (142 mM NaCl, 5mM KCl, 1 mM MgCl2, 1mM CaCl2, 5.6 mM glucose and 25 mM HEPES, pH 7.2) containing 2% ethanol and incubated for 30 min at 37˚C. The samples were spun down at 14000 rpm for 15 min at 4˚C and the supernatant containing

ESA saved. Pellets were washed once in 1x PBS and saved. The ESA and pellet fractions were suspended in 4x Laemelli blue buffers and subjected to Western blot as described above. The blots were probed with mouse anti-MIC2, mouse anti-GRA1 and rabbit anti-eno2 antibodies. Quantification has been performed using the ImageJ software.

### 3.10 In vivo virulence test

A group of 8 female mice Balb/C (Janvier Labs®) aged of 8 to 10 weeks were injected intraperitoneally with 250 parasites of the RHΔKu80 or the HOOK-KO strain. Two independent experiments were performed. A similar experiment was performed using RHΔhxgprtΔKu80 Tati and cMycHOOK-iKD ±ATc. In the latter case, the daily drinking water of the mice is supplemented with 0.2mg/mL of ATc and 5% sucrose to allow quenching of the expression of cMyc-HOOK protein.

The survival of the mice is monitored daily for two weeks. The mice are then euthanized when the endpoints validated by the ethics committee are reached.

### 3.11 Statistics

Means and SEM / SD were calculated in GraphPad (Prism). *P*-values were calculated using the Student's *t*-test assuming equal variance, unpaired samples and using two-tailed distribution.

## 4 Microscopy

### 4.1 Transmission electron microscopy (TEM)

After infection of a confluent HFF monolayer, cells containing replicating shield-1 induced Rab11A-WT and Rab11A-DN expressing parasites were detached with a scraper, spun down and fixed with 1% glutaraldehyde in 0.1 M sodium cacodylate pH 6.8 overnight at 4°C. Cells were post-fixed with 1% osmium tetroxide and 1.5% potassium ferricyanide for 1 h, then with 1% uranyl acetate for 45 min, both in distilled water at RT in the dark. After washing, cells were dehydrated in graded ethanol solutions then finally infiltrated with epoxy resin and cured for 48 hs at 60°C. Sections of 70–80 nm thickness on formvar-coated grids were observed with a Hitachi H7500 TEM (Elexience, France), and images were acquired with a 1 Mpixel digital camera from AMT (Elexience, France).

## 4.2 Scanning Electron microscopy (SEM)

Parasites were seeded on BSA coated-glass coverslips for 15 min at 37°C before being fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate for 30 min. After washing, cells were treated with 1% osmium tetroxide in water, in the dark for 1 hour. Cells were dehydrated with increasing ethanol concentration baths. After two pure ethanol baths, cells were air-dried with hexamethyldisilazan (HMDS). Finally, dry coverslips were mounted on stubs and coated with 5 nm platinum (Quorum Technologies Q150T, Milexia, France) and cells were imaged at 2 kV by a secondary electron detector with a Zeiss Merlin Compact VP SEM (Zeiss, France).

The circularity and aspect ratio (AR) parameters were calculated using the ImageJ software. First, each parasite contour was manually delineated on the SEM images and the pluggin "Analyze"> "Set measurements"> Shape descriptor" was applied for all defined ROI (individual parasites, n = 70) to extract the circularity and aspect ratio parameters. Circularity describes how close an object is to a true circle and is calculated using the formula: circularity = $4D*(\text{area}/\text{perimeter}^2)$. A circularity value of 1 indicates a perfect circle. As the value approaches 0, it indicates an increasingly elongated shape. The aspect ratio describes the proportional relationship of an object's width to its height and is calculated using the formula: AR = major axis/minor axis.

## 4.3 Videomicroscopy

Time-lapse video microscopy was conducted in LabTek chambers installed on an Eclipse Ti inverted confocal microscope (Nikon France Instruments, Champigny sur Marne, France) with a temperature and CO2-controlled stage and chamber (Okolab), equipped with two Prime 95B Scientific Cameras (Photometrics, UK) and a CSU W1 spinning disk (Yokogawa, Roper Scientific, France). The microscope was piloted using MetaMorph software (Universal Imaging Corporation, Roper Scientific, France). A live-SR module (Gataca Systems, France) was added to the system to improve the obtained resolutions. Exposure time of 500 ms was used for the simultaneous acquisition of the GFP and mCherry channels, in dual camera mode (with band pass filters 525/50 nm and 578/105 nm, dichroic mirror at 560 nm, and laser excita- tion at 488 nm and 561 nm). Videos were captured at 2frames/second.

## 4.4 Automatic tracking and vesicle co-distribution using the Imaris software

Automatic tracking of vesicles using the Imaris software (Bitplane, Oxford Instruments) was applied on the recorded videos retrieved from the GFP and mcherry channels of SAGΔG- PI-GFP / mcherryRab11A-WT expressing parasites. We first used the tool "Spot detector" for selecting-filtering spot size and intensity values for each channel. Next, we manually removed detection of false GFP-positive spots (notably detected in the vacuolar space due to the secretion of the SAGΔGPI protein). The tool "Track Manager" was used to manually correct the obtained tracks when required and to extract the xy positions of a given spot over time enabling to calculate the Mean Square Displacement (MSD) using MATLAB (see below). The tool "spot co-localization" was used to calculate the percentage of co-distribution between DG and Rab11A-postive vesicles. A distance of 300 nm between the spots was selected corresponding to the average size of the vesicles. At a given time point and for the entire vacuole, the number of all detected green spots, as well as the number of green spots co-distributing with the red spots were extracted to calculate the co-distribution percentage. This was repeated over 5 consecutive time points every 2 s for the first 10 s of recording to avoid bleaching of the fluorescent signals. The mean co-distribution percentage over these 5 time points was calculated per vacuole. The mean +/- SD of 10 vacuoles was then calculated.

## 4.5 Manual tracking and mathematical modeling with MATLAB

When indicated, the manual tracking plugin from the ImageJ software (https://imagej-nih-gov/ij/) was applied on the images obtained with the MetaMorph software to extract in time the spatial xy positions of the fluorescent vesicles. In order to track and model the type of motion of the vesicle, images were processed in MATLAB (www.mathworks.com) by applying *fit* function ('poly1' or 'poly2' options).

MSD was calculated thanks to a MATLAB script according to the formula:

$$MSD(n\Delta t) = \frac{1}{N-n}\sum_{i=1}^{N-n}(d_{i+n} - d_i)^2$$

MSD curves were fitted according to the formula:

- $MSD = 4Dt+v^2t$ (with D the Diffusion Coefficient and v the velocity), for directed motion
- $MSD = 4Dt$ (with D the Diffusion Coefficient), for normal diffusion.

# 5 Biochemistry

## 5.1 Total protein extract and Western Blot:

Parasites were lysed in a lysis buffer (NaCl 150mM, TrisHCl 20mM, EDTA 1mM, 1% Triton X100, protease and phosphatase inhibitors) for 30 minutes on ice, then the lysate was centrifuged for 15 min at 14000 rpm to remove cell debris. Next, the lysate was mixed with 4X Laemmli loading buffer (240mM Tris-HCl pH 6.8; 8% SDS; 40% sucrose; 0.04% bromophenol blue and 400mM DTT) and total proteins were subjected to electrophoresis in a 12% polyacrylamide gel. By a standard western blot procedure, the proteins were transferred onto a nitrocellulose membrane (Amersham$^{TM}$Protran$^{TM}$ 0.45µm NC). The membrane was blocked with 5% milk (non-fat milk powder dissolved in TBST buffer: 20mM Tris pH7.5, 150mM NaCl, and 0.1% Tween20) and probed with primary antibodies diluted in the blocking buffer. The primary antibodies were followed by species specific secondary antibodies conjugated to HRP. The antibody incubations were followed by thorough washing using the TBST buffer. The membranes were visualized using ECL Western blotting substrate (Pierce).

The sources of antibodies (Abs) included: rabbit anti-HA monoclonal Ab (mAb) (Cell Signaling Technology), mouse anti-cMyc MAb (Thermo Scientific), rabbit anti-Enolase2 (Eno2) (in house), mouse anti-Rab11A (in house), mouse anti-Ty (D. Soldati-Favre).

## 5.2 Immunoprecipitation

For immunoprecipitation assays, a minimum of $5x10^8$ parasites of the respective strains cMycHOOK- iKD /FTS-HA and cMycHOOK- iKD (control) or HOOK-HA parasites were lysed on ice for 30 minutes in 500 µl of modified RIPA buffer (50mM TrisHCl pH8.0, 2mM EDTA, 75mM NaCl, 0.65% NP40, 0.005%SDS, protease inhibitors) and centrifuged at 14 000 rpm for 10 minutes to eliminate cell debris. 20µl of lysate were kept for WB (boiled in 4x buffer then kept at -20°C). Then 30µl of pre-washed anti-cMyc coated agarose beads (Pierce$^{TM}$) or anti-HA coated agarose beads (Pierce$^{TM}$) are added to the supernatant overnight. The next day, 20µl of supernatant were kept for WB (boiled in 4x buffer then kept at -20°C). Then, after five washes of 10 minutes each with cold modified RIPA buffer, bound proteins were eluted by

boiling the samples in 2 X laemmeli buffer. Samples were subsequently subjected to SDS PAGE and western blotting or mass spectrometry analysis.

## 5.3 GST pull-down

The full length Rab11A was GST tagged by cloning into a pGEX6p3 vector (Pharmacia) using the respective restriction sites mentioned in the table 5 of primers listed above. The plasmids expressing GST-Rab11B, GST-Rab5A and GST-Rab7 were previously generated in the lab and described in (Sangaré et al., 2016). Expression of GST–Rabs in BL21 competent cells was achieved by induction with 1mM IPTG at 37°C for 4 h. Bacteria lysates expressing all GST recombinants and GST alone (control) were bound to 100µl of Protino Glutathione agarose 4B beads (Machery Nagel) in GST-lysis/binding buffer (Tris HCl (pH 7.6) 50mM, EDTA 1mM, EGTA1mM, 2- mercaptoethanol 10mM, NaCl 150mM, TritonX-100 0.5%, and 0.5mM PMSF) overnight at 4°C. The beads were washed 5 times with wash buffer A (Tris HCl (pH 7.6) 50mM, 2- mercaptoethanol 10mM, NaCl 500mM, Triton 0.5% and 0.5mM PMSF) and 3 times with wash buffer B (Tris HCl (pH 7.6) 20mM, NaCl 150mM, NP40 0.65%, SDS 0.005%, 0.5mM PMSF) sequentially. Beads containing 150µg of the recombinant proteins and the control GST protein were incubated with a lysate from 0.4 billion wildtype RHΔKu80 or HOOK-HA intracellular parasites, overnight at 4°C. Parasites were lysed using modified RIPA (TrisHCl (pH8.0) 50mM, EDTA 2mM, NaCl 75mM, NP40 0.65%, SDS 0.005%, PMSF 0.5mM). After 3 washes with the lysis buffer, the proteins bound to the beads were eluted with 1x Laemelli blue buffer by boiling. The samples were subject to western blot and/or mass spectrometric analysis.

## 5.4 Mass spectrometry and proteomic analysis

After denaturation at 100°C in 5% SDS, 5% βmercaptoethanol, 1 mM EDTA, 10% glycerol, 10 mM Tris buffer pH 8 for 3 min, protein samples were fractionated on a 10% acrylamide SDS-PAGE gel. The electrophoretic migration was halted as soon as the protein sample entered 1 cm into the separating gel. The gel was quickly stained with Coomassie Blue, and five bands, containing the entire sample, was cut. In gel digestion of gel cuts was performed as previously

described (Miguet, L. et al., 2009). An UltiMate 3000 RSLCnano System (Thermo Fisher Scientific) was utilized for separation of the protein digests. Peptides were consequently fractionated onto a commercial C18 reversed phase column (75 µm×150 mm, 2 µm particle, PepMap100 RSLC column, Thermo Fisher Scientific, temperature 35 °C). Trapping was performed during 4 min at 5 µl/min, with solvent A (98 % H2O, 2% ACN and 0.1 % FA). Elution was performed using two solvents A (0,1 % FA in water) and B (0,1 % FA in ACN) at a flow rate of 300 nl/min. Gradient separation was 3 min at 5% B, 37 min from 5 % B to 30% B, 5 min to 80% B, and maintained for 5 min. The column was equilibrated for 10 min with 5% buffer B prior to the following sample analysis. The eluted peptides from the C18 column were analyzed by Q-Exactive instruments (Thermo Fisher Scientific). The electrospray voltage was 1.9 kV, and the capillary temperature was 275 °C. Full MS scans were acquired in the Orbitrap mass analyzer over m/z 300–1200 range with resolution 35,000 (m/z 200). The target value was 5.00E+05. Ten most intense peaks with charge state between 2 and 4 were fragmented in the HCD collision cell with normalized collision energy of 27%, and tandem mass spectrum was acquired in the Orbitrap mass analyzer with resolution 17,500 at m/z 200. The target value was 1.00E+05. The ion selection threshold was 5.0E+04 counts, and the maximum allowed ion accumulation times were 250 ms for full MS scans and 100 ms for tandem mass spectrum. Dynamic exclusion was set to 30s.

## 5.5 Proteomic data analysis

Raw data collected during nanoLC-MS/MS analyses were processed and converted into *.mgf peak list format with Proteome Discoverer 1.4 (Thermo Fisher Scientific). MS/MS data was interpreted using search engine Mascot (version 2.4.0, Matrix Science, London, UK) installed on a local server. Searches were performed with a tolerance on mass measurement of 0.2 Da for precursor and 0.2 Da for fragment ions, against a composite targetdecoy database (50620 total entries) built with 3 strains of Toxoplasma gondii ToxoDB.org database (strains ME49, GT1 and VEG, release 12.0, September 2014, 25264 entries) fused with the sequences of recombinant trypsin and a list of classical contaminants (46 entries). Cysteine carbamidomethylation, methionine oxidation, protein N-terminal acetylation and cysteine

propionamidation were searched as variable modifications. Up to one trypsin missed cleavage was allowed.

# Results

# Chapter III - Results

## 1 Rab11A regulates dense granule transport and secretion during *Toxoplasma gondii* invasion of host cells and parasite replication

In the first part of my PhD, we aimed to characterize the role of *Tg*Rab11A in the secretion of dense granules during parasite intracellular replication. In particular, we were interested in characterizing which step of this secretory pathway is regulated by *Tg*Rab11A: vesicle anchoring and movement on the parasite cytoskeleton tracks or/and tethering/fusion of the vesicles to the plasma membrane. This part of my project allowed completing the study of *Tg*Rab11A functions that was initiated by a PhD student in the lab (Kannan Venugopal) and a paper entitled "**Rab11A regulates the constitutive secretory pathway during *Toxoplasma gondii* invasion of host cells and parasite replication"**, in which I share the first authorship, has been recently published in *Plos Pathogens*. The results obtained are presented here as published in the article.

### 1.1 *Tg*Rab11A localizes to dynamic cytoplasmic vesicles

First, to investigate *T. gondii* Rab11A localization, we raised a polyclonal antibody in mice, which recognized a unique protein of the expected size of 25kDa in a total extract of type I RHΔKu80 parasites (Figure 1A). We performed immunofluorescence assays (IFA) in fixed RHΔKu80 tachyzoites. *Tg*Rab11A displayed distinct localizations depending on the cell cycle stage. During the G1 phase, *Tg*Rab11A was localized in cytoplasmic vesicles and as previously described (Agop-Nersesian et al., 2009), a signal was also detected at the Golgi/Endosome-Like Compartment (ELC) region (Figure 1B). IFA confirmed the co-distribution of *Tg*Rab11A with the TGN marker *Tg*Sortilin-like Receptor (*Tg*SORTLR) (S1 Figure). Consistent with this observation, *Tg*Rab11A was found to be mostly localized adjacent to the Rab5A signal defining the early ELC, previously shown to be tightly associated with the TGN (Venugopal et al., 2017) (S1 Figure). During cytokinesis, the Golgi localization of *Tg*Rab11A was also detected in emerging daughter cells, together with a strong enrichment of the protein at the apical tip of

the growing buds, reflecting a possible Rab11A-dependent transport of newly synthesized material between these two locations (Figure 1B and S1B Figure). *Tg*Rab11A also accumulated at the basal pole of the parasite at the end of cytokinesis (Figure 1B).

In order to get further insights into the dynamic localization of *Tg*Rab11A, we used the previously established transgenic ddFKBP-myc-mCherryRab11A-RHΔKu80 parasites (from here designated as mcherryRab11A-WT parasites) (Agop-Nersesian et al., 2009; Andenmatten et al., 2013). In this strain, the expression of *Tg*Rab11A fused to a mCherry tag is under the control of an N-terminal ddFKBP tag, which allows regulation of recombinant protein levels by the inducer Shield-1. Using super resolution live imaging of parasites expressing the Inner Membrane Complex protein IMC3-YFP and mCherryRab11A-WT, we clearly observed bi-directional trajectories of *Tg*Rab11A-positive vesicles between the basal and apical poles of the parasite both within the parasite cytosol (Figure 1C and S1 Movie) and along the parasite cortex delineated by the IMC3-YFP staining (Figure 1C and S2 Movie). During cytokinesis, videomicroscopy highlights the presence of *Tg*Rab11A at the Golgi area of daughter cells and the transport of *Tg*Rab11A-positive vesicles along the newly formed daughter bud scaffold (S3 Movie). In addition, consistent with our IFA imaging, we detected a dynamic localization of *Tg*Rab11A at the basal pole of replicating parasites (S4 Movie). Interestingly, we also noticed *Tg*Rab11A-positive vesicles and tubular-like structures within the residual body region (Figure 1C, RB). This region has been recently described to harbour a dense actino-myosin network that interconnects intracellular dividing tachyzoites (Frénal et al., 2017b; Periz et al., 2017), suggesting that *Tg*Rab11A may regulate actin-dependent material exchanges between parasites, or the dynamics of this cell-to-cell connecting network. In line with this observation, after transient expression of actin chromobodies coupled to Emerald GFP (Cb-E) that specifically label filamentous actin (Periz et al., 2017), we visualized *Tg*Rab11A-positive vesicles moving along actin-positive structures at the parasite cortex (Figure 1D, upper panel and S5 Movie) or anchored to dynamic F-actin structures within the parasite cytosol (Figure 1D, lower panel and S6 Movie). As previously observed (Periz et al., 2017), we also detected dynamic F-actin structures at the Golgi/ ELC area that co-distribute with the *Tg*Rab11A signal (S5 Movie). To investigate whether *Tg*Rab11A-positive vesicle movements depend on the actin cytoskeleton, we treated IMC3-YFP/ mcherryRab11A-WT tachyzoites with cytochalasin D (CD) for 30 min before recording parasites by live imaging.

Depolymerizing actin filaments by CD led to the formation of cytosolic and cortical *Tg*Rab11A-positive clusters that in contrast to non-treated parasites, displayed confined trajectories as illustrated by the tracking of their displacement (Figure 1E “tracking”, S7 Movie).

Collectively, these data demonstrated that *Tg*Rab11A-positive vesicle movement is dependent on actin cytoskeleton activity and that *Tg*Rab11A might participate in (i) vesicle budding from the TGN/ELC, (ii) cargo transport between the apical and basal poles of the parasite and (iii) material exchange between the replicating parasites via release of vesicles at the basal pole.

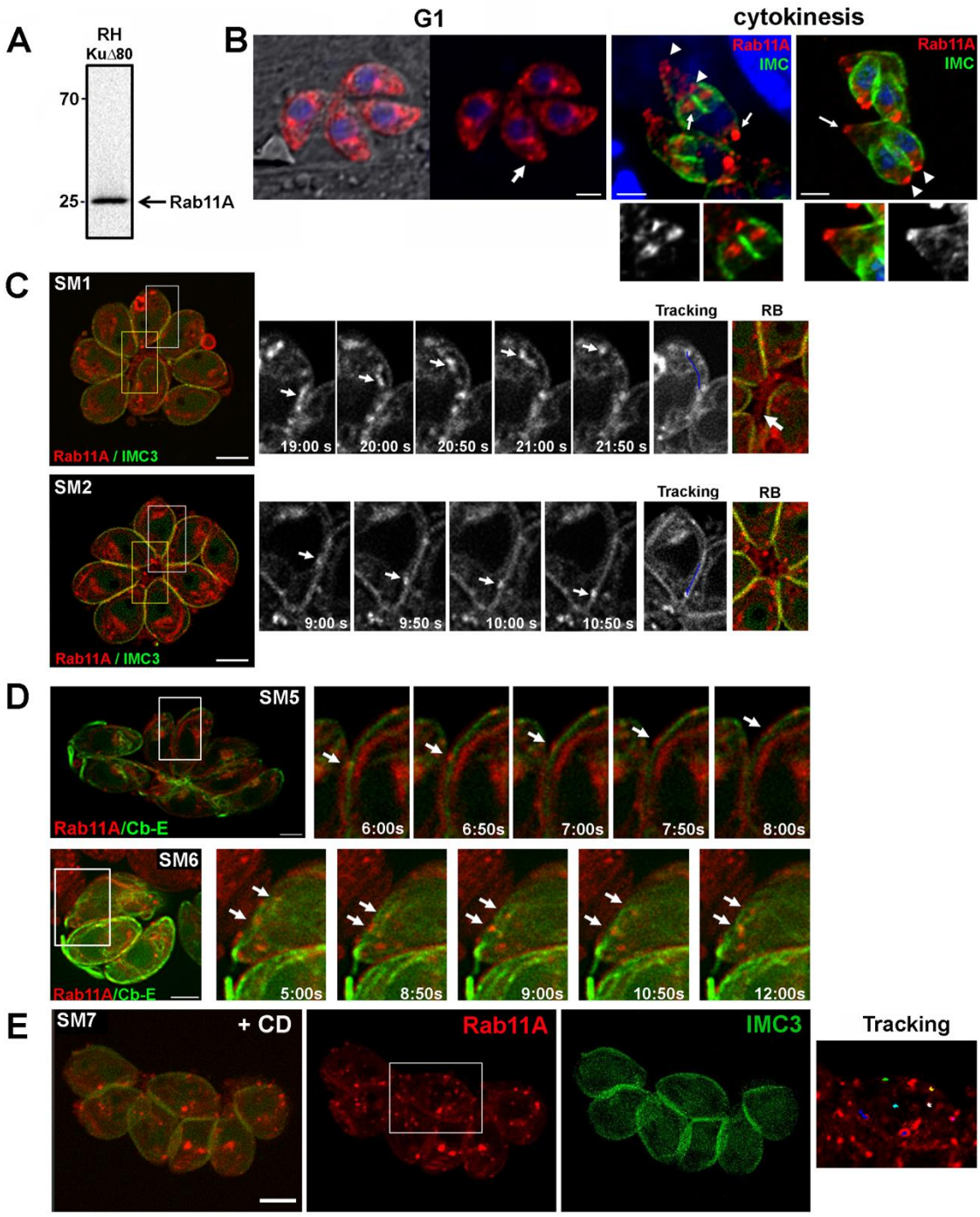
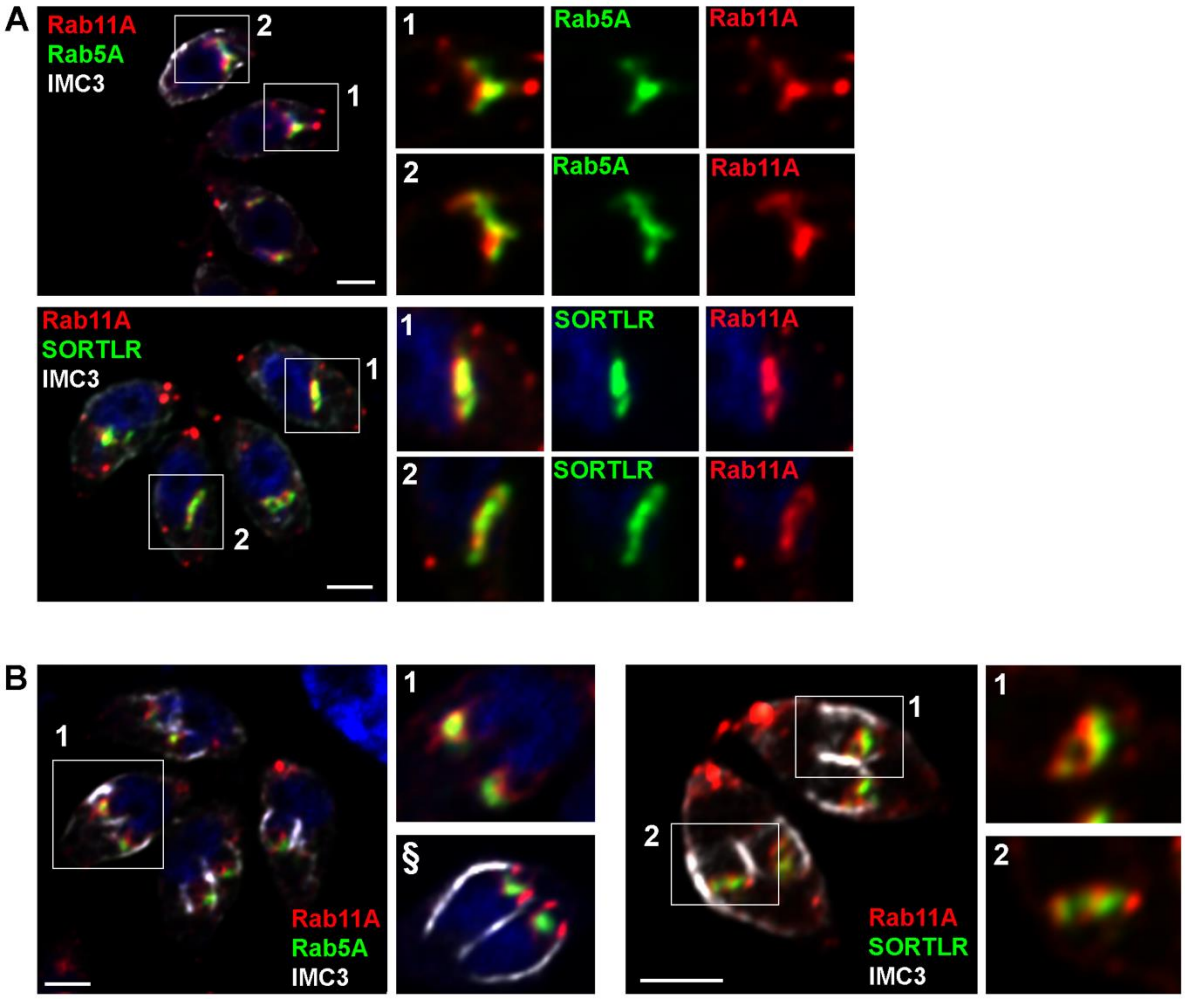



**Figure 1. A-** Western blot analysis with specific anti-Rab11A antibodies detects a unique band at 25kDa in a RHΔKU80 parasite lysate. **B-** Analysis of *Tg*Rab11A localization in fixed RHΔKU80 parasites using antibodies recognizing Rab11A, IMC3 and ROP2/3, as indicated. Bars: 1 µm. **C-** Sequences of images extracted from S1 Movie and S2 Movie (left images, white frames) showing the dynamic bi-directional movement of Rab11A-positive vesicles in the cytosol (upper sequence) and along the parasite cortex (lower sequence) of mcherryRab11A-WT and IMC3-YFP expressing parasites. Tracking of vesicle trajectory is also shown. Images on the right show a zoom of the residual body (RB) region indicated by a yellow frame in the corresponding vacuoles. The arrow indicates *Tg*Rab11A-positive tubular-like structures connecting the parasites Bars: 2 µm. **D-** Sequences of images extracted from S5 Movie and S6 Movie (left images, white frames) showing the dynamic movement of *Tg*Rab11A-positive vesicles along the parasite cortex (upper sequence) and in the cytosol (lower sequence) of mcherryRab11A-WT and Cb-Emerald GFP (Cb-E) expressing parasites. Bars: 2 µm. **E-** Images extracted from movie S7

Movie. mcherryRab11A-WT and IMC3-YFP expressing parasites were treated with cytochalasin D (CD) for 30 min before being recorded. *Tg*Rab11A-positive vesicles are detected in clusters displaying confined trajectories (right image: “tracking”). Bar: 2 µm.




**S1 Figure.** IFA showing the localization of *Tg*Rab11A (red), the TGN marker *Tg*SORTLR and the ELC marker *Tg*Rab5A (green) in fixed RHΔKU80 parasites during the G1 phase of the cell cycle (A) and cytokinesis (B). Parasite contours or daughter cells buds are revealed after detection of the protein IMC3 (white). Zooms of the areas indicated by white frames (1, 2) and corresponding to the Golgi/ELC region of a given parasite are also shown. The image B§ originating from a separate vacuole illustrates the localization of *Tg*Rab11A at the tip of the forming daughter cell buds. Bars: 2 µm.

## 1.2 *Tg*Rab11A-positive vesicles dynamically co-distribute with DGs

The DG-mediated secretory pathway is considered in *T. gondii* to be the default constitutive secretory pathway based on the observation that soluble SAG1 protein truncated of its GPI anchor (SAG1ΔGPI) is transported within DGs before being released into the vacuolar space(Grant and Donaldson, 2009; Heaslip et al., 2016). Interestingly, the dynamic motion of *Tg*Rab11A-positive vesicles was similar to recently described actin and myosin F-dependent

movements of DGs (Heaslip et al., 2016) and *Tg*Rab11A is known as a regulator of exocytosis in other eukaryotic systems (Welz et al., 2014).

In order to explore dense granule dynamics in relation to *Tg*Rab11A, we expressed SAG1ΔGPI-GFP in mcherryRab11A-WT parasites. Using live imaging, we confirmed that DG content was efficiently released, as illustrated by the localization of the GFP signal in the vacuolar space (Figure 2A). GFP-positive DGs detected in the parasite cytosol displayed a significant and dynamic co-distribution with mcherryRab11A-WT positive vesicles (Figure 2B and 2C). In replicating parasites, 33,7% of the DG population co-distributed over time with *Tg*Rab11A-positive vesicles, while 26,1% of *Tg*Rab11A-positive vesicles co-distributed with DGs. This shows that *Tg*Rab11A-positive vesicles and DGs are distinct intracellular compartments that transiently interact with each other. Consistent with this notion, fluorescent signal intensity profiles indicated that GFP-positive DGs and mcherryRab11A-positive vesicles are closely apposed (Figure 2A and 2B). This is also clearly visualized in S8 Movie (Figure 2D), in which a DG is observed docked onto a *Tg*Rab11A-positive vesicle, the latter being anchored at the periphery of the parasite, and both compartments are simultaneously transported along the parasite cortex (Figure 2D). We tracked this GFP-positive DG motion (Figure 2E and 2F; S9 Movie and S10 Movie) and fitted the recorded xy positions over time using mathematical models of “directed” or “diffusive” motion (see M&M) (Wang et al., 2014). We confirmed that the DG trajectory 2 is consistent with “directed” motion (fitted curve, Figure 2F) characteristic of a vesicle moving along cytoskeleton tracks, in contrast to the trajectories 1 and 3, characteristic of “confined” diffusive motions (Wang et al., 2014). This together with the observed inhibition of *Tg*Rab11A-positive vesicle and DG movements upon CD treatment (Figure 1D) (Heaslip et al., 2016), suggests that *Tg*Rab11A promotes DG transport by mediating their anchoring along actin filaments, at least at the parasite cortex.

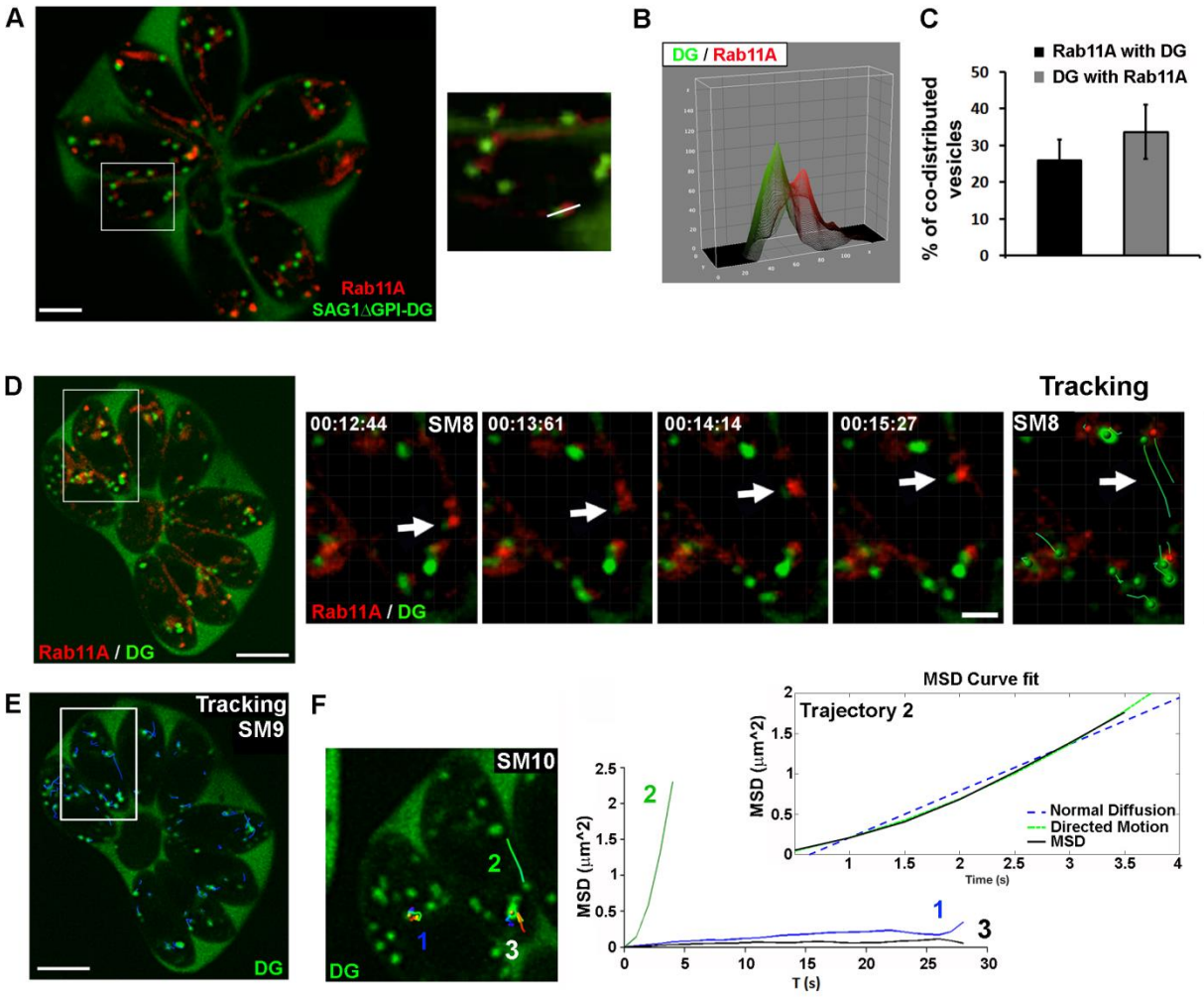



**Figure 2. A-** Image extracted from a time-lapse acquisition illustrating the release of SAGΔGPI protein (green) into the vacuolar space of mcherryRab11A-WT and SAGΔGPI-GFP expressing parasites, as well as the co-distribution in the parasite cytosol of SAGΔGPI-GFP positive DG (green) and mcherryRab11A-WT positive vesicles (red). The right insert shows a zoom of the region indicated by a white frame in the full vacuole. Bar: 2 µm. **B-** Fluorescence intensity profiles plotted over the distance of the GFP and mcherry signals along the line indicated in A (insert). **C-** Percentage of co-distribution between the total population of SAGΔGPI-GFP-positive DGs and mcherryRab11A-WT-positive vesicles of a given vacuole averaged over 5 consecutive time points (n = 10 vacuoles). Data show mean ± SD. **D-** Sequences of images extracted from S8 Movie (region indicated by a white fame in the full vacuole) showing the joint motion of a *Tg*Rab11A-positive vesicle (red) and a SAGΔGPI-positive DG (green) along the parasite cortex, as illustrated by their tracking (S8B Movie). Time is indicated in seconds. **E-** Automated tracking of all DG trajectories within the vacuole (S9 Movie). **F-** Three trajectories (1, 2, 3) (S10 Movie) in the region indicated by a white frame in E- were analyzed by plotting the Mean Square Displacement (MSD) over ΔT (s) using the Imaris software. Trajectory N˚2 (black line) corresponding to the track shown in -D (S8 Movie) fitted a mathematical model of "directed" motion (green line) defined by the equation MSD = 4Dt+v2 t 2 while trajectories 1 and 3 display confined motions.

## 1.3 *Tg*Rab11A promotes DG exocytosis

To assess whether *Tg*Rab11A regulates DG transport, docking or the later step of fusion at the PM, we used a previously established parasite strain that over-expresses in a rapidly

inducible manner an inactive GDP locked version of *Tg*Rab11A fused to the mCherry fluorescent reporter (DDmCherrycmycRab11A-DN-RHΔKu80; from hereon called mCherryRab11A-DN and distinguished from mCherryRab11A-WT) (Agop-Nersesian et al., 2009; Andenmatten et al., 2013). By WB, we confirmed that both *Tg*Rab11A-WT and *Tg*Rab11A-DN proteins were expressed in similar amounts after 4 h induction with Shield-1 (Figure 3A). First, we monitored DG release in fixed Rab11A-WT and Rab11A-DN intracellular tachyzoites following gentle saponin permeabilization, which improved detection of secreted GRA proteins localized in the vacuolar space and at the PVM. To rule out any indirect effect of the previously described cytokinesis defect on DG secretion in Rab11A-DN parasites (Agop-Nersesian et al., 2009), we pre-treated freshly egressed extracellular tachyzoites for 1 h with Shield-1 before seeding them on a fibroblast monolayer and analysed DG secretion 2h and 4h after parasite invasion (Figure 3B). We observed a drastic block of GRA1 and GRA3 secretion in Rab11A-DN parasites in contrast to Rab11A-WT in which both proteins were typically released in the vacuolar space or decorated the PVM (Figure 3B and 3C). A similar observation holds for additional GRA proteins (GRA2, GRA5 and GRA16) as shown in S2 Fig. Notably, in contrast to Rab11A-WT parasites, GRA16-positive DGs were also retained within Rab11A-DN parasite cytosol and accordingly GRA16 no longer reached the host cell nuclei 16h post-infection (Bougdour et al., 2013) (S2B Figure).

To further analyse the role of *Tg*Rab11A in DG secretion, we also expressed SAG1ΔGPI-GFP in mcherryRab11A-DN parasites. In contrast to Rab11A-WT parasites, Rab11A-DN parasites were impaired in their ability to release SAGΔGPI-GFP into the PV space (Figure 3D). Consequently, DGs were densely packed in the cytosol, which impaired reliable automatic tracking of all vesicles and therefore the quantification of the percentage of “directed” versus “diffusive” or “confined” trajectories in the total DG population. Nonetheless, DGs appeared to mostly display diffusive and confined motions (Figure 3E: tracking of 4 DG and their resulting trajectories shown in S11 Movie, which were further analysed in Figure 3F). In particular, the accumulation of DGs observed at the altered interface between the two segregating daughter cells accounted for a local quasi static behavior as illustrated by their confined trajectories (Figure 3F: trajectories 2, 3 and SM11). Rare longer trajectories could be detected along the cortex of the parasites (such as illustrated for trajectory 1), however they never fitted, with good probability, a model of directed motion. In agreement, analysis of

cortical DG trajectories in Rab11A-WT and Rab11A-DN parasites revealed a significant increase in the coefficient of diffusion of Rab11A-DN trajectories, suggesting a role for *Tg*Rab11A in regulating DG directed transport along the parasite cytoskeleton (Figure 3G). Finally, we performed an experiment in which we washed out 0.5 µM- (S12 Movie) or 1 µM- (S13 Movie) Shield-1 pre-induced Rab11A-DN parasites in order to arrest the expression of the Rab11A-DN protein. We clearly observed, 4h after Shield-1 removal, a strong accumulation of GFP-positive DGs at the parasite PM together with the re-initialization of their content release (S12 Movie), and a pronounced signal at the defective interface between dividing parasites (S13 Movie). This observation suggests that *Tg*Rab11A may be required for DG docking/tethering at the PM.

Collectively; these data indicate that *Tg*Rab11A regulates both the directed transport of DG along cytoskeleton tracks (Figure 1D and Figure 2D, 2E and 2F) and their exocytosis into the PV space.

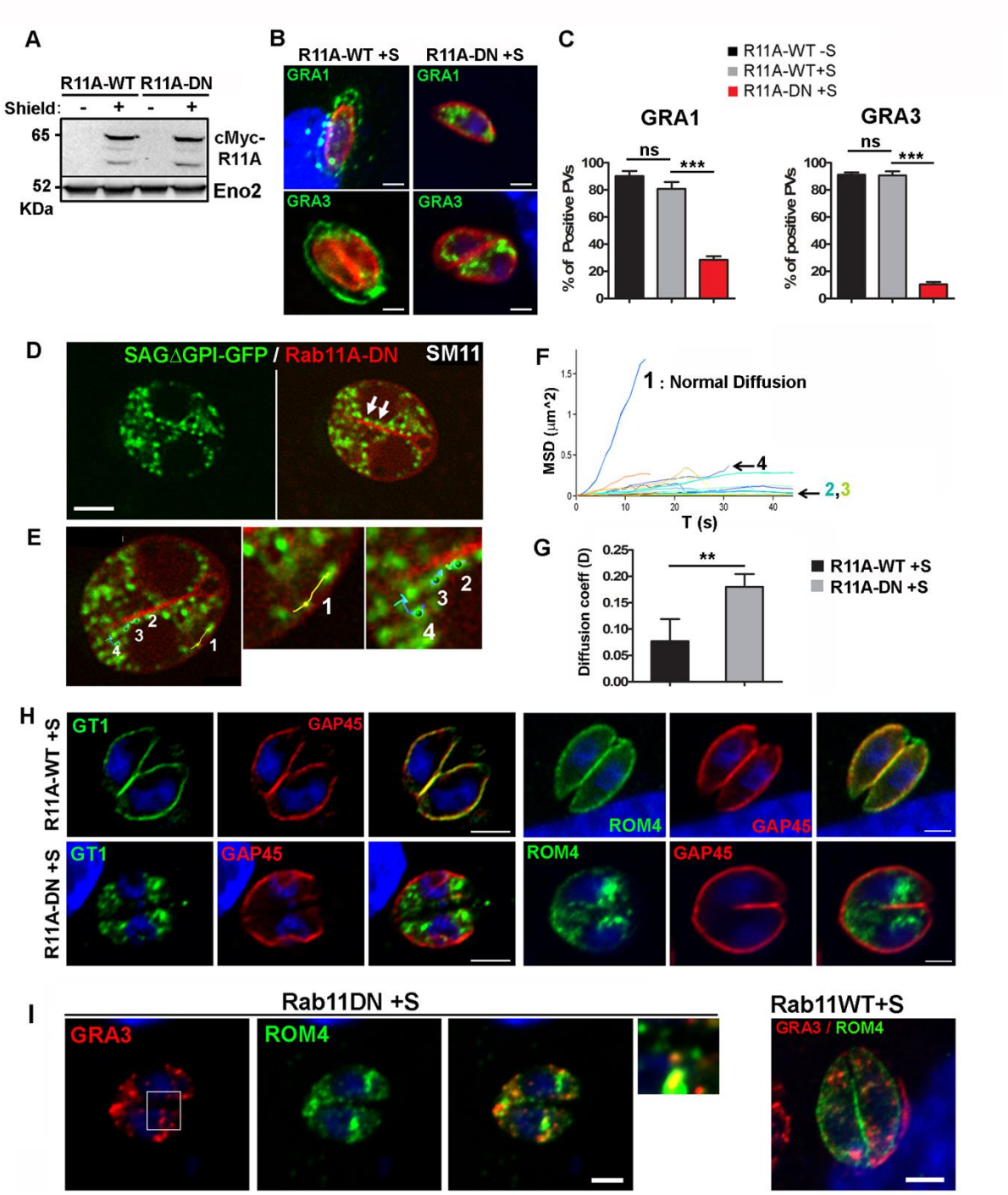
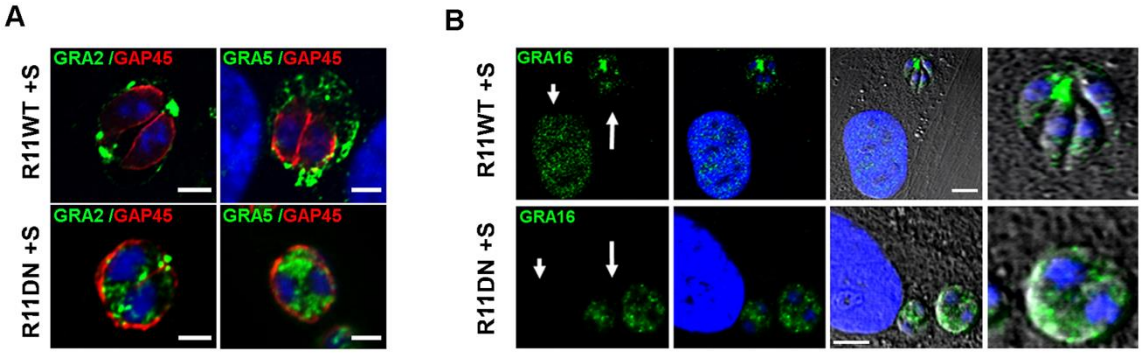



**Figure 3. A-** Western blot analysis using anti-Rab11A antibodies showing Rab11A-WT and Rab11A-DN proteins in similar amounts after 4 h of Shield-1 induction (+S) of intracellular tachyzoites. Eno2 is used as a loading control. **B-** Immunofluorescence assay (IFA) showing the dense granule proteins GRA1 and GRA3 (green) retained in intra-cytosolic vesicles following 2 h (upper panel) and 4 h (lower panel) of Shield-1 induction of Rab11A-DN parasites, while being efficiently released into the vacuolar space and at the vacuole membrane in similarly induced Rab11A-WT parasites. The parasite cortex is delineated by the glideosome protein GAP45 (red). Bars: 1 µm. **C-** Percentage of vacuoles positive for GRA1 and GRA3 secretion in Rab11A-WT and Rab11A-DN parasites induced (+S) or not (-S) with Shield-1. Data show mean ± SEM of three independent experiments (unpaired Student's t-test, GRA1: ***p<0.0001 and GRA3: ***p=0.0008). **D-** Image extracted from S11 Movie illustrating DG movements in mcherryRab11A-DN (red) / SAGΔGPI-GFP expressing parasites. DGs accumulate in the parasite

cytosol or remain stationary along the segregating membrane of daughter cells (arrows). Bar: 2 µm. **E-** Images extracted from S11 Movie showing the tracking of 4 DGs and their resulting trajectories, which were analyzed in F-. **F-** Tracking of DGs in Rab11A-DN expressing parasites indicates mostly confined (as exemplified for DG trajectories 2, 3) and diffusive (trajectories 1, 4) motions. **G-** Mean diffusion coefficient (D) calculated from 10 cortical trajectories manually tracked in Shield-1 induced Rab11A-WT and Rab11A-DN parasites. Data show mean ± SD (unpaired Student's t-test **p<0.01. **H-** IFA showing the glucose transporter GT1 and Romboïd protein ROM4 (green) retained in intra-cytosolic vesicles in Shield-1 induced Rab11A-DN parasites, while being efficiently delivered at the plasma membrane in induced Rab11A-WT parasites. The parasite cortex is delineated by GAP45 (red). Bars: 2 µm. **I-** IFA showing the localization of the proteins GRA3 (red) and ROM4 (green) in distinct vesicles in Shield-1 (+ S) induced Rab11A-DN expressing parasites during parasite replication. In Rab11A-WT expressing parasites, GRA3 and ROM4 localized at the vacuolar membrane and at the parasite plasma membrane, respectively. Bars: 2 µm.




**S2 Figure.** A-Immunofluorescence assay showing the dense granule proteins GRA2 and GRA5 (green) retained in intra-cytosolic vesicles in Shield-1-induced (+S) Rab11A-DN expressing parasites, while being efficiently released into the vacuolar space and at the vacuole membrane in induced Rab11A-WT expressing parasites. The parasite cortex is delineated by GAP45 (red). Bars: 2 µm. B- Fluorescence images showing the dense granule protein GRA16 (green) retained in intra-cytosolic vesicles in Shield-1-induced Rab11A-DN expressing parasites, while being secreted and translocated into the host cell nuclei (small arrows) in induced Rab11A-WT expressing parasites. Bars: 5 µm.

## 1.4 *Tg*Rab11A regulates transmembrane protein localization at the PM

Based on our previous study (Agop-Nersesian et al., 2009), we proposed that *Tg*Rab11A is required for the delivery of vesicles containing SAG1 and probably other surface proteins, from the endosomal network to the plasmalemma of daughter cells, where new PM is synthesized, similar to its function described in other eukaryotes. This prompted us to investigate whether during replication *Tg*Rab11A might regulate the localization of other surface proteins in *T. gondii*. We transiently transfected Rab11A-WT and -DN parasites with plasmids encoding the transmembrane HA-tagged Glucose transporter 1 (GT1) (Pomel et al., 2008), or the Ty-tagged rhomboïd protease 4 (ROM4) (Buguliskis et al., 2010). In contrast to the rhomboid protease ROM1 that localizes to micronemes, ROM4 was found to be targeted

to the tachyzoite PM, suggesting that it is transported through the constitutive pathway (Buguliskis et al., 2010) (Brossier et al., 2005). Similar to DGs, GT1 and ROM4 proteins were retained in intracellular vesicles and were no longer delivered to the parasite PM (Figure 3H). In addition, we took advantage of the impaired exocytosis activity in Rab11A-DN parasites to study whether different populations of secretory vesicles may co-exist during parasite replication. Co-localization studies in fixed Rab11A-DN parasites showed that ROM4 and GRA3 partially co-localize, but were also detected in distinct vesicular compartments (Figure 3I). This may reflect a distinct timing of protein synthesis and vesicle release from the Golgi to the PM. However, this observation also suggests the existence of different regulatory pathways for the trafficking of protein localized at the PM vs proteins secreted into the vacuolar space. In particular, transmembrane proteins may be actively recycled during parasite division, as suggested in a previous study on the retromer subunit *Tg*VPS35 (Sangaré et al., 2016), and more recently during extracellular parasite motility (Gras et al., 2019). Thus, *Tg*Rab11A may not only play a role in the regulation of DG protein release into the vacuolar space but also in the trafficking of proteins localized at the PM during parasite replication.

Importantly, unlike GRA protein secretion, DG biogenesis was not impaired in Rab11A-DN parasites as assessed by transmission electron microscopy (Figure 4). In addition, supporting a major disturbance in DG exocytosis, the IVN could not be detected in the drastically reduced vacuolar space characterized by the PVM being closely apposed to the parasite PM (Figure 4B and 4C). We also detected the previously described defect in daughter cell segregation (Agop-Nersesian et al., 2009) (Figure 4C, arrows). Presumably, in addition to the contribution of the mother cell plasma membrane, delivery of new membrane is required to complete daughter cell segregation at the end of cytokinesis and this process may be regulated by *Tg*Rab11A. In line, the requirement for de novo lipid synthesis to complete daughter cell segregation has been already demonstrated in other studies (Amiar et al., 2020).

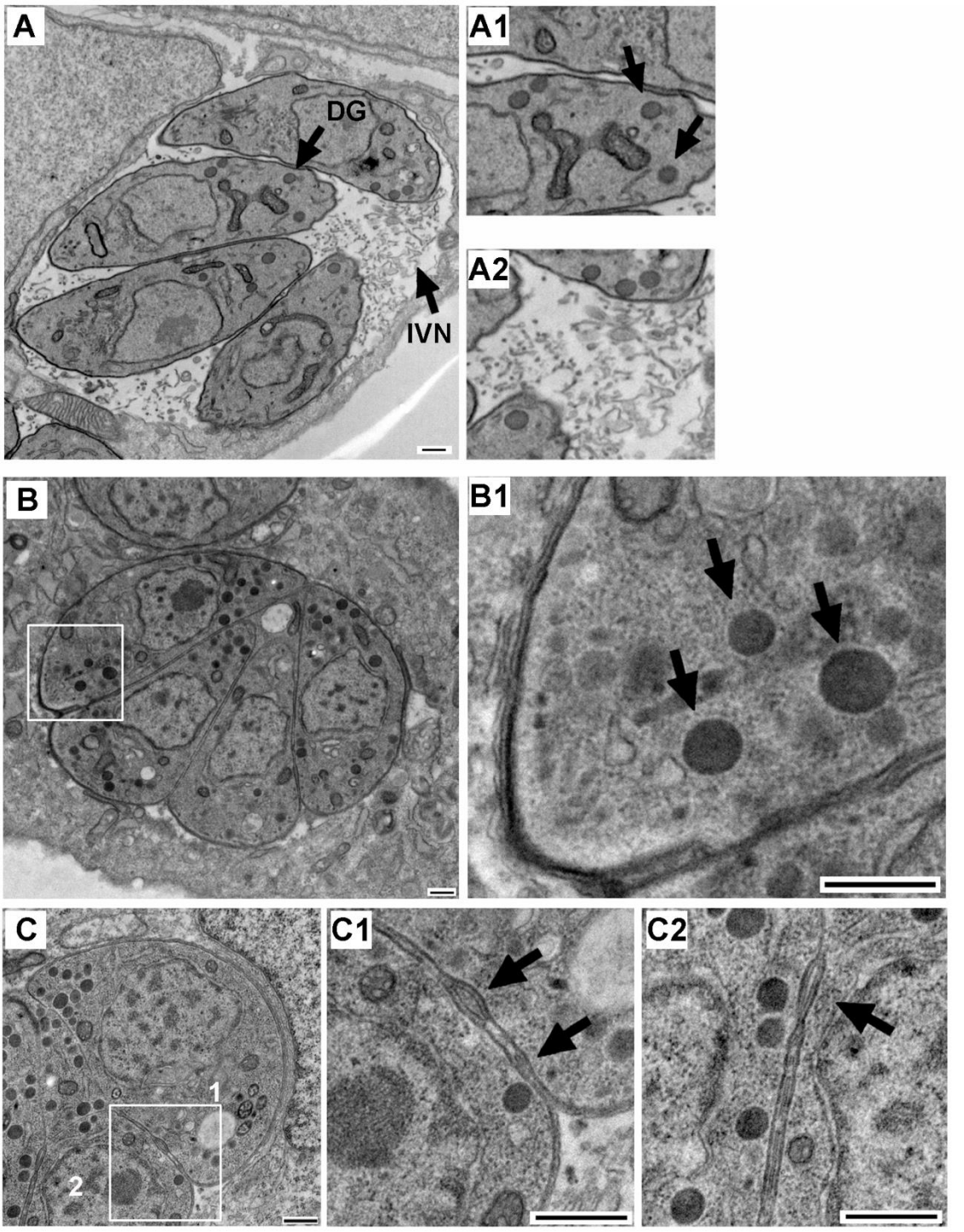
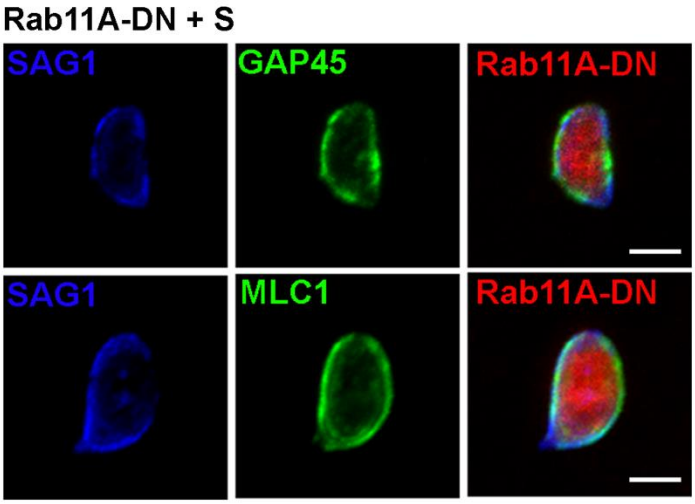



**Figure 4.** Electron micrographs of infected host cells harboring Shield-1 induced Rab11A-WT replicating parasites (**A**), in which dense granules (**A1**) and the IVN (**A2**) are visualized. Shield-1 induced Rab11A-DN parasites (**B-C**) accumulate dense granules (B and B1: a zoom of the region indicated by a white frame in (**B**) and the IVN is not detected in the drastically reduced vacuolar space (**B**). Rab11A-DN expressing parasites also display a previously described defect in membrane segregation between daughter cells (**C**). A zoom of the regions 1 and 2 is shown in C1 and C2. Bars: 500nm.




**S3 Figure.** A-Immunofluorescence assay showing the cortical localization of SAG1, GAP45 and MLC1 in Shield-1-induced extracellular adherent Rab11A-DN expressing parasites. Bars: 2 µm.

## 1.5 *Tg*Rab11A regulates adhesion and motility of extracellular parasites

A role for *Tg*Rab11A in parasite invasion has been previously demonstrated (Andenmatten et al., 2013). To explore which steps of parasite entry (e.g. adhesion, motility, and invasion) were altered, we treated extracellular Rab11A-WT and -DN parasites with Shield-1 for 2 h before monitoring their ability to adhere to host cells. We found that Rab11A-DN tachyzoites were severely impaired in surface attachment to human fibroblast (HFF) monolayers compared to Rab11A-WT parasites (Figure 5A). Furthermore, parasites that successfully adhered exhibited a strong defect in motility, as quantified by the percentage of parasites displaying a SAG1-positive trail deposit (Figure 5B). Importantly, compared to Rab11A-WT parasites, the morphology of adherent motile Rab11A-DN parasites was altered, the latter being wider and shorter, losing their typical arc shape (Figure 5C). Analysis of individual parasites imaged by Scanning EM (n = 70 for WT and DN) confirmed that Rab11A-DN parasites display a significant increase in circularity and accordingly, a decrease in the aspect ratio (AR: major axis/minor axis) (Figure 5D). Of note, conoid extrusion was only slightly decreased in Shield-1 induced Rab11A-DN compared to Rab11A-WT (Figure 5E). Along this line, changes in morphology were not correlated with major perturbations in formation and organization of the sub-pellicular microtubule network in Rab11A-DN parasites compared to Rab11A-WT (Figure 5F).

An impaired recruitment of late glideosome components at daughter cell buds has been previously reported in dividing Rab11A-DN parasites (Agop-Nersesian et al., 2009) and could account for the motility defect. However, we induced Rab11A-DN protein expression in non–

dividing extracellular parasites and accordingly we did not observe any significant defect in the localization of GAP45 and Myosin Light Chain 1 (MLC1) at the cortex of extracellular parasites (S3 Figure). This indicates that the morphological defect observed in Rab11A-DN parasites is not correlated with a significant perturbation of glideosome component localization.

The microneme protein MIC2, a transmembrane protein released at the PM of the parasite, promotes parasite motility and adhesion (Gras et al., 2017; Whitelaw et al., 2017). First, we confirmed by IFA that MIC2-positive micronemes were detected at the apical pole of extracellular induced Rab11A-DN parasites, indicating no defect in their formation and localization (S4A Figure). Secretion of microneme proteins by extracellular parasites can be triggered by ethanol, a step followed by their release from the parasite PM after cleavage by proteases. Notably, ROM4 has been shown to promote MIC2 trimming at the parasite PM (Brossier et al., 2005). Since ROM4 was no longer present at the PM of replicating Rab11A-DN parasites, we investigated whether a similar defect could be observed in 2h Shield-1 induced extracellular Rab11A-DN parasites. As previously observed for glideosome components, ROM4 localization at the PM was not perturbed in extracellular induced Rab11A-DN parasites (S4B Figure). Next, we performed excretion/secretion assays to assess the transport of the MIC2 protein to the parasite PM and its subsequent release into the culture medium. Western blot quantification of the Excreted-Secreted Antigen (ESA) fractions demonstrated a significant reduction in MIC2 release upon induction of microneme exocytosis by ethanol (Figure 5G). Accordingly, a slight increase in MIC2 protein levels was observed in the pellet fraction, also indicating that the decrease in MIC2 secretion is not due to a defect in protein synthesis. As observed by IFA, a reduced level of constitutive GRA1 secretion was also detected by WB, which correlated with GRA1 accumulation in the parasite pellet fraction (Figure 5G). Together, these data suggest that the defect of extracellular Rab11A-DN parasites in host cell adhesion and motility may be due to impaired MIC2 delivery to the PM.

Lastly, Rab11A-DN parasites that successfully adhered to the surface of host cells, displayed only a mild defect in host cell invasion (Figure 5H). This was supported by the observation of a correctly formed RON4-positive junction by invading Rab11A-DN parasites (Figure 5H).

Collectively, these data demonstrate that *Tg*Rab11A promotes parasite invasion by regulating parasite motility and adhesion to host cells, but not the formation of the moving junction.

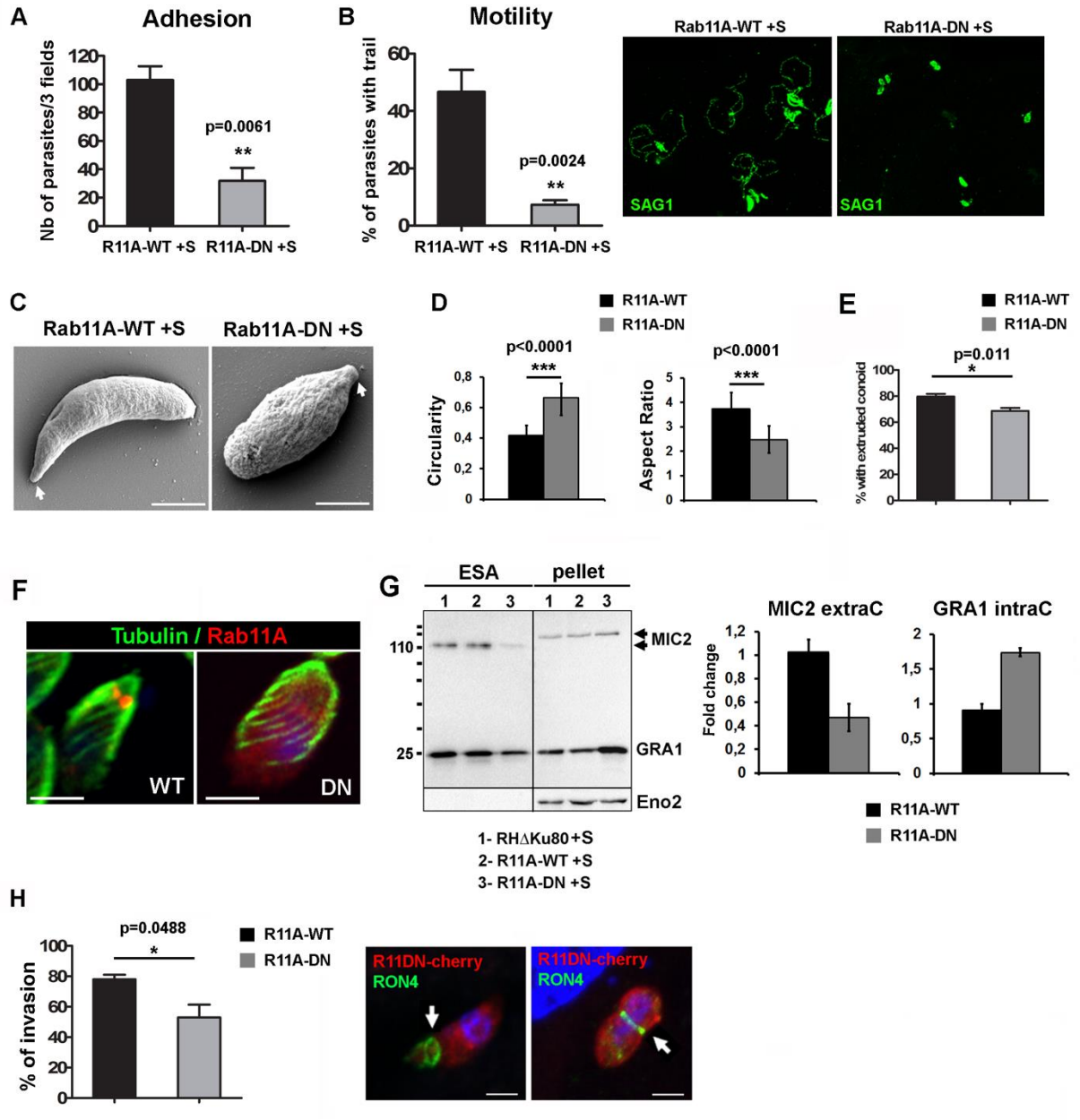
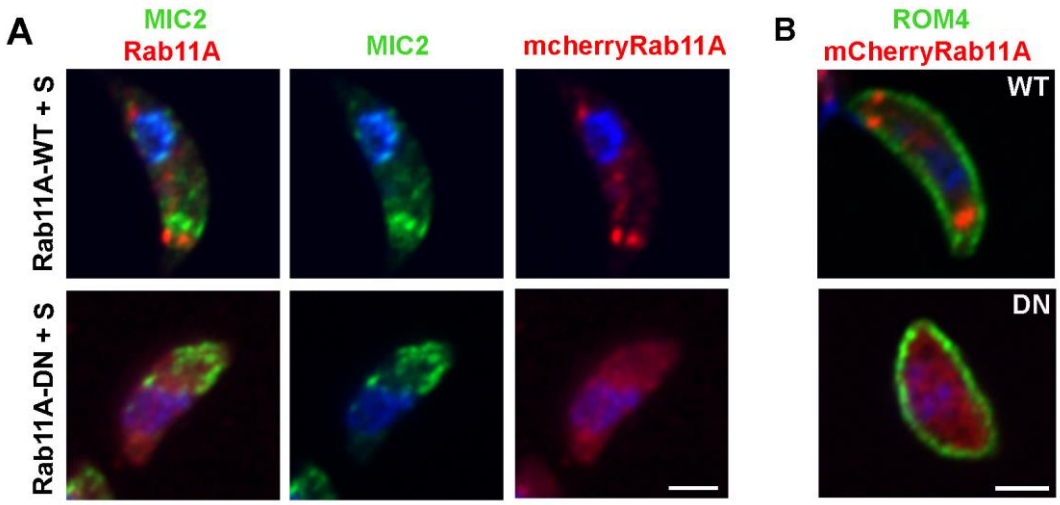



**Figure 5. A-** Quantification of Shield-1 induced extracellular Rab11A-WT and Rab11A-DN parasites adhering to host cells. Data indicate the number of parasites / 3 fields and show mean ± SEM of three independent experiments (unpaired Student's t-test **p = 0.0061). **B-** Quantification of the percentage of Shield-1 induced extracellular Rab11A-WT and Rab11A-DN parasites displaying a SAG1-positive trail deposit (green) as illustrated in the right images. Data show mean ± SEM of three independent experiments (unpaired Student's t-test **p = 0.0024). **C-** Scanning Electron Micrographs (SEM) of Shield-1 induced extracellular Rab11A-WT and Rab11A-DN parasites, which were allowed to move for 15 min on BSA-coated coverslips before fixation. Arrows indicate the apical pole of the parasite. Bars: 2 µm. **D-** The histograms indicated the mean Circularity and Aspect Ratio (major axis / minor axis) of Shield-1 induced extracellular Rab11A-WT and Rab11A-DN parasites imaged by SEM (n

= 70 parasites for each condition; unpaired Student's t-test ***p<0.0001). **E-** Histogram showing the percentage of Shield-1 induced Rab11A-WT and Rab11A-DN extracellular parasites displaying an extruded conoid (unpaired Student's t-test *p = 0.011). **F-** IFA showing the localization of the sub-pellicular microtubule network (green) in Shield-1 induced Rab11A-WT and Rab11A-DN (red) parasites. Bars: 2 µm. **G-** Western blot analysis of excreted-secreted antigen assays (ESA) performed with Shield-1 induced (+S) extracellular RHΔKU80, Rab11A-WT and Rab11A-DN expressing parasites revealed a defect in MIC2 and GRA1 protein secretion. Eno2 was used as a loading control. Secreted MIC2 proteins (ESA fraction) and intracellular GRA1 proteins (pellet fraction) were quantified from 3 independent experiments and expressed as fold-change compared to induced RHΔKU80 parasites. **H-** Quantification of the percentage of Shield-1 induced extracellular Rab11A-WT and Rab11A-DN expressing parasites, which have invaded host cells. Data show mean ± SEM of three independent experiments (unpaired Student's t-test *p = 0,0488). Fluorescence images show Shield-1 induced mcherryRab11A-DN (red) invading host cells, as illustrated by the moving junction positive for RON4 (green). Bars: 1 µm.




**S4 Figure.** Immunofluorescence images showing a similar localization of apical MIC2-positive micronemes (A) and of the plasma membrane protein ROM4 (B) in Shield-1 induced Rab11A-WT and Rab11A-DN parasites. Bars: 2 µm.

## 1.6 *Tg*Rab11A-positive vesicles accumulate at the apical pole during parasite motility and host cell invasion

The active role of *Tg*Rab11A in parasite motility and adhesion led us to explore the localization of *Tg*Rab11A in motile extracellular and invading parasites. Live imaging of mcherryRab11A-WT revealed an unexpected polarized accumulation of *Tg*Rab11A-positive vesicles at two main foci localized at the apical tip of extracellular adhering and motile parasites (Figure 6A, S14 Movie). Apical accumulation of *Tg*Rab11A appeared to be prolonged during host cell invasion (Figure 6B, S15 Movie). Apically polarized localization of *Tg*Rab11A in invading

parasites was further confirmed in fixed parasites after labelling of the moving junction with RON4 (Figure 6C).

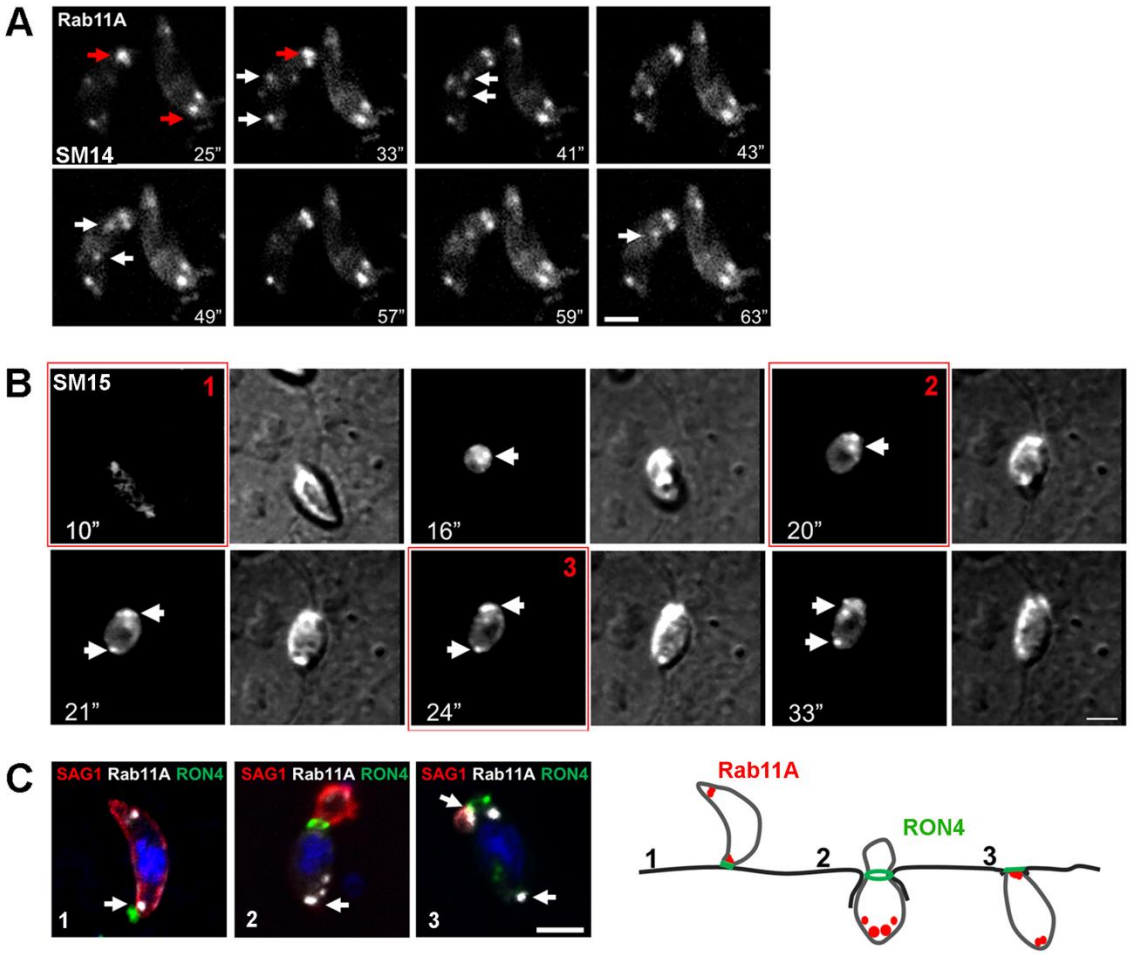



**Figure 6. A-** Sequences of images extracted from S14 Movie showing the polarized recruitment of mcherryRab11A-positive vesicles (white arrows) towards two main foci localized at the tip of adhering parasites (red arrows). Time is indicated in seconds. Bar: 2 µm. **B-** Sequences of images extracted from S15 Movie showing a similar polarized localization of mcherryRab11A-positive vesicles (white arrows) during host cell invasion. At the end of parasite entry, *Tg*Rab11A was also detected at the rear pole of the parasite. Time is indicated in seconds. Bar: 2 µm. **C-** Fluorescence images of RHΔKU80 parasites fixed at three different steps of the host cell invasion process, as indicated in the right scheme. The moving junction is labeled with RON4 (green) and the membrane protein SAG1 was used to label the extracellular portion of the invading parasite (red). Bar: 2 µm.

## 1.7 *Tg*Rab11A regulates polarized secretion of DG content during parasite motility and host cell invasion

Next, we assessed whether *Tg*Rab11A regulates the transport of DGs, not only during parasite replication (Figure 3) but also during parasite motility and invasion. Similarly, to the live

imaging data (Figure 6), we found *Tg*Rab11A at two foci localized at the apex of extracellular parasites that had been allowed to move on coverslips prior to fixation (Figure 7A). These *Tg*Rab11A foci co-localized with the DG protein GRA1 suggesting that *Tg*Rab11A may regulate apical transport and/or anchoring of DGs at the apical pole of motile extracellular parasites. A similar co-recruitment of *Tg*Rab11A and DGs at two apical foci was observed during host cell invasion (Figure 7B, white arrows and S5A Figure). Most importantly, we observed a complete inhibition of this polarized DG apical localization in extracellular motile Rab11A-DN parasites (Figure 7A) and during host cell invasion (Figure 7B and S5A Figure). This demonstrates that *Tg*Rab11A regulates the apical accumulation of DGs during the early steps of parasite motility and entry into host cells.

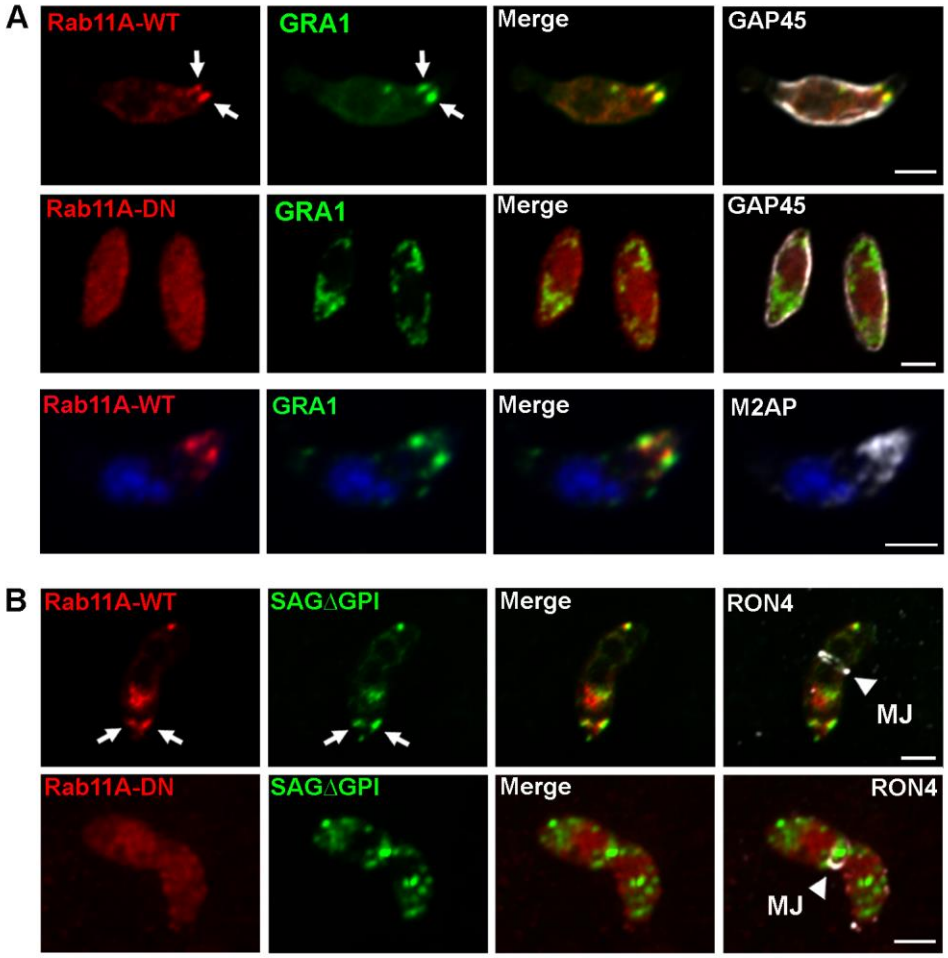
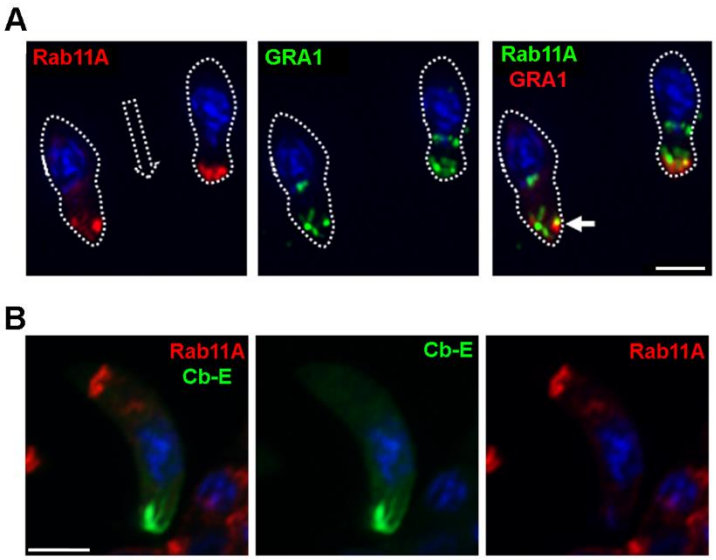



**Figure 7. A-** Immunofluorescence images showing the co-localization of mcherryRab11A (red) and GRA1-positive DGs (green) at two apical foci localized near the apical boundary of the Inner Membrane Complex (labeled with antiGAP45 antibodies) in motile extracellular induced Rab11A-WT (upper raw). This apically polarized accumulation is no longer detected in induced Rab11A-DN expressing parasites (middle raw). The parasite apical pole is indicated by the presence of the microneme protein M2AP (lower raw). Bars: 2 µm. **B-** A similar apical and focalized co-localization

between *Tg*Rab11A and SAGΔGPI-GFP-positive DGs (white arrows) is observed during host cell invasion confirmed by the detection of the RON4-positive moving junction. DG apical accumulation is no longer observed in invading Rab11A-DN. Bars: 2 µm.




**S5 Figure.** A- Immunofluorescence images showing the co-localization of the mcherryRab11Apositive signal (red) and GRA1-positive DG (green) at two apical foci in invading Rab11A-WT parasites. B- Immunofluorescence images showing the apical localization of mcherryRab11A (red) and the enrichment of actin (Cb-E, green) at the posterior pole of motile extracellular Rab11A-WT parasites. Bars: 2 µm.

# Discussion

In this study, we unraveled an essential role of *Tg*Rab11A in the delivery of transmembrane proteins to the parasite PM and the release of DG proteins into the vacuolar space during intracellular replication.

In other eukaryotic systems, *Tg*Rab11A localizes to the endocytic recycling compartment (ERC) and has been implicated in trafficking of internalized receptors from the ERC to the PM (Mercier and Cesbron-Delauw, 2015). *Tg*Rab11A also localizes to the TGN compartment, where it regulates transport of material from this compartment to the ERC, or to the PM (Chen et al., 1998). Similarly, during *T. gondii* cytokinesis, *Tg*Rab11A mostly localizes at the Golgi of daughter cells, and at the tip of growing buds, suggesting a polarized transport of de novo synthetized material between these two locations during daughter cell emergence. Interestingly, a similar apically polarized localization of *Tg*Rab11A was also evident during extracellular parasite motility. Thus, one may envision that components of the apical complex, a microtubule-rich structure from which emanates subpellicular microtubules

(Leung et al., 2017), may control Rab11A-dependent recruitment of specific cargos at the apical pole of the parasite. In particular, RING2, a component of the apical polar ring, was shown to function in constitutive and cGMP-stimulated secretion of microneme proteins (Katris et al., 2014). Recently, two other components of the apical polar ring, APR1 and the Kinesin A, have also been reported to regulate MIC2 secretion (Leung et al., 2017). Hence, it will be of interest to investigate whether *Tg*Rab11A interacts with components of the apical polar ring to promote recruitment and/or exocytosis of micronemes and DG during extracellular motility and invasion. Moreover, videomicroscopy recordings of mCherryRab11A-WT in intracellular parasites revealed highly dynamic *Tg*Rab11A-positive vesicles displaying bidirectional trajectories between the apical and the basal poles, with an accumulation at the basal pole of replicating parasites. This suggests that *Tg*Rab11A may contribute to the formation of the residual body, an organized structure that interconnects parasites during replication, or in the regulation of parasite exchanges, the two processes being likely tightly correlated. In this context, we observed *Tg*Rab11A-positive vesicles and tubular-like structures in the region of the residual body. This region was recently reported to harbor a dense actin-myosin network that connects the parasites within the PV ensuring synchronous divisions (Frénal et al., 2017b; Periz et al., 2017). Thus, Rab11A may also contribute to the regulation of this actin network function and dynamics. Indeed, in plants dysregulated Rab11A activity affects actin organization in the apical region of growing pollen tubes (de Graaf et al., 2005, p.). Supporting the hypothesis of a specific interaction between *Tg*Rab11A and the actino-myosin cytoskeleton, depolymerizing actin filaments alters *Tg*Rab11A-positive vesicle displacements. A role for the complex Myosin Vb-FIP2-Rab11A in promoting actin-mediated transport of vesicles has been previously observed in mammalian cells (Chu et al., 2009; Horgan and McCaffrey, 2009; Schafer et al., 2014). So far, no homologues of Rab11-family interacting proteins (FIPs) have been identified in *T. gondii* and *Plasmodium*. Nonetheless, *P. falciparum* Rab11A was reported to directly interact with the myosin light chain 1 (MLC1/MTIP), which therefore links Rab11A-mediated vesicular transport to unconventional myosins and the actin cytoskeleton (Agop-Nersesian et al., 2009). As actin depolymerization resulted in the formation of both cytosolic and peripheral *Tg*Rab11A-positive static clusters, it's possible that distinct myosins regulate different steps of *Tg*Rab11A/DG transport e.g. MyoF in the cytosol and from the TGN (Heaslip et al., 2016),

MyoA at the parasite cortex where the glideosome is located (Agop-Nersesian et al., 2009), and MyoJ in the cell-to-cell connecting network (Frénal et al., 2017b). Further studies using parasite strains deleted for these molecular motors will address this question.

Co-distribution studies indicated that *Tg*Rab11A-positive vesicles associate with dense granules in a dynamic manner. However, we did not observe *Tg*Rab11A at the limiting membrane of DG. Rather, these two compartments appear to transiently dock one with each other enabling joint transient motions that were particularly evident at the cortex of the parasite. Indeed, tracking of the trajectories of both *Tg*Rab11A-positive vesicles and DG revealed that *Tg*Rab11A-positive vesicles promoted DG anchoring at the parasite cortex and their rapid "directed" transport. This mode of transport called "hitchhiking" has been recently described in different cell types and has emerged as a novel mechanism to control organelle movement (Salogiannis and Reck-Peterson, 2017). During this process, the "hitchhiker" benefits from distinct molecular motors present at the surface of the "vehicle". In addition, endosomes represent multifunctional platforms that receive specific signals and could drive transport of hitchhiker cargo to particular regions of the cell. Notably, co-movement of cargo may facilitate interactions at membrane contact sites important for organelle maturation, fusion and/or material exchange. Related to this last aspect, we found that over-expression of Rab11A-DN led to a complete block in DG secretion. We observed that restoration of *Tg*Rab11A functions by washing out Shield-1 correlated with an accumulation of *Tg*Rab11A-positive vesicles at the parasite plasma membrane suggesting a role for *Tg*Rab11A in vesicle docking/tethering at the PM, which remains to be formally demonstrated. In other eukaryotic systems, Rab11A is known to promote vesicle docking and fusion at the PM via its interaction with the exocyst complex and SNARE proteins, respectively (Welz et al., 2014). However, homologues of the different exocyst complex subunits could not be identified in *T. gondii* (Carruthers, 2013). Thus, unexplored mechanisms of Rab11A-mediated vesicle docking at the PM may exist in *T. gondii* and Rab11A-interacting SNAREs remain to be identified. One may envision that *Tg*Rab11A drives DGs to sites that favor exocytosis by promoting interactions with regulatory factors involved in vesicle fusion.

Benefiting from the fast and efficient induction of the Rab11A-DN protein expression in extracellular parasites, we confirmed the previously described defect in host cell invasion (McNamara et al., 2013). Of note, our numerous attempts to generate parasites expressing

C-terminal tagged *Tg*Rab11A failed, and notably, our attempts to apply the rapidly inducible AID knock-down system also failed (Brown et al., 2018). This is likely due to the fact that the C-terminal domain of the Rabs contains one or two cysteines recognized by geranylgeranyl-transferases to induce their isoprenylation, a modification required for their association with membranes. The impaired cell invasion of Rab11A-DN expressing parasites results from a strong defect in parasite adhesion to host cells. Indeed, parasites that successfully adhered to host cells were only mildly perturbed in host cell entry. Moreover, secretion of MIC2, an adhesin essential for parasite adhesion and motility was reduced upon dysregulation of *Tg*Rab11A activity. Secretion of the GPI-anchored protein SAG1 is also altered in Rab11A-DN expressing parasites (Agop-Nersesian et al., 2009). Thus, it's likely that the altered secretion of these two host cell adhesins contributes to the decrease in adhesion and motility of Rab11A-DN parasites. Consistent with a role of *Tg*Rab11A in the regulation of surface protein trafficking, we also found a strong defect in the localization of the romboïd protease ROM4 and the glucose transporter GT1 at the PM, indicating a broader role of *Tg*Rab11A in the regulation of surface protein trafficking. Presumably, distinct exocytic pathways exist in *T. gondii*, such as described in other organisms. In particular, whether a distinct endosome recycling compartment is present in *T. gondii* requires further exploration. Previous studies highlighted that *T. gondii* has functionally repurposed its endocytic system to serve as secretory pathway of this fast replicating intracellular parasite (Carruthers, 2013; Venugopal and Marion, 2018). In this context, the TGN appears to be a hybrid compartment to which the endosomal markers (Rab5 and Rab7) are tightly associated (Venugopal et al., 2017). Therefore, one might envision that material internalized from the PM reaches this hydrid TGN/ELC compartment before being re-directed to other target membranes, such as the rhoptries, the PM, and the degradative vacuole (VAC). Such a recycling process has been recently observed during extracellular parasite motility (Brossier et al., 2005). Recycling of mother material during daughter cell emergence may also follow this indirect secretory pathway, while de novo synthetized proteins may traffic directly from the TGN to the PM.

Finally, during extracellular parasite motility and invasion, imaging of both live and fixed parasites revealed an unexpected polarized accumulation of *Tg*Rab11A-positive vesicles towards two main foci located just beneath the conoid. In mammalian cells, Rab11A-dependent polarized secretion towards the leading edge of motile cells is essential to

promote persistent migration (Fletcher and Rappoport, 2010). This process not only provides additional membrane ensuring the extension of the leading edge, but also contributes to the translocation of regulatory factors involved in actin and microtubule cytoskeleton activity. In *T. gondii*, apical delivery of some effectors may regulate actin and microtubule cytoskeleton activity and thereby parasite motility. Such regulatory mechanisms have been demonstrated for the lysine methyltransferase, AKMT (Apical complex lysine (K) methyltransferase) localized at the conoid (Heaslip et al., 2011). It has been also recently shown that the DG protein GRA8 contributes to parasite motility by regulating conoid extrusion and organization of the microtubule network (Díaz-Martín et al., 2019, p. 8). Thus, future research will aim to identify the cargos that are apically delivered in a Rab11A-dependent manner and their putative role in regulating parasite motility. Interestingly, the apical accumulation of DGs that we observed in extracellular motile parasites has been previously described during parasite invasion (Labruyere et al., 1999). Thus, an alternative explanation would be that the parasite "prepares its arrival" at the host cell, anticipating the burst of DG secretion that occurs during invasion by promoting their anchoring at the apical pole. In such a scenario, a second signal (vacuole closure?) would then trigger their fusion and content release into the vacuolar space. Importantly, at present we cannot explain the defect in morphology we observed in extracellular motile Rab11A-DN parasites. This may be linked to a dysregulation of the actin cytoskeleton activity or be related to a defect in the dynamics of an endo-exocytosis activity required for parasite forward movement, both potentially leading to shape deformation of the moving parasite. Along with the first hypothesis, actin staining (Cb-E transfected parasites) in extracellular parasites that were allowed to move on coated coverslips before fixation revealed a strong accumulation of actin at the basal pole of the parasite (S5B Fig), such as recently described in invading parasites (Del Rosario et al., 2019).

Therefore, identifying *Tg*Rab11A interactors will be an important future goal, as it will improve our understanding of the mechanisms regulating the distinct exocytic pathways in *T. gondii*. In particular, it will be important to characterize the molecular mechanisms involved in anchoring *Tg*Rab11A-positive vesicle to actin or microtubule molecular motors, and of a possible process of vesicle docking/tethering at the PM, both during parasite motility and intracellular replication. Finally, exploring a putative functional interaction between

*Tg*Rab11A and the apical complex may lead to the discovery of novel regulated secretory mechanisms essential to ensure parasite virulence.

# 2 Implication of the *Toxoplasma gondii* HOOK-FTS-HIP complex in microneme secretion

In the second part of my thesis project, we aimed to characterize the mechanisms involved in the *Tg*Rab11A-dependent exocytic events that we observed at the apical tip of *T. gondii*. In particular, we were interested in the characterization of a novel *Tg*Rab11A binding partner, *Tg*HOOK that we identified by IP and GST-pull down. This led to the identification and the functional characterization of a novel regulatory complex: *Tg*HOOK-*Tg*FTS-HIP complex. This part of my PhD was performed in close collaboration with the laboratory of Dominique Soldati-Favre, notably David Dubois (Geneva University).

## 2.1 The adaptor molecule *Tg*HOOK, a novel partner of *Tg*Rab11A

Previously, we demonstrated that *Tg*Rab11A regulates dense granule secretion and plasma membrane protein delivery in *T. gondii* during intracellular replication (Venugopal et al., 2020). To further dissect the molecular mechanisms regulating this *Tg*Rab11A-dependent secretory activity, we aimed to find partner molecules. For that, Kannan Venugopal (KV) first performed a co-immunoprecipitation (co-IP) with a cMyc tagged version of *Tg*Rab11A and identified a HOOK domain containing protein (TGGT1_289100) as a putative partner, that we called *Tg*HOOK. To verify this interaction, KV performed a GST-pull down using a GDP-bound inactive and GTPγs-bound active form of *Tg*Rab11A protein as baits. After mass spectrometry analysis, TGGT1_289100 was found as a preferential partner of the GTPγS bound form of *Tg*Rab11A compared to its GDP bound inactive form. (Figure 1A). To functionally characterize this novel protein, KV generated a stable knock-in parasite line of *Tg*HOOK with an HA tag tethered to its C-terminus, which we address here on as HOOK-HA strain (figure 1B). Using a parasitic lysate of the HOOK-HA strain, a GST Rab pull down assay was performed. WB analysis of bound partners showed an interaction between *Tg*HOOK and GTPγS Rab11A and to a lesser

extend active GTPγS Rab11B, but not between *Tg*HOOK and GTPγS Rab5 or GTPγS Rab7, localized at early and late endosomes respectively (Figure 1C).

A

| | | | Total number of peptides | | | Total number of spectra | | |
|---|---|---|---|---|---|---|---|---|
| Protein name | Protein accession numbers | MW (Da) | GST-GTPγS | GST-Rab11A GDP | GST-Rab11A GTPγS | GST-GTPγS | GST-Rab11A GDP | GST-Rab11A GTPγS |
| Ras-related protein Rab11 | TGGT1_289680 | 24997 | 19 | 30 | 27 | 764 | 2090 | 1459 |
| hypothetical protein (HOOK domain containing protein) | TGGT1_289100 | 78764 | | | 16 | | | 21 |

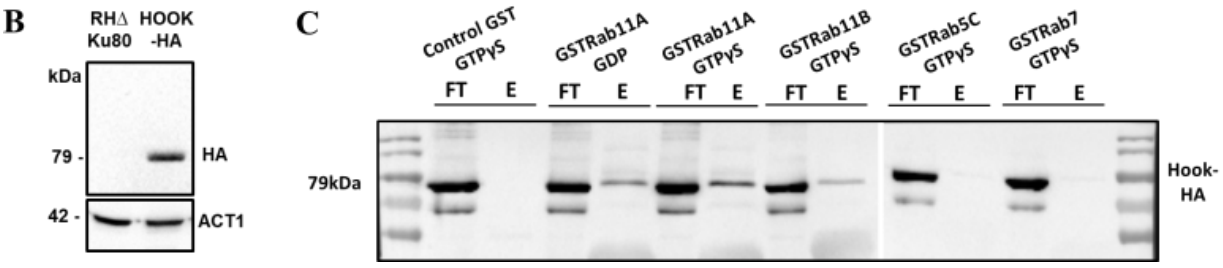



**Figure 1 – *Tg*HOOK, a potential partner for Rab11A.** (**A**) Table showing the mass spectrometry results for the GST pull down experiment using a GDP-bound inactive or GTP- bound active *Tg*Rab11A. *Tg*HOOK strongly interacts with the GTPγS-bound active Rab11A, with a high amount of unique peptides. (**B**) Knock-In parasites expressing HA tagged *Tg*HOOK. A western blot revealing the expression of a single band with the predicted molecular weight of 79KDa, corresponding to the HA-tagged version of *Tg*HOOK, which was not detected in the RHΔKu80 parental strain. Act1: Actin, loading control (**C**) Western blot showing the results of the GST Rab-pull down performed using HOOK-HA parasitic lysate. *Tg*HOOK was preferentially pull-downed (E) with GST- Rab11A and to a lesser extend to GST-Rab11B. FT: Flowthrough; E: Elution (from beads).

## 2.2 *Tg*HOOK localizes at the apical pole in *T. gondii*

To examine the intracellular localization of *Tg*HOOK, we used the C-terminal endogenously tagged strain HOOK-HA. We found that *Tg*HOOK is localized in vesicles spread throughout the cytoplasm but enriched at the apical region of the parasite with a pattern of distribution similar to microneme proteins (Figure 2A) and also accumulated at the very apical tip of the parasite, where the conoid is located. Based on a study carried out on novel cell cycle regulators, this protein was previously found to co-localize with the microneme protein MIC2, therefore it was named MIC18 (Butler et al., 2014). However, *Tg*HOOK lacks a signal peptide, one of the main characteristics of MIC proteins. Yet the authors found that this protein

contains a HOOK domain known for its role in binding to microtubules, and thus suggested a role in the translocation of MIC proteins along the subpellicular microtubules from the Golgi to the apex of the parasite (Butler et al., 2014). However, using SIM (Structured Illuminated Microscopy) in intracellular replicating parasites, we found that *Tg*HOOK does not co-localize with any of the tested MIC proteins, as here illustrated for MIC2 (Figure 2B). In addition, we found that *Tg*HOOK accumulates just beneath centrin2 (Cen2) and calmodulin-like 1 (CAM1) proteins, two components of the conoid, suggesting that *Tg*HOOK is located at or near the apical polar ring (Figure 2C). Moreover, although we found an interaction between Rab11A and *Tg*HOOK by GST-pull down, only a very partial co-localization was observed between Rab11A positive vesicles and *Tg*HOOK, often localized at the basal pole of the parasite (Figure 2D).

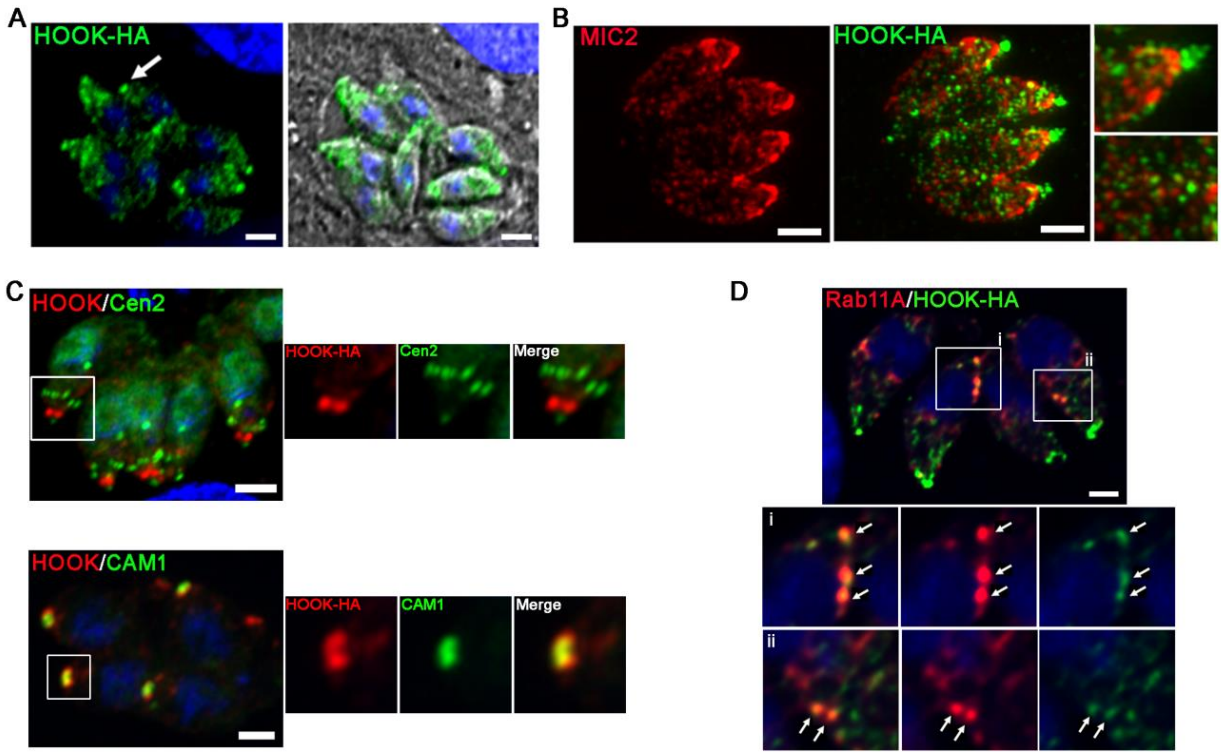



**Figure 2 - *Tg*HOOK-HA localization.** Immunofluorescence assay (IFA) performed in intracellular parasites showing that *Tg*HOOK localizes at the apical tip (arrows) and in numerous vesicles enriched at the apical region of the parasite (**A**), *Tg*HOOK does not colocalize with MIC2 proteins (**B**), and accumulates at the conoid region, just beneath centrin2 (Cen2) and calmodulin-like 1(CAM1) proteins (**C**). (**D**) IFA experiment showing that *Tg*HOOK partially co-localizes with Rab11A positive vesicles in intracellular replicating parasites. Scale bar = 2µm.

## 2.3 *Tg*HOOK contributes to parasite motility and host cell adhesion, and modestly to invasion and egress

In order to investigate the role of *Tg*HOOK, a Tetracycline inducible knock-down (iKD) line for the protein was generated in the lab (iKD HA-HOOK). The group of D. Soldati-Favre (University of Geneva) found an association between *Tg*HOOK and the microneme protein AMA1 using the BirA proximity assay (Chen et al., 2015; Hehl et al., 2000; Nadipuram et al., 2016). In this context, they had previously generated a cMycHOOK-iKD parasite line as well as direct HOOK-KO parasites using the CRISPR-Cas9 system. Therefore, a collaboration was established between our labs and the functional characterization of the *Tg*HOOK mutant lines was performed. They demonstrated that the HOOK-KO parasites exhibit a strong impairment in the lytic cycle as shown by the smaller lysis plaques formed by these parasites compared to the parental line (RHΔKu80) when grown 7 days on a fibroblast monolayer (Figure 3A). This strong alteration in the lytic cycle was correlated with a slight defect in intracellular growth rate (Figure 3B) as well as a moderate defect in host cell invasion (Figure 3C) and egress capacity (Figure 3D). We also measured a defect in parasite motility (Figure 3E) and host cell attachment that likely contributes to the defect of invasion we noticed (Figure 3F). Overall, the strongest defect was seen for parasite egress.

These combined mild alterations in all the steps of the lytic cycle should lead to a defect of virulence *in vivo*. Accordingly, mice infected with the HOOK-KO strain showed a delay in death (10 to 11 days post-infection) compared to the control parental strain that were able to kill the mice within 7 days post-infection (Figure 3G). This difference was not drastic but statistically significant, indicating that the depletion of the *Tg*HOOK protein has an effect on the parasite virulence in mice.

An impairment in parasite egress, motility and host cell adhesion strongly suggests a defect in microneme secretion (Gras et al., 2017). Surprisingly, MIC protein secretion (including MIC2 and AMA1) by extracellular parasites upon induction with ethanol or the Ca2+ ionophore BIPPO was not affected in HOOK-KO and cMycHOOK-iKD parasites (D. Soldati's lab, data not shown). Accordingly, no detectable obvious defect in microneme localization (and thus biogenesis) could be observed during intracellular replication by immunofluorescence assay (IFA) (Figure 3H). We also did not detect any defect in rhoptry biogenesis and localization

(Figure 3H). In addition, we previously demonstrated that *Tg*Rab11A is essential to promote DG secretion; however no defect in the biogenesis and secretion of this compartment was detected by IFA in intracellular replicating parasites (Figure 3H). The lab of D. Soldati-Favre also confirmed that secretory organelle biogenesis and positioning were not affected by electron microscopy (EM) (not shown). Similar defects in host cell invasion, attachment and parasite motility were obtained using the tetracycline inducible knock-down cMyc-HOOK-iKD parasite line, as well as a decreased virulence in mice (Figure 4).

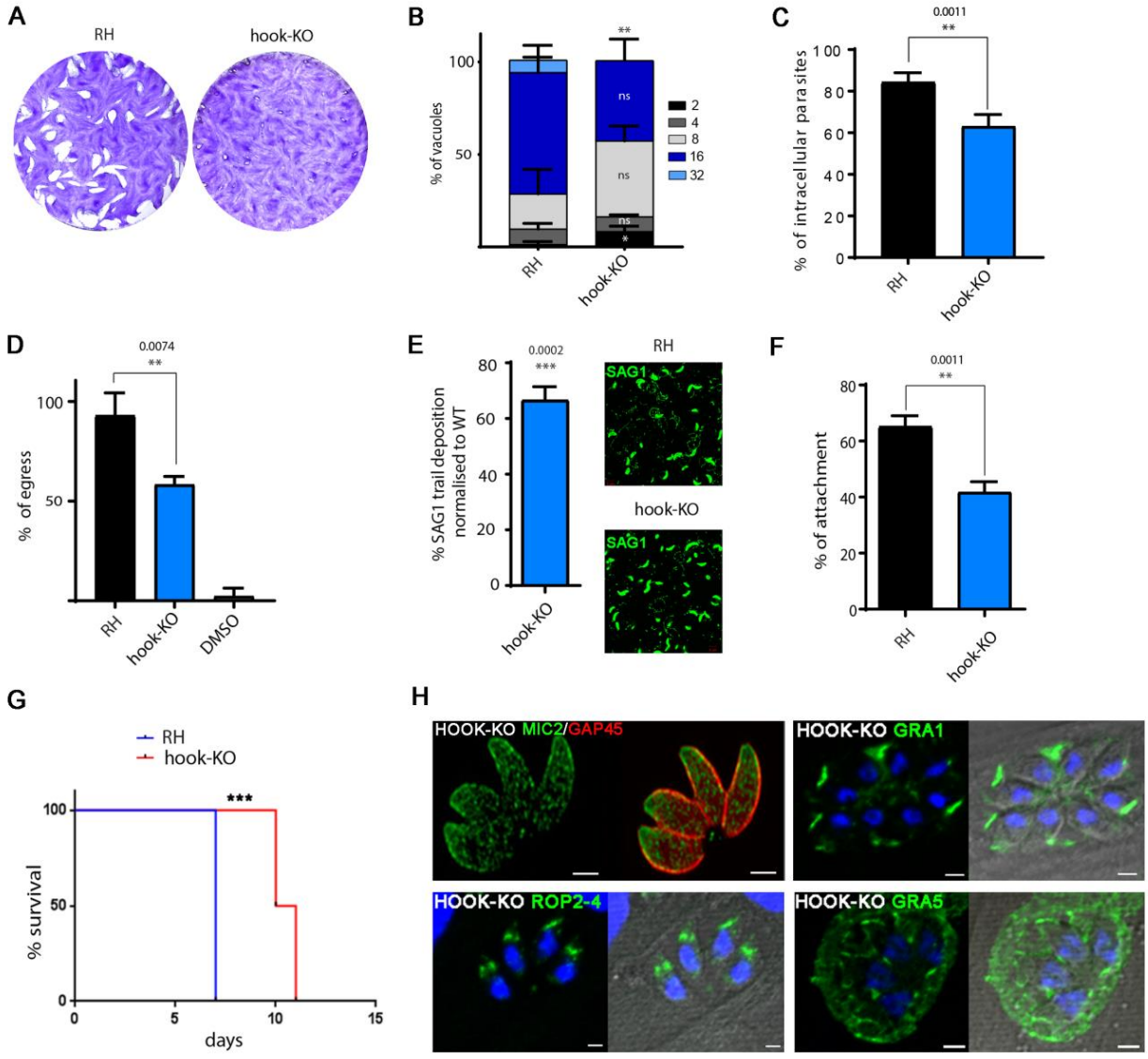



**Figure 3 – HOOK-KO phenotypical characterization.** (**A**) HOOK-KO parasites display a significant impairment in the lytic cycle as shown by the smaller lysis plaques compared to the parental line (RHKu80) when grown 7 days on fibroblast monolayer. (**B**) HOOK-KO parasites display a slight delay in intracellular growth rate. The number of parasites per vacuole was counted 30 hours post-infection. HOOK-KO parasites show a moderate decrease in their invasion capacity (p=0.0011) (**C**) and BIPPO-

induced egress (p=0.0074) (**D**); but significant motility (**E**) and host cell attachment (F) defects compared to control parasites. Data are presented as mean ± SEM. n= 3 independent experiments. Student T-test. (**G**) Survival of mice infected with parental RHΔKu80 strain and HOOK-KO was determined. Mice were infected with 250 parasites intraperitoneally. Log rank Mantel-Cox Test ***p<0,001. (**H**) No detectable defect in MIC2, GRA1, GRA5, and RO2-4 localization was detected for HOOK-KO parasites in intracellular replicating parasites by IFA. Scale bar = 2µm.

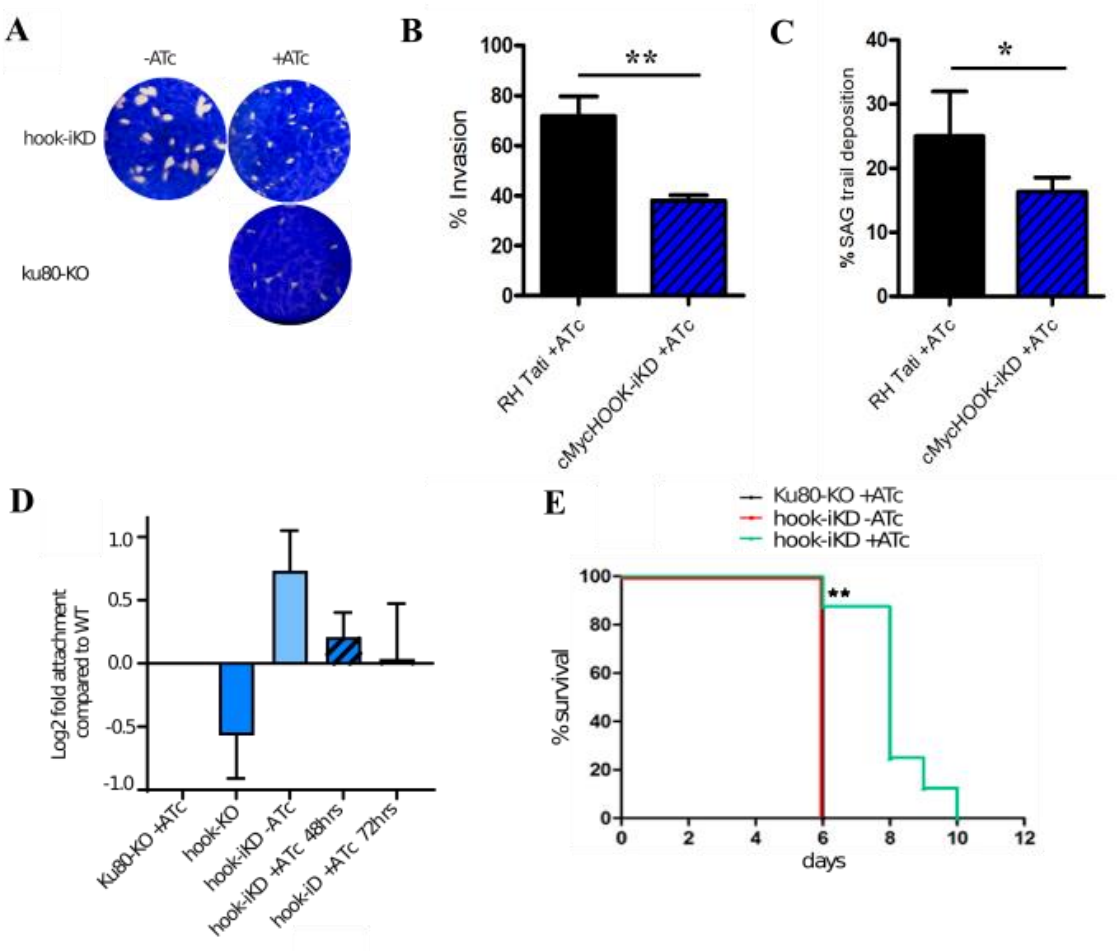



**Figure 4 – cMycHOOK-iKD phenotypical characterization.** (**A**) cMycHOOK-iKD parasites induced with ATc (+ATc) show a significant impairment in the lytic cycle as shown by the smaller lysis plaques compared to the cMycHOOK-iKD –ATc strain and the parental line (RH Tati) when grown 7 days on fibroblast monolayer. cMycHOOK-iKD +ATc parasites display moderate defects in their invasion capacity (**B**), motility (**C**) and attachment compared to cMycHOOK-iKD –ATc parasites and the parental strain (**D**). Data are presented as mean ± SEM. n= 3 independent experiments. Student T-test. (**E**) Survival of mice infected with parental RHΔKu80Tati strain pre-treated with ATc and cMycHOOK-iKD pre-treated or not with ATc. Mice were infected with 250 parasites intraperitoneally. Log-rank Mantel-Cox **p=0,001

Moreover, HOOK proteins were shown to regulate early and late endosome trafficking in other eukaryotes (Introduction, section 6.5.1). In *T. gondii*, the Rab5 and Rab7-positive ELC

are closely associated with the TGN and serve in a secretory pathway for apical secretory organelle biogenesis. Therefore, we investigated whether the deletion of *Tg*HOOK would affect these compartments. We did not observe any detectable defect in Rab5 and Rab7-positive compartment localization and morphology in HOOK-KO intracellular parasites by IFA (Figure 5A). The localization of the TGN marker *Tg*SORTLR was also not altered.

In addition, live imaging of HOOK-KO parasites expressing *Tg*Rab11A-mcherry showed that Rab11A- positive vesicle movement was not perturbed in HOOK-KO intracellular replicating parasites (Figure 5B). Importantly, based on the apical accumulation of *Tg*HOOK, we envisioned that *Tg*HOOK may be involved in the apical localization of Rab11A-positive vesicles in extracellular motile parasites for instance by mediating vesicle anchoring at/near the apical polar ring before cargo secretion (Venugopal et al., 2020). First, although *Tg*HOOK and Rab11A showed an apical localization near the conoid, the two proteins did not co-localize at the apical tip of motile parasites (Figure 5C). However, in cMycHOOK-iKD parasites induced with ATc, Rab11A (and GRA1) appeared less focalized at the two foci beneath the conoid as we normally observed in control parasites (Figure 5D). Of note, Rab11A and GRA1 proteins still co-localize but both signals despite being enriched at the apical pole appeared more spread. However, this phenomenon was only observed in about 60% of the total parasite population. Thus, these results suggest that *Tg*HOOK could contribute to Rab11A apical localization in extracellular parasites, but detailed quantifications of the fluorescent signals are required to thoroughly address this aspect. Moreover, it will be important to examine whether distinct Rab11A-dependent trafficking activities other than GRA and MIC secretion are perturbed upon *Tg*HOOK depletion in intracellular replicative parasites (see discussion section).

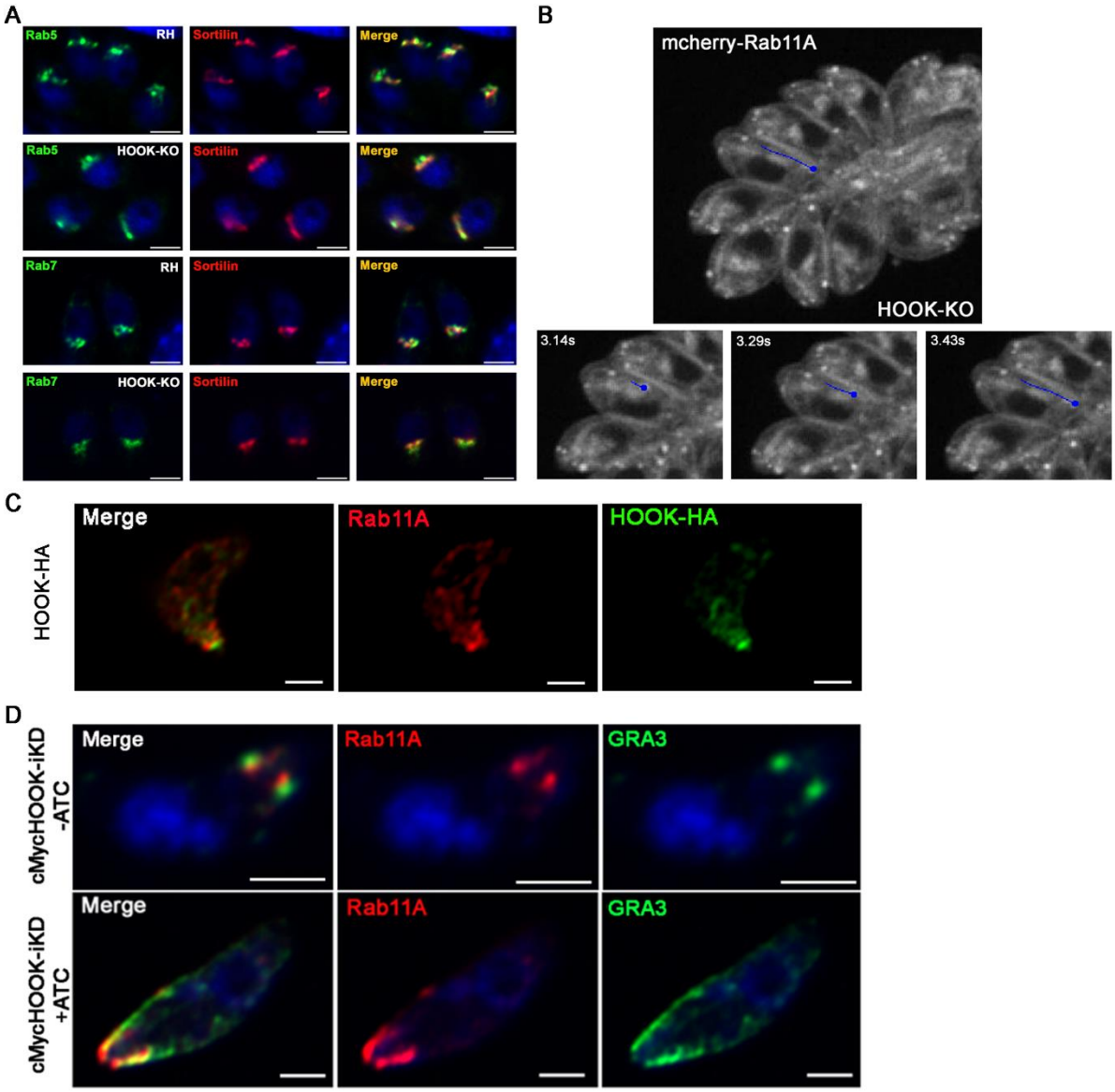



**Figure 5 – HOOK-KO and endosomal compartment localization.** (**A**) IFA experiments showing that no defect in the localization of Rab5 and Rab7-positive compartments in HOOK-KO parasites compared to control parasites. (**B**) Snapshot from a video recording of mcherryRab11A/HOOK-KO parasites revealing no major alterations in Rab11A-positive compartment distribution and Rab11A-positive vesicle movement upon HOOK depletion, as illustrated by the tracked trajectory showing the fast directional motion of a Rab11A-positive vesicle. (**C**) IFA showing that *Tg*Rab11A and *Tg*HOOK co-distribute at the apex of extracellular motile parasites but do not co-localize (**D**) Upper panel: Rab11A and GRA1 co-localize at two foci beneath the conoid in extracellular cMycHOOK-iKD –ATc parasites that have been allowed to move on coated coverslips before fixation; lower panel: altered accumulation of Rab11A and GRA1, less focalized in cMycHOOK-iKD +ATc in extracellular parasites. Scale bar = 2µm.

## 2.4 Identification of *Tg*HOOK associated proteins, *Tg*FTS and *Tg*HIP

The data we obtained so far did not allow to clearly identify which trafficking processes are regulated by *Tg*HOOK. Thus, to further investigate the functional role of *Tg*HOOK, we performed co-immunoprecipitation assays using HOOK-HA protein as bait followed by mass spectrometry analysis. Three specific proteins, a ubiquitin-conjugating enzyme subfamily protein (TGGT1_264050) and two hypothetical proteins with unknown functions (TGGT1_306920 and TGGT1_316650), were reproducibly identified among the proteins showing the highest number of peptides/spectra suggesting the formation of a complex between *Tg*HOOK and these proteins (Figure 6). Interestingly, TGGT1_264050 presented homologies with the Foot Toes protein described in other Eukaryotes, known to be a tight interactor of *Tg*HOOK and a member of the FHF complex, thus it was called *Tg*FTS. TGGT1_306920 did not show homologies with known proteins and was called *Tg*HIP (*Tg*HOOK Interacting Protein). The third protein, TGGT1_316650 has been reported, using the hyperLOPIT method, to be associated with distinct subcellular compartments such as the cytosol and the 19S proteasome subunit (Barylyuk et al., 2020) and has been previously found in IP performed in the lab, which used other baits than *Tg*HOOK, suggesting a putative non-specific binding. Thus, as a first step, we discarded this protein for further deeper functional analysis.

| | | | Total number of peptides | |
|---|---|---|---|---|
| Protein name | Protein accession numbers | Protein molecular weight (Da) | Control RHΔku80 | HOOK-HA |
| hypothetical protein : HOOK | TGGT1_289100 | 78,764 | | 40 |
| hypothetical protein | TGGT1_316650 | 135,264 | | 24 |
| hypothetical protein : HIP | TGGT1_306920 | 49,799 | | 15 |
| ubiquitin-conjugating enzyme subfamily protein : FTS | TGGT1_264050 | 34,431 | | 8 |
| ribosomal protein RPS7 | TGGT1_239100 | 22,588 | 1 | 4 |
| rhoptry kinase family protein ROP40 (incomplete catalytic triad) | TGGT1_291960 | 57,905 | 1 | 4 |

| | | | Total number of peptides | |
|---|---|---|---|---|
| Protein name | Protein accession numbers | Protein molecular weight (Da) | Control RHΔku80 | HOOK-HA |
| hypothetical protein : HOOK | TGGT1_289100 | 78,764 | | 44 |
| hypothetical protein | TGGT1_316650 | 135,264 | | 31 |
| rhoptry kinase family protein ROP37 (incomplete catalytic triad) | TGGT1_294560 | 83,586 | | 17 |
| ubiquitin-conjugating enzyme subfamily protein : FTS | TGGT1_264050 | 34,431 | | 7 |
| hypothetical protein : HIP | TGGT1_306920 | 49,799 | | 8 |
| putative protein disulfide isomerase-related protein (provisional) | TGGT1_249270 | 59,882 | | 4 |

**Figure 6 – HOOK-HA immunoprecipitation.** Two independent HOOK-HA IP experiments were performed and the tables indicate the mass spectrometry analysis results. *Tg*FTS and *Tg*HIP were identified as preferential interactors of *Tg*HOOK, with high amount of unique peptides.

As reported in the ToxoDB, *Tg*HOOK, *Tg*FTS and *Tg*HIP display a low fitness score, -3.25, -2.25 and -3.1 respectively, according to the Genome-Wide CRISPR Screen, suggesting that these proteins might be essential for the parasite lytic cycle (Figure 7A). In addition, mRNA expression study (ToxoDB) shows that *Tg*HOOK, *Tg*FTS, and *Tg*HIP exhibit a similar profile of expression, which is dependent on the cell cycle and picks in M phase similarly to microneme and rhoptry proteins as well as proteins regulating daughter cell cytokinesis (Figure 7B). Similar to mammalian HOOK, *Tg*HOOK contains 3 conserved regions: an N-terminal globular microtubule binding domain, a central coiled-coil domain known to promote dimerization of HOOK proteins, as well as a more divergent C-terminal domain involved in cargo binding (Figure 7C). *Tg*FTS has an ubiquitin-conjugating enzyme domain similarly to the Foot Toes protein described in other Eukaryotes (Figure 7C). However, *Tg*HIP does not contain any conserved functional domain. Xu et al studied mammalian FTS, an inactive variant of an E2 ubiquitin conjugating enzyme domain-containing protein, and identified FTS-associated proteins in order to elucidate its cellular function. They demonstrated that the β-sheet surface of the ubiquitin conjugating domain of FTS interacts with all three human Hook

proteins (Hook1, Hook2, and Hook3) via a conserved helix in the C-terminus of Hook proteins, to form a stable complex. They also identified an uncharacterized FTS-Hook Interacting Protein (FHIP) and demonstrated that FHIP interacts with the HOOK– FTS complex to form a tightly bound complex referred to as FHF (Xu et al., 2008). Thus, our findings suggest that a similar stable complex may exist in *T. gondii* and could regulate apically polarized secretory events at the conoid, which is a microtubule-based structure where dynein accumulates (Hu et al., 2002b). We thus got interested and decided to further study these two proteins.

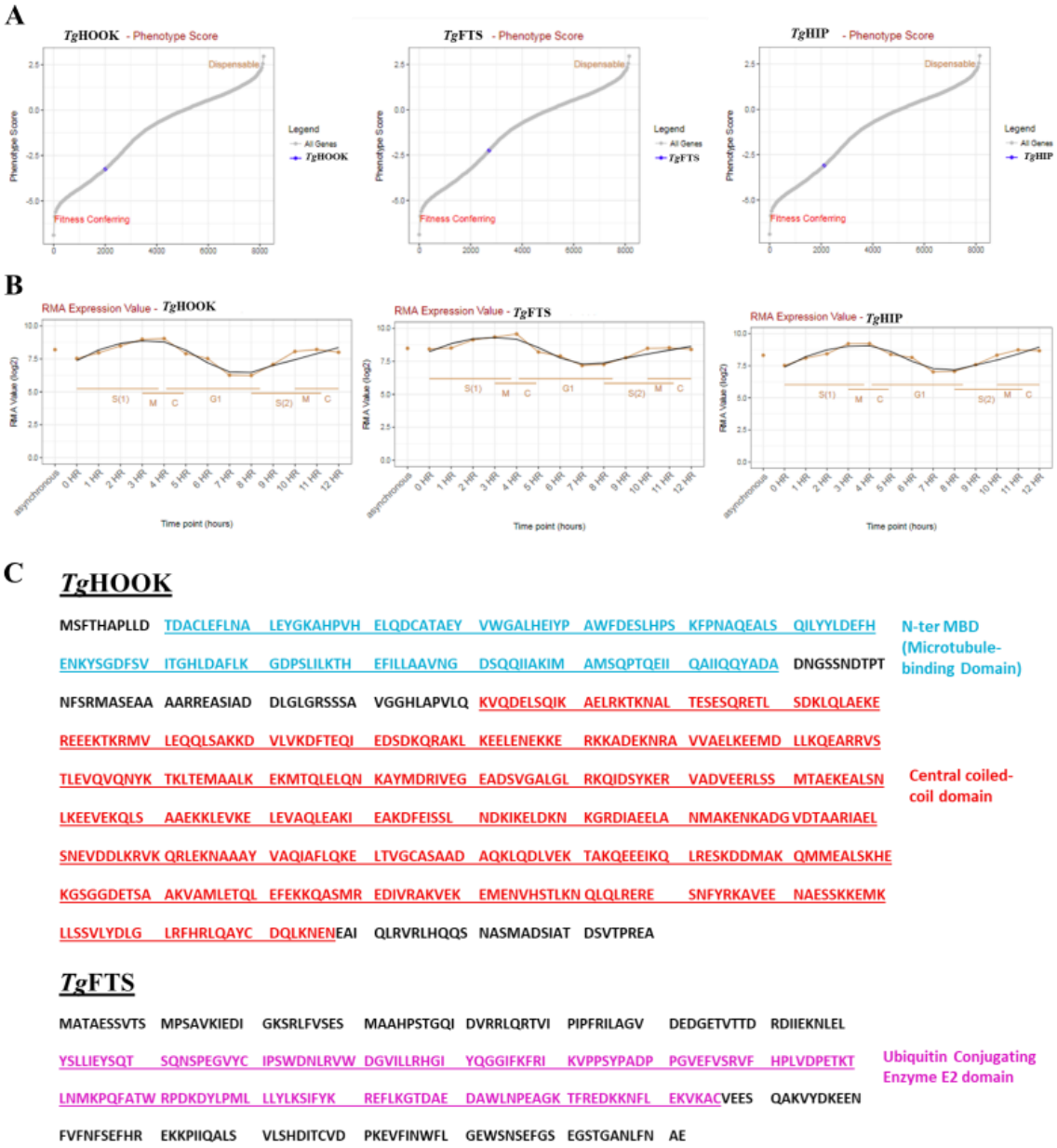



**Figure 7 – *Tg*HOOK, *Tg*FTS, and *Tg*HIP presentation.** (**A**) Fitness score of *Tg*HOOK, *Tg*FTS, and *Tg*HIP during *T. gondii* lytic cycle obtained by the Genome-Wide CRISPR Screen. (**B**) *T. gondii* RH cell cycle microarray expression profiles of *Tg*HOOK, *Tg*FTS, and *Tg*HIP determined hourly after thymidine

synchronization. (**C**) A schematic representation of *Tg*HOOK and *Tg*FTS protein sequences showing the detected functional domains

## 2.5 *Tg*FTS and *Tg*HIP accumulate at the apical tip of intracellular replicating and extracellular parasites

To address the localization of these uncharacterized proteins, we generated parasite strains expressing a 2Ty tagged APR1 (Apical polar ring 1) in knock-in lines expressing cMyc tagged FTS (FTS-cMyc / APR1-2Ty) or HA tagged HOOK (HOOK-HA / APR1-2Ty). D. Dubois generated an endogenously tagged Ty-HIP parasite line. In addition, in order to co-localize *Tg*HOOK and *Tg*FTS, we attempted to generate a stable double Knock-in (KI) line expressing both proteins, *Tg*HOOK and *Tg*FTS, by transfecting a pLic vector encoding *Tg*FTS-cMyc (C-terminal tagging) in HOOK-HA parasites and inversely by transfecting a pLic plasmid encoding *Tg*HOOK-HA (C-terminal tagging) in FTS-cMyc parasites. However, we did not succeed. According to what has been previously established in mammals, FTS protein interacts with the C-terminal domain of HOOK proteins (Xu et al., 2008). Therefore, this failure might be due to the fact that both proteins carry a tag fused to their C-terminal end, which may impair their interaction, which we demonstrated later on as being crucial for *Tg*FTS stability (see section 6). Accordingly, we succeeded in generating a stable Knock-In line expressing a *Tg*FTS protein with an HA-tag fused to its C-terminus in the cMycHOOK-iKD line, which harbors the cMyc tag at the N-terminal end of the *Tg*HOOK protein (that we refer to as cMycHOOK iKD/FTS-HA). The generated strains were verified by western blot (WB) (Figure 8A).

In intracellular replicating parasites, *Tg*HOOK, *Tg*FTS, and *Tg*HIP localize predominantly at the apical tip of the parasite, with *Tg*HOOK and *Tg*FTS accumulating just beneath the apical polar ring APR1 (Figure 8B), although *Tg*FTS also display a vesicular pattern enriched at the apical region of the parasite similar to *Tg*HOOK. However, HIP does not present an abundant cytoplasmic vesicular pattern observed for the two other proteins, suggesting a predominant functional role at the conoid. Similar accumulated apical localizations were detected for the three proteins when examining extracellular parasites, which have been allowed to glide on coverslips before fixation and analysis by IFA (motility assay: M&M) (Figure 8C, upper panels). In agreement with our HOOK-HA IP results showing an interaction between *Tg*HOOK and *Tg*FTS, in extracellular motile parasites *Tg*HOOK and *Tg*FTS co-localized at the apical tip, at the conoid (Figure 8C). However, *Tg*HOOK seems to localize slightly beneath the *Tg*FTS signal.

Strikingly, in intracellular parasites, *Tg*FTS and *Tg*HOOK proteins are detected in cytosolic vesicles that do not co-localize (Figure 8D), suggesting that these proteins may interact only transiently upon specific regulatory signals, notably at the conoid. Of note, the lab of D. Soldati-Favre currently investigates the co-localization between *Tg*HOOK and HIP.

Together, these results suggest the hypothesis that a functional *Tg*HOOK-*Tg*FTS-HIP complex may be formed at the apical tip of the parasite and may be implicated in the regulation of apically polarized secretion of cargo proteins, although *Tg*HOOK deletion did not result in impaired secretion of MIC proteins in extracellular parasites.

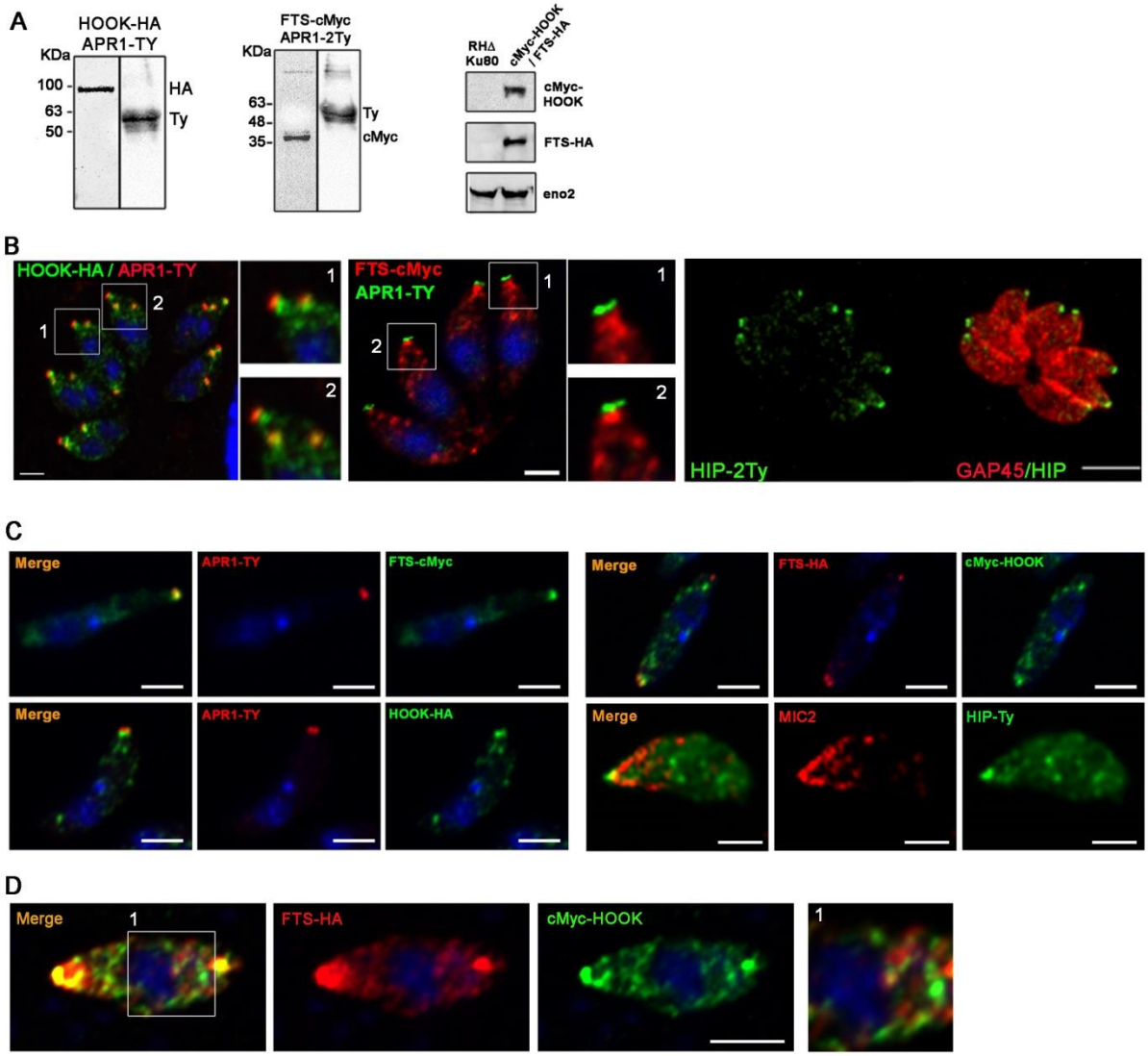



**Figure 8 – *Tg*FTS and *Tg*HIP localization.** (**A**) Western blot revealing a band corresponding to the HA-tagged version of *Tg*HOOK at 79KDa, and a band corresponding to the Ty-tagged version of APR1 (marker of the apical polar ring) at the expected size of 52KDa **(left)**. Western blot revealing a band corresponding to the cMyc-tagged version of *Tg*FTS at 35KDa, and a band corresponding to the Ty-tagged version of APR1 at the expected size of 52KDa **(center)**. Western blot revealing the expression

of a band corresponding to the cMyc-tagged version of *Tg*HOOK at 79KDa, and a band corresponding to the HA-tagged version of *Tg*FTS at 35KDa **(right)**. (**B**) IFA experiments performed using intracellular replicating parasites showing that *Tg*HOOK, *Tg*FTS, and *Tg*HIP localize essentially at the apical tip of the parasite. (**C**) IFA experiments performed using extracellular motile parasites showing that *Tg*HOOK and *Tg*FTS co-localize at the conoid region of the parasite. (**D**) IFA experiments showing that *Tg*HOOK-positive vesicles and *Tg*FTS-positive vesicles detected in the cytosol of intracellular replicating parasites do not co-localize.

## 2.6 *Tg*FTS and *Tg*HOOK interact together; and HOOK depletion leads to FTS degradation

In order to further assess the interaction between *Tg*HOOK and *Tg*FTS proteins, the cMycHOOK iKD/FTS-HA was used for Co-IP assays. A Co-IP experiment using anti-HA and anti-cMyc agarose beads, followed by a Western Blot, was performed three times with the double KI line and the cMycHOOK iKD line as a negative control. We reproducibly found that *Tg*FTS-HA was immunoprecipitated with cMyc*Tg*HOOK using anti-cMyc beads and that cMycT*g*HOOK was immunoprecipitated with *Tg*FTS-HA using anti-HA beads, further supporting our mass spectrometry data and showing that *Tg*HOOK interacts with *Tg*FTS (Figure 9A).

In addition, we performed WB using the cMycHOOK iKD/FTS-HA parasite line induced or not with ATc to examine whether depletion of *Tg*HOOK could impact on *Tg*FTS expression. Our results showed that upon ATc treatment of replicating parasites for 48 hours, *Tg*HOOK `expression was totally repressed as expected but we also observed that *Tg*FTS expression was reduced by 2 fold (Figure 9B). These results suggest that both proteins not only interact together but also that the interaction of *Tg*HOOK with *Tg*FTS is crucial to stabilize the *Tg*FTS protein, which comes in agreement with the formation of functional HFH complex. Of note, similar results were previously described in (Xu et al., 2008), where they found that HOOK depletion leads to FTS protein degradation.

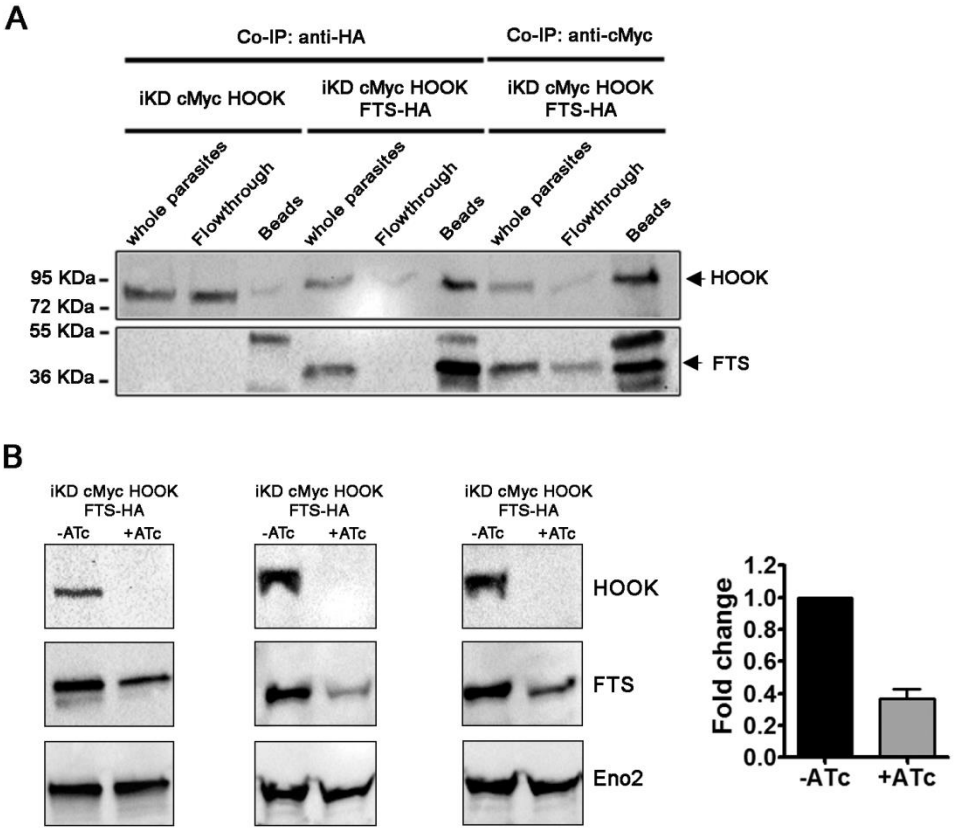



**Figure 9 – *Tg*FTS interacts with *Tg*HOOK.** (**A**) Western blot showing the results obtained by the anti-HA and anti-cMyc co-immunoprecipitation performed with cMycHOOK-iKD/FTS-HA strain and cMycHOOK-iKD strain as negative control. Two bands corresponding to the cMyc-tagged version of *Tg*HOOK at 79KDa, and a band corresponding to the HA-tagged version of *Tg*FTS at the expected size 35KDa were observed in the bead fraction, showing the interaction between *Tg*HOOK and *Tg*FTS. (**B**) Western blot showing that FTS-HA abundance is reduced by ~2fold upon the downregulation of cMycHOOK protein in cMycHOOK/FTS-HA iKD parasites induced with ATc , Enolase 2 (Eno2) was used as a loading control.

## 2.7 *Tg*FTS and *Tg*HIP promote microneme proteins secretion

To address the function of *Tg*FTS and HIP proteins and decipher their biological role, we tried to generate an ATc inducible knock-down parasite line for *Tg*FTS by the promotor replacement strategy (see M&M) but failed to obtain positively transfected parasites. Meanwhile, David Dubois generated an Auxin Inducible Degron (AID) system for the proteins *Tg*FTS and *Tg*HIP, in which a C-terminal miniAID (mAID) tag was added to the endogenous locus of *Tg*FTS and *Tg*HIP in a Transport Inhibitor Response 1 (Tir1) expressing strain to target the protein for proteosomal degradation upon the addition of auxin indole-3-acetic acid (IAA). *Tg*FTS and *Tg*HIP depletion led to an impairment in the parasite lytic cycle as shown by the

smaller lysis plaques formed by these mutant parasites induced with IAA compared to the parental line (Tir1) when grown 7 days on fibroblast monolayer (Figure 10A). Moreover, parasites lacking *Tg*FTS and *Tg*HIP showed a severe defect in invasion (Figure 10B), parasite motility (Figure 10C), and host cell attachment (Figure 10D). However, egress was mildly affected and only in the case of HIP depletion. In sharp contrast to *Tg*HOOK but in agreement with the defects in parasite egress, motility and host attachment, David Dubois demonstrated that both mutants were impaired in MIC2 secretion upon induction with 2% ethanol (Figure 10E). Indeed, parasites depleted for *Tg*FTS lead to 85% decrease in MIC2 secreted in the Extracellular Secreted Antigens (ESA) fraction compared to the stimulated parental (Tir1) +IAA. Whereas, HIP-mAID +IAA parasites resulted in 57% reduction in MIC2 released in ESA. Together, these results suggest that the significant impairment in motility and adhesion is most presumably linked to a defect in microneme secretion. Importantly, he also showed that microneme biogenesis and localization was not affected by both IFA and EM, supporting the hypothesis that the *Tg*HOOK/*Tg*FTS/HIP complex does not regulate microneme transport from the TGN/ELC towards the apical pole of the parasite upon their biogenesis during daughter cell formation. Thus, as discussed further in the following discussion chapter of this thesis, it is likely that the complex regulates the last step of microneme protein secretion by either regulating microneme anchoring to the apical polar ring or their transport within the conoid before fusion with the plasma membrane of the parasite and this during parasite egress and subsequent invasion of host cells.

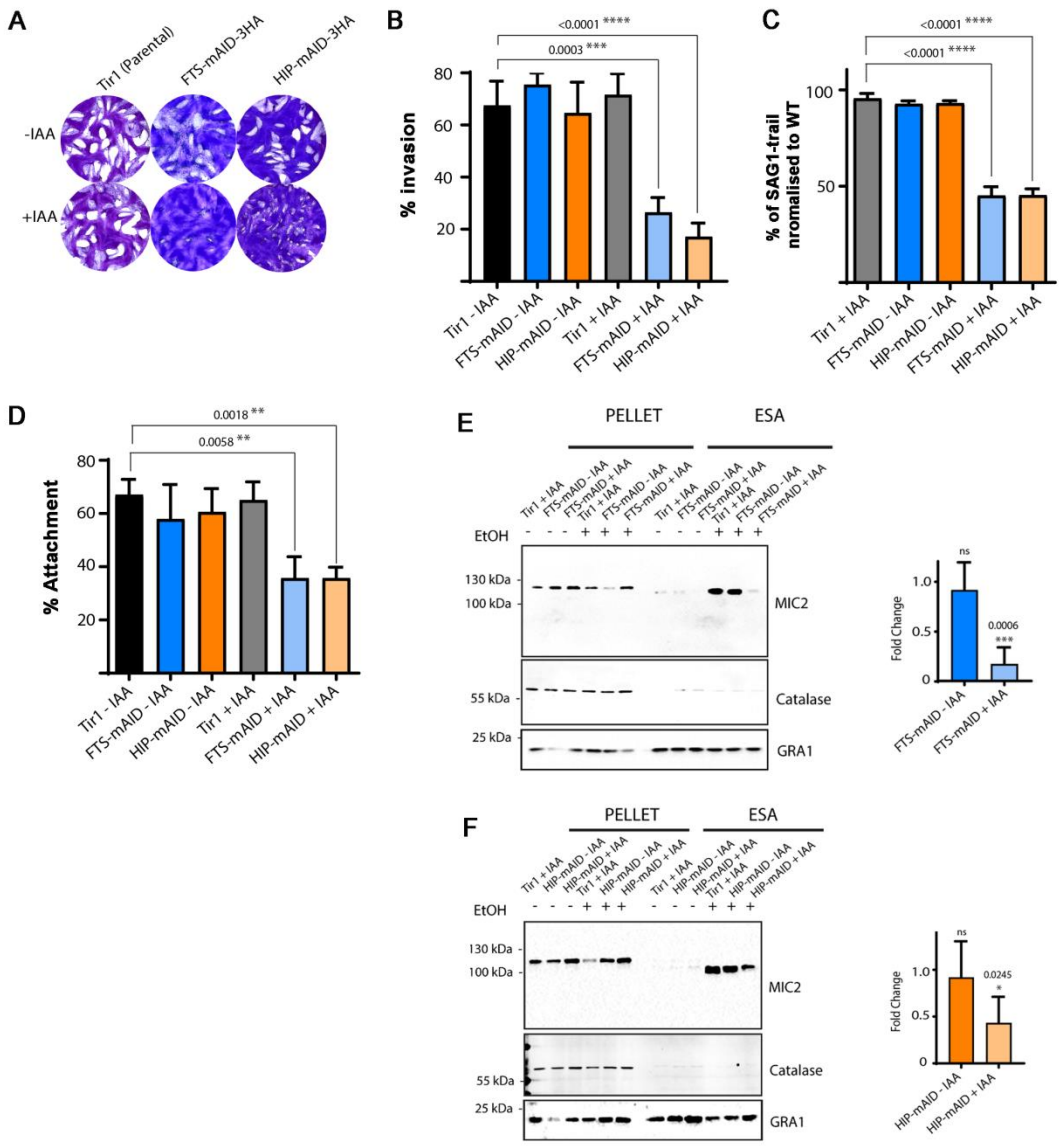



**Figure 10 – *Tg*FTS and *Tg*HIP phenotypical characterization.** (**A**) FTS mAID and HIP mAID +IAA show a significant impairment in the lytic cycle as shown by the smaller lysis plaques compared to the untreated parasites or the parental line (TirA) when grown 7 days on fibroblast monolayer. FTS mAID and HIP mAID +IAA display severe defects in invasion (**B**), motility (**C**), and host cell attachment (**D**). Data are presented as mean ± SEM. n= 3 independent experiments. Student T-test. (**E**-**F**) Western blot showing that *Tg*FTS & *Tg*HIP depleted parasites were severely impaired in microneme secretion when stimulated with 2% ethanol (EtOH) for 30 min. ESA: Extracellular Secreted Antigens. Catalase: loading control for parasite number and integrity. GRA1 refers to the constitutive secretion of dense granules. Data are presented as mean ± SEM. n= 3 independent experiments. Student T-test.

# Discussion and perspectives

# Chapter IV – Discussion and Perspectives

## 1 *Tg*HOOK interacts with *Tg*FTS and HIP to form a stable HFH complex implicated in the process of microneme secretion

This project provided insights on a novel endosomal trafficking pathway in *T. gondii*. We first identified a novel partner of *Tg*Rab11A, containing a unique HOOK-domain, that we called *Tg*HOOK. In other eukaryotes, Hook proteins were shown to be adaptor molecules for the molecular motor dynein and to play a role in vesicular transport by anchoring vesicles to microtubule tracks via their interaction with Rab proteins (Krämer and Phistry, 1996; Maldonado-Báez and Donaldson, 2013; Sunio et al., 1999). *Tg*HOOK is localized in numerous cytoplasmic vesicles, enriched at the apical region of the parasite, and accumulating at the conoid just beneath the apical ring. Deletion of *Tg*HOOK resulted in a defect in extracellular parasite motility and adhesion, and a moderate alteration in host cell invasion and egress. In *T. gondii*, the MTOC from which emerge the subpellicular microtubules is located at the apical tip of the parasite (Apical Polar Ring) and accordingly, the dynein light chain 1 (DLC1/DLC8a) also accumulates at this location, indicating that the "–" end of the microtubules is likely positioned at the apical pole and not in the perinuclear region in contrast to mammalian cells. In addition, it has been demonstrated that micronemes are located adjacently to the subpellicular microtubules, and that microtubule disruption leads to the alteration of micronemes distribution along cortical microtubules (Leung et al., 2017), suggesting a microtubule-based transport for these organelles that could be *Tg*HOOK-dependent. However, no discernable change in microneme protein stability or microneme organelle biogenesis and localization was detectable in HOOK-KO parasites. Thus, *Tg*HOOK does not seem to be involved in MIC protein trafficking from the TGN/ELC to form mature organelles, neither in mature organelle transport towards the apical region of the parasite.
Thus, the unaltered microneme localization at the apical region of TgHOOK deficient parasites, also observed for *Tg*FTS and HIP deficient parasites did not comfort this hypothesis.

*Tg*HOOK, *Tg*FTS and HIP were found to only co-localize at the apical tip of the parasite just beneath or at the apical polar ring (APR), suggesting that a functional *Tg*HFH complex may be

assembled at this location to promote regulated microneme secretion upon host cell egress and host cell recognition (adhesion/invasion). Importantly, it has been demonstrated that microneme secretion occurs in a highly regulated manner, correlated with intracellular fluctuations in $Ca^{2+}$ (Arrizabalaga and Boothroyd, 2004; Lovett et al., 2002). Previously, it has been proposed that the intraconoidal microtubules might anchor micronemes to promote their content release at the conoid (Carruthers and Sibley, 1997; Nichols and Chiappino, 1987). Moreover, Del Carmen et al, suggested that the conoid protrudes and retracts during microneme secretion (Carmen et al., 2009). Thus, two hypotheses were proposed. Some postulate that the conoid protrusion / retraction process could possibly allow the replenishment of micronemes stored on subpellicular microtubules under the APR. Micronemes would be subsequently docked onto intraconoidal microtubules prior to exocytosis. Others speculate that microneme secretion requires the protrusion of the conoid in order to dock and fuse to the plasma membrane at the level of the posterior polar ring where they are released (Dubois and Soldati-Favre, 2019; Paredes-Santos et al., 2012). Recently, it has been shown that *Tg*DLC8a similarly to the *Tg*HFH complex, is involved in microneme secretion and invasion, indicating that it might be a candidate for the intraconoidal transport of micronemes towards the site of exocytosis, a process called the apical replenishment of micronemes (Lentini et al., 2019). Based on our findings, we can propose two roles for the members of the *Tg*HFH complex. In the first model, the *Tg*HFH complex ensures the anchoring of a minimal subset of micronemes to the apical polar ring (APR), a process necessary for their subsequent translocation and transport within the conoid (in a *Tg*HFH-independent process) upon a secretion signal. In the second model, *Tg*HOOK may interact with microtubule anchored motors (such as dynein), and the *Tg*HFH complex promotes the transport of micronemes within the conoid towards the vicinity of the plasma membrane where exocytosis occurs.

To investigate whether *Tg*HOOK and *Tg*FTS associate with microtubule structures, including the conoid, we treated HOOK-HA and FTS-cMyc extracellular parasites with deoxycholate (DOC), which allows to only preserve microtubule structures, including the conoid and subpellicular microtubules. After performing IFA experiments, we could not observe any labelling of these structures by *Tg*HOOK and *Tg*FTS, suggesting that the *Tg*HFH complex does not permanently associate with microtubules, consistent with a role in vesicular trafficking; a

process correlated with transient associations of the cargos with the cytoskeleton tracks. Moreover, we assessed *Tg*HOOK localization in the DLC8a mutant strain generated by the lab of D. Soldati-Favre (cMycDLC8a-iKD +ATc) but we could not find any defect in *Tg*HOOK and Rab11A apical localization in extracellular motile parasites, suggesting that their apical transport/localization is independent on dynein. Still, at present, we cannot conclude that there is no interaction between DLC8a and a component of the complex, since they might interact to some degree, directly or as a larger complex, at the apex of the parasite. Thus, it will important to complete this result by performing co-IP to confirm the lack of interaction between *Tg*HOOK and DLC8a.

To further examine whether the *Tg*HFH complex could regulate the intraconoidal microneme transport, it will be also interesting to perform electron microscopy analysis in an attempt to localize *Tg*HFH positive micronemes within the conoid comparing WT and DLC8a KO strains. We tried to examine MIC2-positive microneme localization within the conoid in WT and HOOK-KO or *Tg*FTS-mAID induced parasites using SIM microscopy (that provides a 50nm resolution). However, MIC2-positive structures were already difficult to image in the WT parasites, with very distinct pattern/signals between the parasites, which did not allow us to conclude on putative defects in the *Tg*HFH KO lines. Of note, we could locate in some parasites, *Tg*HOOK-positive vesicular structures within the conoid.

Moreover, parasite motility, host cell adhesion and egress defects are usually linked to a defect in microneme secretion, nevertheless, no reduction in microneme exocytosis was observed in HOOK-KO extracellular parasites in sharp contrast to *Tg*FTS and HIP iKO parasites. This suggests a distinct role for *Tg*HOOK in the regulation of this process, which appears less prominent than the two other members of the complex. Along these lines, conversely to *Tg*FTS and HIP, which localization is mainly restricted to the apical tip of the parasite, *Tg*HOOK localization appears to be more cytosolic with an abundant vesicular-like pattern also localized at the basal pole of the parasite, where it often co-localizes with Rab11A. In addition, *Tg*HOOK deficient parasites display multiple defects in all steps of the lytic cycle. Thus, it is likely that *Tg*HOOK is implicated in distinct trafficking processes at different steps of the parasite cell cycle, dependent on its distinct binding partners. This also implies that *Tg*HOOK is likely implicated in the trafficking of intracellular compartments other than micronemes,

during parasite intracellular replication in a HIP and *Tg*FTS independent process. Of importance is the mild defect in parasite replication specific to HOOK-KO parasites, also a hallmark of Rab11A deficient parasites, suggesting that *Tg*HOOK-*Tg*Rab11A interaction may regulate some steps of the cytokinesis process, as previously described for *Tg*RAb11A. Supporting this hypothesis, both proteins co-localize at the conoid of budding daughter cells (see section 3).

Importantly, the impairment in microneme secretion observed in *Tg*FTS and HIP deficient parasites should be also associated with a defect in parasite egress (Gaji et al., 2015; Lourido et al., 2012; McCoy et al., 2012; Nagamune et al., 2008). However, unexpectedly, *Tg*HOOK is the only member of the *Tg*HFH complex leading to a moderate defect in egress. This suggests that *Tg*FTS and HIP depleted parasites are most probably capable of sufficient microneme secretion to enable the initial steps of egress, whereas the downstream process of invasion is severely impacted. Thus, why HOOK-KO parasites are defective in egress but display a very mild defect in invasion, in contrast to the two other members of the complex, is difficult to explain and would require further elucidation of *Tg*HOOK functions and the molecular mechanisms involved. Therefore, further investigations of the molecular mechanisms regulating the *Tg*HFH complex would be required, for instance by identifying specific binding partners of *Tg*FTS and HIP by IP experiments and mass spectrometry identification of bound partners.

## 2 Topology of the *Tg*HFH complex

The co-IP and WB experiments performed using cMycHOOK-iKD/FTS-HA induced or not with ATc revealed a tight interaction between *Tg*HOOK and *Tg*FTS impacting on the stability of *Tg*FTS, consistent with the formation a stable *Tg*HFH complex at the tip of the parasite, the only location where the three proteins were found co-localized by IFA. To further investigate the interaction between the members of the complex, the lab of D. Soldati-Favre performed several Co-IP assays coupled to MS, using HIP-mAID-HA/HOOK-Ty strain. HIP pull-down in the absence of IAA revealed a close association between *Tg*HOOK and HIP, but not with *Tg*FTS. Conversely, the whole complex was fished by IP when HOOK was pulled-down in the absence

of IAA. However, HOOK pull-down in the presence of IAA revealed an unaltered association between *Tg*HOOK and *Tg*FTS, suggesting that the rest of complex remains stable even in the absence of HIP. In line with these findings, FTS pull-down, in the absence of IAA using FTS-mAID-HA strain, proved the existence of the *Tg*HOOK-*Tg*FTS-HIP complex. These results indicate that the abundance of *Tg*HOOK impact the stability of *Tg*FTS whereas *Tg*HOOK-*Tg*FTS stability is independent of HIP; which is consistent with the literature where it has been demonstrated that HOOK binds both FTS and FHIP via its C-terminus (Mattera et al., 2020; Xu et al., 2008). It is surprising that by IP *Tg*FTS and HIP were found as preferential partners of *Tg*HOOK although these three proteins only co-localize at the apical tip and *Tg*HOOK seems to be involved in several functions (and thus trafficking events) compared to the two other members of the complex. This suggests that these three proteins form a transient but tight complex with a strong affinity between the partners.

# 3 *Tg*HOOK interacts with *Tg*Rab11A to regulate different vesicle trafficking processes

**Apical delivery of cargos**

Rab11 plays a role in both the constitutive and regulated secretion in other eukaryotic systems (Li et al., 2007; Sato et al., 2008; Urbé et al., 1993). In addition, Rab11 binds to the exocyst complex subunit Exo70 to regulate exocytic events in mammalian cells (Takahashi et al., 2012). However, the exocyst complex components are not encoded in the genome of *T. gondii* and in Apicomplexa as a whole (Klinger et al., 2013). Therefore, the question remains opened on how exocytosis is regulated in *T. gondii*. By co-IP and pull-down assays, we identified *Tg*HOOK as a unique partner of active GTP-bound active form of *Tg*Rab11A. According to the literature, HOOK proteins act as adaptors between vesicular cargos and the molecular motor dynein, regulating thus the endosomal traffic (Olenick et al., 2016). In the first part of my thesis project, we showed that *Tg*Rab11A could be a potential regulator of apically polarized secretory events during parasite motility and host cell invasion (Venugopal et al., 2020). Notably, we found a defect in MIC protein secretion and in the apical accumulation of GRA proteins in extracellular *Tg*Rab11A deficient parasites, resulting in a

strong alteration of parasite adhesion, motility, and host cell invasion. Thus, we hypothesized that the interaction between *Tg*Rab11A and *Tg*HOOK could be implicated in the microtubule-dependent secretion of cargos during the early steps of host cell invasion, notably microneme and GRA proteins. In *Tg*HOOK depleted parasites, the Rab11A and GRA1 signal appeared less focalized at the two foci beneath the conoid compared to control extracellular parasites. However, this pattern was not observed in all *Tg*HOOK depleted parasites, and this mild defect needs to be further investigated and thoroughly quantified. These results suggest that *Tg*HOOK could contribute at least partially to the Rab11A apical accumulation. Since we have previously demonstrated that Rab11A-positive vesicle movement seems to be mainly actin-dependent (Venugopal et al., 2020), *Tg*HOOK would be rather implicated in vesicle anchoring to the conoid before their exocytosis.

In addition, since *Tg*Rab11A interacts with *Tg*HOOK, it would be possible that *Tg*Rab11A regulates the process of MIC secretion in extracellular parasites by working in close association with the *Tg*HFH complex. Although no MIC protein secretion defect could be monitored by ESA in HOOK-KO extracellular parasites, these parasites showed altered parasite egress and parasite motility/adhesion. Thus, for the moment, it is difficult to determine the trafficking mechanisms that can explain those functional defects, but it is possible that *Tg*HOOK contributes mildly to MIC secretion compared to *Tg*FTS and HIP and that this slight defect is not observed by ESA. Of note, we also did not prove yet any specific interaction between *Tg*Rab11A and the two other members of the complex since we did not find *Tg*FTS and HIP in the GST pull down using GTP-bound active Rab11A.

**Distinct trafficking processes regulated by the *Tg*Rab11A-*Tg*HOOK complex?**

Moreover, we observed a partial co-localization between *Tg*HOOK and *Tg*Rab11A-positive vesicles often found in close proximity to the plasma membrane of the parasite and at the basal pole of intracellular replicating parasites (sometimes within the connecting membrane network present in the residual body), suggesting that *Tg*HOOK and *Tg*Rab11A may interact to regulate distinct trafficking processes than apical MIC protein secretion. According to the literature, mammalian HOOK1 was described to be implicated in the recycling of specific clathrin-independent endocytic (CIE) cargos via endosomes decorated with Rab11 and Rab22 (Maldonado-Báez and Donaldson, 2013). Thus, similarly, *Tg*HOOK could be involved in cargo

recycling in a Rab11A-dependent manner in *T. gondii,* notably the recycling of mother material during daughter cell cytokinesis. Such recycling process has been described during daughter cell formation for the IMC and the micronemes (Ouologuem and Roos, 2014; Periz et al., 2019). It is important to note that a putative Rab11A-dependent recycling activity has never been demonstrated so far in *T. gondii*. Thus, it would be interesting to examine Rab11A-positive vesicle and microneme motion in dividing HOOK-KO parasites using live imaging. Furthermore, a recent study (Gras et al., 2019) showed that extracellular motile parasites can internalize material, notably lipids, which would be further targeted to different intracellular compartments, including the Golgi, the rhoptries and the VAC, suggesting a recycling process as described in mammals. Therefore, it would be also interesting to examine whether this recycling process of internalized material towards the rhoptries and putatively the plasma membrane could be regulated by *Tg*Rab11A in a *Tg*HOOK-dependent manner.

# Bibliography

# Bibliography


Adjogble, K.D.Z., Mercier, C., Dubremetz, J.-F., Hucke, C., Mackenzie, C.R., Cesbron-Delauw, M.-F., Däubener, W., 2004. GRA9, a new Toxoplasma gondii dense granule protein associated with the intravacuolar network of tubular membranes. Int J Parasitol 34, 1255–1264. https://doi.org/10.1016/j.ijpara.2004.07.011

Agop-Nersesian, C., Egarter, S., Langsley, G., Foth, B.J., Ferguson, D.J.P., Meissner, M., 2010. Biogenesis of the Inner Membrane Complex Is Dependent on Vesicular Transport by the Alveolate Specific GTPase Rab11B. PLoS Pathog 6. https://doi.org/10.1371/journal.ppat.1001029

Agop-Nersesian, C., Naissant, B., Rached, F.B., Rauch, M., Kretzschmar, A., Thiberge, S., Menard, R., Ferguson, D.J.P., Meissner, M., Langsley, G., 2009. Rab11A-Controlled Assembly of the Inner Membrane Complex Is Required for Completion of Apicomplexan Cytokinesis. PLoS Pathog 5. https://doi.org/10.1371/journal.ppat.1000270

Ahn, H.-J., Kim, S., Kim, H.-E., Nam, H.-W., 2006. Interactions between secreted GRA proteins and host cell proteins across the parasitophorous vacuolar membrane in the parasitism of Toxoplasma gondii. Korean J Parasitol 44, 303–312. https://doi.org/10.3347/kjp.2006.44.4.303

Aivazian, D., Serrano, R., Pfeffer, S., 2006. TIP47 is a key effector for Rab9 localization. The Journal of cell biology 173, 917–26. https://doi.org/10.1083/jcb.200510010

Ajioka, J.W., Fitzpatrick, J.M., Reitter, C.P., 2001. Toxoplasma gondii genomics: shedding light on pathogenesis and chemotherapy. Expert Rev Mol Med 2001, 1–19. https://doi.org/10.1017/S1462399401002204

Albert, S., Gallwitz, D., 1999. Two new members of a family of Ypt/Rab GTPase activating proteins. Promiscuity of substrate recognition. J Biol Chem 274, 33186–33189. https://doi.org/10.1074/jbc.274.47.33186

Albert, S., Will, E., Gallwitz, D., 1999. Identification of the catalytic domains and their functionally critical arginine residues of two yeast GTPase-activating proteins specific for Ypt/Rab transport GTPases. EMBO J 18, 5216–5225. https://doi.org/10.1093/emboj/18.19.5216

Alexander, D.L., Mital, J., Ward, G.E., Bradley, P., Boothroyd, J.C., 2005. Identification of the Moving Junction Complex of Toxoplasma gondii: A Collaboration between Distinct Secretory Organelles. PLoS Pathog 1. https://doi.org/10.1371/journal.ppat.0010017

Allan, B.B., Moyer, B.D., Balch, W.E., 2000. Rab1 recruitment of p115 into a cis-SNARE complex: programming budding COPII vesicles for fusion. Science 289, 444–448. https://doi.org/10.1126/science.289.5478.444

Alvarez, C.A., Suvorova, E.S., 2017. Checkpoints of apicomplexan cell division identified in Toxoplasma gondii. PLoS Pathog 13. https://doi.org/10.1371/journal.ppat.1006483

Amiar, S., Katris, N.J., Berry, L., Dass, S., Duley, S., Arnold, C.-S., Shears, M.J., Brunet, C., Touquet, B., McFadden, G.I., Yamaryo-Botté, Y., Botté, C.Y., 2020. Division and Adaptation to Host Environment of Apicomplexan Parasites Depend on Apicoplast Lipid Metabolic Plasticity and Host Organelle Remodeling. Cell Reports 30, 3778-3792.e9. https://doi.org/10.1016/j.celrep.2020.02.072

Andenmatten, N., Egarter, S., Jackson, A.J., Jullien, N., Herman, J.-P., Meissner, M., 2013. Conditional genome engineering in Toxoplasma gondii uncovers alternative invasion mechanisms. Nat Methods 10, 125–127. https://doi.org/10.1038/nmeth.2301

Anderson-White, B., Beck, J.R., Chen, C.-T., Meissner, M., Bradley, P.J., Gubbels, M.-J., 2012. Cytoskeleton assembly in Toxoplasma gondii cell division. Int Rev Cell Mol Biol 298, 1–31. https://doi.org/10.1016/B978-0-12-394309-5.00001-8

Anderson-White, B.R., Ivey, F.D., Cheng, K., Szatanek, T., Lorestani, A., Beckers, C.J., Ferguson, D.J.P., Sahoo, N., Gubbels, M.-J., 2011. A family of intermediate filament-like proteins is sequentially assembled into the cytoskeleton of Toxoplasma gondii. Cell Microbiol 13, 18–31. https://doi.org/10.1111/j.1462-5822.2010.01514.x

Aridor, M., Bannykh, S.I., Rowe, T., Balch, W.E., 1995. Sequential coupling between COPII and COPI vesicle coats in endoplasmic reticulum to Golgi transport. Journal of Cell Biology 131, 875–893. https://doi.org/10.1083/jcb.131.4.875

Arrizabalaga, G., Boothroyd, J.C., 2004. Role of calcium during Toxoplasma gondii invasion and egress. Int. J. Parasitol. 34, 361–368. https://doi.org/10.1016/j.ijpara.2003.11.017

Arvan, P., Castle, D., 1998. Sorting and storage during secretory granule biogenesis: looking backward and looking forward. Biochem J 332, 593–610.

Attias, M., Miranda, K., De Souza, W., 2019. Development and fate of the residual body of Toxoplasma gondii. Experimental Parasitology 196, 1–11. https://doi.org/10.1016/j.exppara.2018.11.004

Baden, L.R., Katz, J.T., Franck, L., Tsang, S., Hall, M., Rubin, R.H., Jarcho, J., 2003. Successful toxoplasmosis prophylaxis after orthotopic cardiac transplantation with trimethoprim-sulfamethoxazole. Transplantation 75, 339–343. https://doi.org/10.1097/01.TP.0000044864.99398.F1

Bahadoran, P., Aberdam, E., Mantoux, F., Buscà, R., Bille, K., Yalman, N., Saint-Basile, G., Casaroli-Marano, R., Ortonne, J.-P., Ballotti, R., 2001. Rab27a A Key to Melanosome Transport in Human Melanocytes. The Journal of cell biology 152, 843–50. https://doi.org/10.1083/jcb.152.4.843

Balch, W.E., Glick, B.S., Rothman, J.E., 1984. Sequential intermediates in the pathway of intercompartmental transport in a cell-free system. Cell 39, 525–536. https://doi.org/10.1016/0092-8674(84)90459-8

Bargieri, D.Y., Andenmatten, N., Lagal, V., Thiberge, S., Whitelaw, J.A., Tardieux, I., Meissner, M., Ménard, R., 2013. Apical membrane antigen 1 mediates apicomplexan parasite attachment but is dispensable for host cell invasion. Nat Commun 4. https://doi.org/10.1038/ncomms3552

Barlowe, C., Orci, L., Yeung, T., Hosobuchi, M., Hamamoto, S., Salama, N., Rexach, M.F., Ravazzola, M., Amherdt, M., Schekman, R., 1994. COPII: A membrane coat formed by Sec proteins that drive vesicle budding from the endoplasmic reticulum. Cell 77, 895–907. https://doi.org/10.1016/0092-8674(94)90138-4

Barragan, A., Brossier, F., Sibley, L.D., 2005. Transepithelial migration of Toxoplasma gondii involves an interaction of intercellular adhesion molecule 1 (ICAM-1) with the parasite adhesin MIC2. Cell. Microbiol. 7, 561–568. https://doi.org/10.1111/j.1462-5822.2005.00486.x

Barragan, A., Sibley, L.D., 2002. Transepithelial Migration of Toxoplasma gondii Is Linked to Parasite Motility and Virulence. J Exp Med 195, 1625–1633. https://doi.org/10.1084/jem.20020258

Barrowman, J., Sacher, M., Ferro-Novick, S., 2000. TRAPP stably associates with the Golgi and is required for vesicle docking. EMBO J 19, 862–869. https://doi.org/10.1093/emboj/19.5.862

Barylyuk, K., Koreny, L., Ke, H., Butterworth, S., Crook, O.M., Lassadi, I., Gupta, V., Tromer, E., Mourier, T., Stevens, T.J., Breckels, L.M., Pain, A., Lilley, K.S., Waller, R.F., 2020. A Comprehensive Subcellular Atlas of the Toxoplasma Proteome via hyperLOPIT Provides Spatial Context for Protein Functions. Cell Host Microbe 28, 752-766.e9. https://doi.org/10.1016/j.chom.2020.09.011

Baum, J., Papenfuss, A.T., Baum, B., Speed, T.P., Cowman, A.F., 2006. Regulation of apicomplexan actin-based motility. Nat. Rev. Microbiol. 4, 621–628. https://doi.org/10.1038/nrmicro1465

Beck, J.R., Chen, A.L., Kim, E.W., Bradley, P.J., 2014. RON5 Is Critical for Organization and Function of the Toxoplasma Moving Junction Complex. PLoS Pathog 10. https://doi.org/10.1371/journal.ppat.1004025

Beck, J.R., Rodriguez-Fernandez, I.A., Leon, J.C. de, Huynh, M.-H., Carruthers, V.B., Morrissette, N.S., Bradley, P.J., 2010. A Novel Family of Toxoplasma IMC Proteins Displays a Hierarchical Organization and Functions in Coordinating Parasite Division. PLOS Pathogens 6, e1001094. https://doi.org/10.1371/journal.ppat.1001094

Behnke, M.S., Fentress, S.J., Mashayekhi, M., Li, L.X., Taylor, G.A., Sibley, L.D., 2012. The Polymorphic Pseudokinase ROP5 Controls Virulence in Toxoplasma gondii by Regulating the Active Kinase ROP18. PLOS Pathogens 8, e1002992. https://doi.org/10.1371/journal.ppat.1002992

Behnke, M.S., Khan, A., Lauron, E.J., Jimah, J.R., Wang, Q., Tolia, N.H., Sibley, L.D., 2015. Rhoptry Proteins ROP5 and ROP18 Are Major Murine Virulence Factors in Genetically Divergent South American Strains of Toxoplasma gondii. PLoS Genet 11. https://doi.org/10.1371/journal.pgen.1005434

Behnke, M.S., Khan, A., Wootton, J.C., Dubey, J.P., Tang, K., Sibley, L.D., 2011. Virulence differences in Toxoplasma mediated by amplification of a family of polymorphic pseudokinases. Proc Natl Acad Sci U S A 108, 9631–9636. https://doi.org/10.1073/pnas.1015338108

Behnke, M.S., Wootton, J.C., Lehmann, M.M., Radke, J.B., Lucas, O., Nawas, J., Sibley, L.D., White, M.W., 2010. Coordinated Progression through Two Subtranscriptomes Underlies the Tachyzoite Cycle of Toxoplasma gondii. PLoS One 5. https://doi.org/10.1371/journal.pone.0012354

Bennett, M.K., Scheller, R.H., 1993. The molecular machinery for secretion is conserved from yeast to neurons. Proceedings of the National Academy of Sciences 90, 2559–2563. https://doi.org/10.1073/pnas.90.7.2559

Berry, L., Chen, C.-T., Reininger, L., Carvalho, T.G., El Hajj, H., Morlon-Guyot, J., Bordat, Y., Lebrun, M., Gubbels, M.-J., Doerig, C., Daher, W., 2016. The conserved apicomplexan Aurora kinase TgArk3 is involved in endodyogeny, duplication rate and parasite virulence. Cell Microbiol 18, 1106–1120. https://doi.org/10.1111/cmi.12571

Besteiro, S., Michelin, A., Poncet, J., Dubremetz, J.-F., Lebrun, M., 2009. Export of a Toxoplasma gondii Rhoptry Neck Protein Complex at the Host Cell Membrane to Form the Moving Junction during Invasion. PLoS Pathog 5. https://doi.org/10.1371/journal.ppat.1000309

Bichet, M., Joly, C., Hadj Henni, A., Guilbert, T., Xémard, M., Tafani, V., Lagal, V., Charras, G., Tardieux, I., 2014. The toxoplasma-host cell junction is anchored to the cell cortex to

sustain parasite invasive force. BMC Biol 12. https://doi.org/10.1186/s12915-014-0108-y

Bielska, E., Schuster, M., Roger, Y., Berepiki, A., Soanes, D.M., Talbot, N.J., Steinberg, G., 2014. Hook is an adapter that coordinates kinesin-3 and dynein cargo attachment on early endosomes. J Cell Biol 204, 989–1007. https://doi.org/10.1083/jcb.201309022

Billker, O., Lourido, S., Sibley, L.D., 2009. Calcium-Dependent Signaling and Kinases in Apicomplexan Parasites. Cell Host Microbe 5, 612–622. https://doi.org/10.1016/j.chom.2009.05.017

Bisio, H., Lunghi, M., Brochet, M., Soldati-Favre, D., 2019. Phosphatidic acid governs natural egress in Toxoplasma gondii via a guanylate cyclase receptor platform. Nat Microbiol 4, 420–428. https://doi.org/10.1038/s41564-018-0339-8

Blader, I., Coleman, B., Chen, C.-T., Gubbels, M.-J., 2015. The lytic cycle of Toxoplasma gondii: 15 years later. Annu Rev Microbiol 69, 463–485. https://doi.org/10.1146/annurev-micro-091014-104100

Blobel, G., Dobberstein, B., 1975. Transfer of proteins across membranes. II. Reconstitution of functional rough microsomes from heterologous components. Journal of Cell Biology 67, 852–862. https://doi.org/10.1083/jcb.67.3.852

Blume, M., Rodriguez-Contreras, D., Landfear, S., Fleige, T., Soldati-Favre, D., Lucius, R., Gupta, N., 2009. Host-derived glucose and its transporter in the obligate intracellular pathogen Toxoplasma gondii are dispensable by glutaminolysis. Proc Natl Acad Sci U S A 106, 12998–13003. https://doi.org/10.1073/pnas.0903831106

Blumenschein, T.M.A., Friedrich, N., Childs, R.A., Saouros, S., Carpenter, E.P., Campanero-Rhodes, M.A., Simpson, P., Chai, W., Koutroukides, T., Blackman, M.J., Feizi, T., Soldati-Favre, D., Matthews, S., 2007. Atomic resolution insight into host cell recognition by Toxoplasma gondii. EMBO J 26, 2808–2820. https://doi.org/10.1038/sj.emboj.7601704

Bohne, W., Heesemann, J., Gross, U., 1994. Reduced replication of Toxoplasma gondii is necessary for induction of bradyzoite-specific antigens: a possible role for nitric oxide in triggering stage conversion. Infect Immun 62, 1761–1767.

Bonifacino, J.S., Glick, B.S., 2004. The mechanisms of vesicle budding and fusion. Cell 116, 153–166. https://doi.org/10.1016/s0092-8674(03)01079-1

Boothroyd, J.C., Dubremetz, J.-F., 2008. Kiss and spit: the dual roles of Toxoplasma rhoptries. Nat. Rev. Microbiol. 6, 79–88. https://doi.org/10.1038/nrmicro1800

Boothroyd, J.C., Grigg, M.E., 2002. Population biology of Toxoplasma gondii and its relevance to human infection: do different strains cause different disease? Current Opinion in Microbiology 5, 438–442. https://doi.org/10.1016/S1369-5274(02)00349-1

Bos, J., Rehmann, H., Wittinghofer, A., 2007. GEFs and GAPs: Critical elements in the control of small G proteins. Cell 129, 865–77. https://doi.org/10.1016/j.cell.2007.05.018

Boucher, L.E., Bosch, J., 2015. The apicomplexan glideosome and adhesins -- structures and function. J Struct Biol 190, 93–114. https://doi.org/10.1016/j.jsb.2015.02.008

Boucrot, E., Pick, A., Camdere, G., Liska, N., Evergren, E., HT, M., Kozlov, M., 2012. Membrane Fission Is Promoted by Insertion of Amphipathic Helices and Is Restricted by Crescent BAR Domains. Cell. https://doi.org/10.1016/j.cell.2012.01.047

Bougdour, A., Durandau, E., Brenier-Pinchart, M.-P., Ortet, P., Barakat, M., Kieffer, S., Curt-Varesano, A., Curt-Bertini, R.-L., Bastien, O., Coute, Y., Pelloux, H., Hakimi, M.-A., 2013. Host cell subversion by Toxoplasma GRA16, an exported dense granule protein that

targets the host cell nucleus and alters gene expression. Cell Host Microbe 13, 489–500. https://doi.org/10.1016/j.chom.2013.03.002

Bradley, P.J., Sibley, L.D., 2007. Rhoptries: an arsenal of secreted virulence factors. Curr Opin Microbiol 10, 582–587. https://doi.org/10.1016/j.mib.2007.09.013

Bradley, P.J., Ward, C., Cheng, S.J., Alexander, D.L., Coller, S., Coombs, G.H., Dunn, J.D., Ferguson, D.J., Sanderson, S.J., Wastling, J.M., Boothroyd, J.C., 2005. Proteomic Analysis of Rhoptry Organelles Reveals Many Novel Constituents for Host-Parasite Interactions in Toxoplasma gondii. J. Biol. Chem. 280, 34245–34258. https://doi.org/10.1074/jbc.M504158200

Braulke, T., Bonifacino, J., 2008. Sorting of lysosomal proteins. Biochimica et biophysica acta 1793, 605–14. https://doi.org/10.1016/j.bbamcr.2008.10.016

Braun, L., Brenier-Pinchart, M.-P., Yogavel, M., Curt-Varesano, A., Curt-Bertini, R.-L., Hussain, T., Kieffer-Jaquinod, S., Coute, Y., Pelloux, H., Tardieux, I., Sharma, A., Belrhali, H., Bougdour, A., Hakimi, M.-A., 2013. A Toxoplasma dense granule protein, GRA24, modulates the early immune response to infection by promoting a direct and sustained host p38 MAPK activation. J Exp Med 210, 2071–2086. https://doi.org/10.1084/jem.20130103

Braun, L., Travier, L., Kieffer, S., Musset, K., Garin, J., Mercier, C., Cesbron-Delauw, M.-F., 2008. Purification of Toxoplasma dense granule proteins reveals that they are in complexes throughout the secretory pathway. Mol Biochem Parasitol 157, 13–21. https://doi.org/10.1016/j.molbiopara.2007.09.002

Breinich, M.S., Ferguson, D.J.P., Foth, B.J., van Dooren, G.G., Lebrun, M., Quon, D.V., Striepen, B., Bradley, P.J., Frischknecht, F., Carruthers, V.B., Meissner, M., 2009. An alveolate specific dynamin is required for the biogenesis of specialised secretory organelles in Toxoplasma gondii. Curr Biol 19, 277–286. https://doi.org/10.1016/j.cub.2009.01.039

Bromley, E., Leeds, N., Clark, J., McGregor, E., Ward, M., Dunn, M.J., Tomley, F., 2003. Defining the protein repertoire of microneme secretory organelles in the apicomplexan parasite Eimeria tenella. Proteomics 3, 1553–1561. https://doi.org/10.1002/pmic.200300479

Brooks, C.F., Francia, M.E., Gissot, M., Croken, M.M., Kim, K., Striepen, B., 2011. Toxoplasma gondii sequesters centromeres to a specific nuclear region throughout the cell cycle. Proc Natl Acad Sci U S A 108, 3767–3772. https://doi.org/10.1073/pnas.1006741108

Brossier, F., Jewett, T.J., Sibley, L.D., Urban, S., 2005. A spatially localized rhomboid protease cleaves cell surface adhesins essential for invasion by Toxoplasma. Proc Natl Acad Sci U S A 102, 4146–4151. https://doi.org/10.1073/pnas.0407918102

Brossier, F., Starnes, G.L., Beatty, W.L., Sibley, L.D., 2008. Microneme Rhomboid Protease TgROM1 Is Required for Efficient Intracellular Growth of Toxoplasma gondii. Eukaryot Cell 7, 664–674. https://doi.org/10.1128/EC.00331-07

Brown, K.M., Long, S., Sibley, L.D., 2018. Conditional Knockdown of Proteins Using Auxin-inducible Degron (AID) Fusions in Toxoplasma gondii. Bio Protoc 8. https://doi.org/10.21769/BioProtoc.2728

Bruce, E.A., Digard, P., Stuart, A.D., 2010. The Rab11 pathway is required for influenza A virus budding and filament formation. J Virol 84, 5848–5859. https://doi.org/10.1128/JVI.00307-10

Buguliskis, J.S., Brossier, F., Shuman, J., Sibley, L.D., 2010. Rhomboid 4 (ROM4) Affects the Processing of Surface Adhesins and Facilitates Host Cell Invasion by Toxoplasma gondii. PLOS Pathogens 6, e1000858. https://doi.org/10.1371/journal.ppat.1000858

Bullen, H.E., Jia, Y., Yamaryo-Botté, Y., Bisio, H., Zhang, O., Jemelin, N.K., Marq, J.-B., Carruthers, V., Botté, C.Y., Soldati-Favre, D., 2016. Phosphatidic Acid-Mediated Signaling Regulates Microneme Secretion in Toxoplasma. Cell Host Microbe 19, 349–360. https://doi.org/10.1016/j.chom.2016.02.006

Bullen, H.E., Tonkin, C.J., O'Donnell, R.A., Tham, W.-H., Papenfuss, A.T., Gould, S., Cowman, A.F., Crabb, B.S., Gilson, P.R., 2009. A Novel Family of Apicomplexan Glideosome-associated Proteins with an Inner Membrane-anchoring Role. J Biol Chem 284, 25353–25363. https://doi.org/10.1074/jbc.M109.036772

Bunnik, E.M., Venkat, A., Shao, J., McGovern, K.E., Batugedara, G., Worth, D., Prudhomme, J., Lapp, S.A., Andolina, C., Ross, L.S., Lawres, L., Brady, D., Sinnis, P., Nosten, F., Fidock, D.A., Wilson, E.H., Tewari, R., Galinski, M.R., Ben Mamoun, C., Ay, F., Le Roch, K.G., 2019. Comparative 3D genome organization in apicomplexan parasites. Proc Natl Acad Sci U S A 116, 3183–3192. https://doi.org/10.1073/pnas.1810815116

Burton, J., Roberts, D., Montaldi, M., Novick, P., Camilli, P.D., 1993. A mammalian guanine-nucleotide-releasing protein enhances function of yeast secretory protein Sec4. Nature 361, 464–467. https://doi.org/10.1038/361464a0

Butcher, B.A., Denkers, E.Y., 2002. Mechanism of Entry Determines the Ability of Toxoplasma gondii To Inhibit Macrophage Proinflammatory Cytokine Production. Infect Immun 70, 5216–5224. https://doi.org/10.1128/IAI.70.9.5216-5224.2002

Butcher, B.A., Fox, B.A., Rommereim, L.M., Kim, S.G., Maurer, K.J., Yarovinsky, F., Herbert, D.R., Bzik, D.J., Denkers, E.Y., 2011. Toxoplasma gondii Rhoptry Kinase ROP16 Activates STAT3 and STAT6 Resulting in Cytokine Inhibition and Arginase-1-Dependent Growth Control. PLoS Pathog 7. https://doi.org/10.1371/journal.ppat.1002236

Butler, C.L., Lucas, O., Wuchty, S., Xue, B., Uversky, V.N., White, M., 2014. Identifying Novel Cell Cycle Proteins in Apicomplexa Parasites through Co-Expression Decision Analysis. PLOS ONE 9, e97625. https://doi.org/10.1371/journal.pone.0097625

Buxton, D., 1993. Toxoplasmosis: the first commercial vaccine. Parasitology Today 9, 335–337. https://doi.org/10.1016/0169-4758(93)90236-9

Caffaro, C.E., Boothroyd, J.C., 2011. Evidence for Host Cells as the Major Contributor of Lipids in the Intravacuolar Network of Toxoplasma-Infected Cells ▿ . Eukaryot Cell 10, 1095–1099. https://doi.org/10.1128/EC.00002-11

Cai, H., Reinisch, K., Ferro-Novick, S., 2007. Coats, tethers, Rabs, and SNAREs work together to mediate the intracellular destination of a transport vesicle. Dev Cell 12, 671–682. https://doi.org/10.1016/j.devcel.2007.04.005

Cai, Y., Chin, H.F., Lazarova, D., Menon, S., Fu, C., Cai, H., Sclafani, A., Rodgers, D.W., De La Cruz, E.M., Ferro-Novick, S., Reinisch, K.M., 2008. The Structural Basis for Activation of the Rab Ypt1p by the TRAPP Membrane-Tethering Complexes. Cell 133, 1202–1213. https://doi.org/10.1016/j.cell.2008.04.049

Calmels, T.P.G., Callebaut, I., Léger, I., Durand, P., Bril, A., Mornon, J.-P., Souchet, M., 1998. Sequence and 3D structural relationships between mammalian Ras- and Rho-specific GTPase-activating proteins (GAPs): the cradle fold. FEBS Letters 426, 205–211. https://doi.org/10.1016/S0014-5793(98)00331-7

Carmen, M.G.D., Mondragón, M., González, S., Mondragón, R., 2009. Induction and regulation of conoid extrusion in Toxoplasma gondii. Cellular Microbiology 11, 967–982. https://doi.org/10.1111/j.1462-5822.2009.01304.x

Carroll, K.S., Hanna, J., Simon, I., Krise, J., Barbero, P., Pfeffer, S.R., 2001. Role of Rab9 GTPase in facilitating receptor recruitment by TIP47. Science 292, 1373–1376. https://doi.org/10.1126/science.1056791

Carruthers, V., Boothroyd, J.C., 2007. Pulling together: an integrated model of Toxoplasma cell invasion. Curr. Opin. Microbiol. 10, 83–89. https://doi.org/10.1016/j.mib.2006.06.017

Carruthers, V.B., 2013. Protein trafficking through the endosomal system prepares intracellular parasites for a home invasion.

Carruthers, V.B., 2002. Host cell invasion by the opportunistic pathogen Toxoplasma gondii. Acta Tropica 81, 111–122. https://doi.org/10.1016/S0001-706X(01)00201-7

Carruthers, V.B., Giddings, O.K., Sibley, L.D., 1999. Secretion of micronemal proteins is associated with toxoplasma invasion of host cells. Cell. Microbiol. 1, 225–235. https://doi.org/10.1046/j.1462-5822.1999.00023.x

Carruthers, V.B., Sibley, L.D., 1997. Sequential protein secretion from three distinct organelles of Toxoplasma gondii accompanies invasion of human fibroblasts. Eur. J. Cell Biol. 73, 114–123.

Carruthers, V.B., Tomley, F.M., 2008. Receptor-ligand interaction and invasion: Microneme proteins in apicomplexans. Subcell Biochem 47, 33–45.

Casanova, J., Wang, xiang yang, Kumar, R., Bhartur, S., Navarre, J., Woodrum, J., YA, A., Ray, G., Goldenring, J., 1999. Association of Rab25 and Rab11a with the Apical Recycling System of Polarized Madin-Darby Canine Kidney Cells. Molecular biology of the cell 10, 47–61. https://doi.org/10.1091/mbc.10.1.47

Cecilia N. Arighi, 2004. Role of the mammalian retromer in sorting of the cation-independent mannose 6-phosphate receptor. https://doi.org/10.1083/jcb.200312055

Cérède, O., Dubremetz, J.F., Bout, D., Lebrun, M., 2002. The Toxoplasma gondii protein MIC3 requires pro-peptide cleavage and dimerization to functionas adhesin. EMBO J 21, 2526–2536. https://doi.org/10.1093/emboj/21.11.2526

Cérède, O., Dubremetz, J.F., Soête, M., Deslée, D., Vial, H., Bout, D., Lebrun, M., 2005. Synergistic role of micronemal proteins in Toxoplasma gondii virulence. J Exp Med 201, 453–463. https://doi.org/10.1084/jem.20041672

Cerutti, A., Blanchard, N., Besteiro, S., 2020. The Bradyzoite: A Key Developmental Stage for the Persistence and Pathogenesis of Toxoplasmosis. Pathogens 9. https://doi.org/10.3390/pathogens9030234

Chanat, E. & Huttner, W. B., 1991. Milieu-induced, selective aggregation of regulated secretory proteins in the trans-Golgi network. J Cell Biol 115, 1505–1519.

Channon, J.Y., Seguin, R.M., Kasper, L.H., 2000. Differential Infectivity and Division of Toxoplasma gondii in Human Peripheral Blood Leukocytes. Infect Immun 68, 4822–4826.

Chardin P, McCormick F, 1999. Brefeldin A: the advantage of being uncompetitive. Cell 97, 153–155. https://doi.org/10.1016/s0092-8674(00)80724-2

Charles Nicolle & Louis Manceaux, 1908. Découverte de l'agent de transmission du typhus par Charles Nicolle [WWW Document]. FranceArchives. URL https://francearchives.fr/de/commemo/recueil-2009/38859 (accessed 3.20.20).

Charron, A.J., Sibley, L.D., 2004. Molecular partitioning during host cell penetration by Toxoplasma gondii. Traffic 5, 855–867. https://doi.org/10.1111/j.1600-0854.2004.00228.x

Chasserot-Golaz, S., Coorssen, J.R., Meunier, F.A., Vitale, N., 2010. Lipid dynamics in exocytosis. Cell. Mol. Neurobiol. 30, 1335–1342. https://doi.org/10.1007/s10571-010-9577-x

Chaturvedi, S., Qi, H., Coleman, D., Rodriguez, A., Hanson, P.I., Striepen, B., Roos, D.S., Joiner, K.A., 1999. Constitutive calcium-independent release of Toxoplasma gondii dense granules occurs through the NSF/SNAP/SNARE/Rab machinery. J Biol Chem 274, 2424–2431. https://doi.org/10.1074/jbc.274.4.2424

Chen, A.L., Kim, E.W., Toh, J.Y., Vashisht, A.A., Rashoff, A.Q., Van, C., Huang, A.S., Moon, A.S., Bell, H.N., Bentolila, L.A., Wohlschlegel, J.A., Bradley, P.J., 2015. Novel Components of the Toxoplasma Inner Membrane Complex Revealed by BioID. mBio 6. https://doi.org/10.1128/mBio.02357-14

Chen, C.-T., Gubbels, M.-J., 2013. The Toxoplasma gondii centrosome is the platform for internal daughter budding as revealed by a Nek1 kinase mutant. J Cell Sci 126, 3344–3355. https://doi.org/10.1242/jcs.123364

Chen, W., Feng, Y., Chen, D., Wandinger-Ness, A., 1998. Rab11 Is Required for Trans-Golgi Network-to-Plasma Membrane Transport and a Preferential Target for GDP Dissociation Inhibitor. Molecular biology of the cell 9, 3241–57. https://doi.org/10.1091/mbc.9.11.3241

Cheng, K.W., Lahad, J., Kuo, W.-L., Lapuk, A., Yamada, K., Auersperg, N., Liu, J., Smith-McCune, K., Lu, K., Fishman, D., Gray, J., Mills, G., 2004. The RAB25 small GTPase determines aggressiveness of ovarian and breast cancers. Nature medicine 10, 1251–6. https://doi.org/10.1038/nm1125

Cherfils, J., Zeghouf, M., 2013. Regulation of small GTPases by GEFs, GAPs, and GDIs. Physiological reviews 93, 269–309. https://doi.org/10.1152/physrev.00003.2012

Christoforidis, S., Miaczynska, M., Ashman, K., Wilm, M., Zhao, L., Yip, S.-C., Waterfield, M.D., Backer, J.M., Zerial, M., 1999. Phosphatidylinositol-3-OH kinases are Rab5 effectors. Nature Cell Biology 1, 249–252. https://doi.org/10.1038/12075

Chu, B.-B., Ge, L., Xie, C., Zhao, Y., Miao, H.-H., Wang, J., Li, B.-L., Song, B.-L., 2009. Requirement of myosin Vb.Rab11a.Rab11-FIP2 complex in cholesterol-regulated translocation of NPC1L1 to the cell surface. J Biol Chem 284, 22481–22490. https://doi.org/10.1074/jbc.M109.034355

Coffey, M.J., Sleebs, B.E., Uboldi, A.D., Garnham, A., Franco, M., Marino, N.D., Panas, M.W., Ferguson, D.J., Enciso, M., O'Neill, M.T., Lopaticki, S., Stewart, R.J., Dewson, G., Smyth, G.K., Smith, B.J., Masters, S.L., Boothroyd, J.C., Boddey, J.A., Tonkin, C.J., 2015. An aspartyl protease defines a novel pathway for export of Toxoplasma proteins into the host cell. eLife 4. https://doi.org/10.7554/eLife.10809

Colicelli, J., 2004. Human RAS superfamily proteins and related GTPases. Science's STKE : signal transduction knowledge environment 2004, RE13. https://doi.org/10.1126/stke.2502004re13

Coombes, J.L., Charsar, B.A., Han, S.-J., Halkias, J., Chan, S.W., Koshy, A.A., Striepen, B., Robey, E.A., 2013. Motile invaded neutrophils in the small intestine of Toxoplasma gondii-infected mice reveal a potential mechanism for parasite spread. Proc Natl Acad Sci U S A 110, E1913–E1922. https://doi.org/10.1073/pnas.1220272110

Coppens, I., Andries, M., Liu, J.L., Cesbron-Delauw, M.F., 1999. Intracellular trafficking of dense granule proteins in Toxoplasma gondii and experimental evidences for a regulated exocytosis. Eur J Cell Biol 78, 463–472. https://doi.org/10.1016/S0171-9335(99)80073-9

Coppens, I., Dunn, J.D., Romano, J.D., Pypaert, M., Zhang, H., Boothroyd, J.C., Joiner, K.A., 2006. Toxoplasma gondii sequesters lysosomes from mammalian hosts in the vacuolar space. Cell 125, 261–274. https://doi.org/10.1016/j.cell.2006.01.056

Courret, N., Darche, S., Sonigo, P., Milon, G., Buzoni-Gâtel, D., Tardieux, I., 2006. CD11c- and CD11b-expressing mouse leukocytes transport single Toxoplasma gondii tachyzoites to the brain. Blood 107, 309–316. https://doi.org/10.1182/blood-2005-02-0666

Couvreur, G., Sadak, A., Fortier, B., Dubremetz, J.F., 1988. Surface antigens of Toxoplasma gondii. Parasitology 97 ( Pt 1), 1–10. https://doi.org/10.1017/s0031182000066695

Curt-Varesano, A., Braun, L., Ranquet, C., Hakimi, M.-A., Bougdour, A., 2016. The aspartyl protease TgASP5 mediates the export of the Toxoplasma GRA16 and GRA24 effectors into host cells. Cell. Microbiol. 18, 151–167. https://doi.org/10.1111/cmi.12498

Cyrklaff, M., Kudryashev, M., Leis, A., Leonard, K., Baumeister, W., Menard, R., Meissner, M., Frischknecht, F., 2007. Cryoelectron tomography reveals periodic material at the inner side of subpellicular microtubules in apicomplexan parasites. J Exp Med 204, 1281–1287. https://doi.org/10.1084/jem.20062405

Darcy, F., Deslee, D., Santoro, F., Charif, H., Auriault, C., Decoster, A., Duquesne, V., Capron, A., 1988. Induction of a protective antibody-dependent response against toxoplasmosis by in vitro excreted/secreted antigens from tachyzoites of Toxoplasma gondii. Parasite Immunol 10, 553–567. https://doi.org/10.1111/j.1365-3024.1988.tb00242.x

Dard, C., Fricker-Hidalgo, H., Brenier-Pinchart, M.-P., Pelloux, H., 2016. Relevance of and New Developments in Serology for Toxoplasmosis. Trends in Parasitology 32, 492–506. https://doi.org/10.1016/j.pt.2016.04.001

Dardé, M.L., 2008. Toxoplasma gondii, “new” genotypes and virulence. Parasite 15, 366–371. https://doi.org/10.1051/parasite/2008153366

de Graaf, B.H.J., Cheung, A.Y., Andreyeva, T., Levasseur, K., Kieliszewski, M., Wu, H., 2005. Rab11 GTPase-Regulated Membrane Trafficking Is Crucial for Tip-Focused Pollen Tube Growth in Tobacco. Plant Cell 17, 2564–2579. https://doi.org/10.1105/tpc.105.033183

de Leon, J.C., Scheumann, N., Beatty, W., Beck, J.R., Tran, J.Q., Yau, C., Bradley, P.J., Gull, K., Wickstead, B., Morrissette, N.S., 2013. A SAS-6-Like Protein Suggests that the Toxoplasma Conoid Complex Evolved from Flagellar Components. Eukaryot Cell 12, 1009–1019. https://doi.org/10.1128/EC.00096-13

de Macedo, C.S., Shams-Eldin, H., Smith, T.K., Schwarz, R.T., Azzouz, N., 2003. Inhibitors of glycosyl-phosphatidylinositol anchor biosynthesis. Biochimie 85, 465–472. https://doi.org/10.1016/s0300-9084(03)00065-8

de Souza, W., 2005. Microscopy and cytochemistry of the biogenesis of the parasitophorous vacuole. Histochem Cell Biol 123, 1–18. https://doi.org/10.1007/s00418-004-0746-1

Deffieu, M.S., Alayi, T.D., Slomianny, C., Tomavo, S., 2019. The Toxoplasma gondii dense granule protein TgGRA3 interacts with host Golgi and dysregulates anterograde transport. Biol Open 8. https://doi.org/10.1242/bio.039818

Del Rosario, M., Periz, J., Pavlou, G., Lyth, O., Latorre-Barragan, F., Das, S., Pall, G.S., Stortz, J.F., Lemgruber, L., Whitelaw, J.A., Baum, J., Tardieux, I., Meissner, M., 2019. Apicomplexan F-actin is required for efficient nuclear entry during host cell invasion. EMBO Rep 20. https://doi.org/10.15252/embr.201948896

Delprato, A., Merithew, E., Lambright, D., 2004. Structure, Exchange Determinants, and Family-Wide Rab Specificity of the Tandem Helical Bundle and Vps9 Domains of Rabex-5. Cell 118, 607–17. https://doi.org/10.1016/j.cell.2004.08.009

Díaz-Martín, R.D., Mercier, C., Gómez de León, C.T., González, R.M., Pozos, S.G., Ríos-Castro, E., García, R.A., Fox, B.A., Bzik, D.J., Flores, R.M., 2019. The dense granule protein 8 (GRA8) is a component of the sub-pellicular cytoskeleton in Toxoplasma gondii. Parasitol Res 118, 1899–1918. https://doi.org/10.1007/s00436-019-06298-7

Díaz, E., Pfeffer, S.R., 1998. TIP47: A Cargo Selection Device for Mannose 6-Phosphate Receptor Trafficking. Cell 93, 433–443. https://doi.org/10.1016/S0092-8674(00)81171-X

Dobrowolski, J. M., Carruthers, V.B., Sibley, L.D., 1997. Participation of myosin in gliding motility and host cell invasion by Toxoplasma gondii. Mol. Microbiol. 26, 163–173. https://doi.org/10.1046/j.1365-2958.1997.5671913.x

Dobrowolski, Janice M., Niesman, I.R., Sibley, L.D., 1997. Actin in the parasite Toxoplasma gondii is encoded by a single copy gene, ACT1 and exists primarily in a globular form. Cell Motility 37, 253–262. https://doi.org/10.1002/(SICI)1097-0169(1997)37:3<253::AID-CM7>3.0.CO;2-7

Dobrowolski, J.M., Sibley, L.D., 1996. Toxoplasma invasion of mammalian cells is powered by the actin cytoskeleton of the parasite. Cell 84, 933–939. https://doi.org/10.1016/s0092-8674(00)81071-5

Docampo, R., 2016. The origin and evolution of the acidocalcisome and its interactions with other organelles. Mol Biochem Parasitol 209, 3–9. https://doi.org/10.1016/j.molbiopara.2015.10.003

Donahue, C.G., Carruthers, V.B., Gilk, S.D., Ward, G.E., 2000. The Toxoplasma homolog of Plasmodium apical membrane antigen-1 (AMA-1) is a microneme protein secreted in response to elevated intracellular calcium levels. Mol. Biochem. Parasitol. 111, 15–30. https://doi.org/10.1016/s0166-6851(00)00289-9

Dou, Z., McGovern, O., Cristina, M., Carruthers, V., 2014. Toxoplasma gondii Ingests and Digests Host Cytosolic Proteins. mBio 5. https://doi.org/10.1128/mBio.01188-14

Dowse, T., Soldati, D., 2004. Host cell invasion by the apicomplexans: the significance of microneme protein proteolysis. Curr. Opin. Microbiol. 7, 388–396. https://doi.org/10.1016/j.mib.2004.06.013

Dowse, T.J., Pascall, J.C., Brown, K.D., Soldati, D., 2005. Apicomplexan rhomboids have a potential role in microneme protein cleavage during host cell invasion. Int. J. Parasitol. 35, 747–756. https://doi.org/10.1016/j.ijpara.2005.04.001

Dubey, J.P., 1997. Tissue cyst tropism in Toxoplasma gondii: a comparison of tissue cyst formation in organs of cats, and rodents fed oocysts. Parasitology 115 ( Pt 1), 15–20. https://doi.org/10.1017/s0031182097008949

Dubey, J.P., Frenkel, J.K., 1972. Cyst-induced toxoplasmosis in cats. J. Protozool. 19, 155–177. https://doi.org/10.1111/j.1550-7408.1972.tb03431.x

Dubey, J.P., Lindsay, D.S., Speer, C.A., 1998. Structures of Toxoplasma gondii Tachyzoites, Bradyzoites, and Sporozoites and Biology and Development of Tissue Cysts. Clin Microbiol Rev 11, 267–299.

Dubey, J.P., Miller, N.L., Frenkel, J.K., 1970. THE TOXOPLASMA GONDII OOCYST FROM CAT FECES. J Exp Med 132, 636–662.

Dubois, D.J., Soldati-Favre, D., 2019. Biogenesis and secretion of micronemes in Toxoplasma gondii. Cell Microbiol 21, e13018. https://doi.org/10.1111/cmi.13018

Dubremetz, J.F., 2007. Rhoptries are major players in Toxoplasma gondii invasion and host cell interaction. Cellular Microbiology 9, 841–848. https://doi.org/10.1111/j.1462-5822.2007.00909.x

Dubremetz, J.F., Achbarou, A., Bermudes, D., Joiner, K.A., 1993. Kinetics and pattern of organelle exocytosis during Toxoplasma gondii/host-cell interaction. Parasitol. Res. 79, 402–408. https://doi.org/10.1007/bf00931830

Dubremetz, J.F., Dissous, C., 1980. Characteristic proteins of micronemes and dense granules from Sarcocystis tenella zoites (Protozoa, Coccidia). Mol. Biochem. Parasitol. 1, 279–289. https://doi.org/10.1016/0166-6851(80)90061-4

Dunn, D., Wallon, M., Peyron, F., Petersen, E., Peckham, C., Gilbert, R., 1999. Mother-to-child transmission of toxoplasmosis: risk estimates for clinical counselling. The Lancet 353, 1829–1833. https://doi.org/10.1016/S0140-6736(98)08220-8

Dwivedi, D., Kumari, A., Rathi, S., Mylavarapu, S.V.S., Sharma, M., 2019. The dynein adaptor Hook2 plays essential roles in mitotic progression and cytokinesis. Journal of Cell Biology 218, 871–894. https://doi.org/10.1083/jcb.201804183

Dzierszinski, F., Mortuaire, M., Cesbron-Delauw, M.F., Tomavo, S., 2000. Targeted disruption of the glycosylphosphatidylinositol-anchored surface antigen SAG3 gene in Toxoplasma gondii decreases host cell adhesion and drastically reduces virulence in mice. Mol. Microbiol. 37, 574–582. https://doi.org/10.1046/j.1365-2958.2000.02014.x

Egarter, S., Andenmatten, N., Jackson, A.J., Whitelaw, J.A., Pall, G., Black, J.A., Ferguson, D.J.P., Tardieux, I., Mogilner, A., Meissner, M., 2014. The Toxoplasma Acto-MyoA Motor Complex Is Important but Not Essential for Gliding Motility and Host Cell Invasion. PLoS One 9. https://doi.org/10.1371/journal.pone.0091819

El Hajj, H., Demey, E., Poncet, J., Lebrun, M., Wu, B., Galéotti, N., Fourmaux, M.N., Mercereau-Puijalon, O., Vial, H., Labesse, G., Dubremetz, J.F., 2006. The ROP2 family of Toxoplasma gondii rhoptry proteins: proteomic and genomic characterization and molecular modeling. Proteomics 6, 5773–5784. https://doi.org/10.1002/pmic.200600187

Elkin, S., Lakoduk, A., Schmid, S., 2016. Endocytic Pathways and Endosomal Trafficking: A Primer. Wiener Medizinische Wochenschrift 166. https://doi.org/10.1007/s10354-016-0432-7

Elsner, M., Hashimoto, H., Nilsson, T., 2003. Cisternal maturation and vesicle transport: join the band wagon! (Review). Mol Membr Biol 20, 221–229. https://doi.org/10.1080/0968768031000114024

Endo, T., Sethi, K.K., Piekarski, G., 1982. Toxoplasma gondii: Calcium lonophore A23187-mediated exit of trophozoites from infected murine macrophages. Experimental Parasitology 53, 179–188. https://doi.org/10.1016/0014-4894(82)90059-5

Etheridge, R.D., Alaganan, A., Tang, K., Lou, H.J., Turk, B.E., Sibley, L.D., 2014. The Toxoplasma pseudokinase ROP5 forms complexes with ROP18 and ROP17 kinases that synergize to control acute virulence in mice. Cell Host Microbe 15, 537–550. https://doi.org/10.1016/j.chom.2014.04.002

Ezougou, C., Ben Rached, F., Moss, D., Lin, J.-W., Black, S., Knuepfer, E., Green, J., Khan, S., Mukhopadhyay, A., Janse, C., Coppens, I., Yera, H., Holder, A., Langsley, G., 2014. Plasmodium falciparum Rab5B Is an N-Terminally Myristoylated Rab GTPase That Is Targeted to the Parasite's Plasma and Food Vacuole Membranes. PloS one 9, e87695. https://doi.org/10.1371/journal.pone.0087695

Farrell, M., Gubbels, M.-J., 2014. The Toxoplasma gondii kinetochore is required for centrosome association with the centrocone (spindle pole). Cell Microbiol 16, 78–94. https://doi.org/10.1111/cmi.12185

Ferguson, D.J., Birch-Andersen, A., Siim, J.C., Hutchison, W.M., 1979. An ultrastructural study on the excystation of the sporozoites of Toxoplasma gondii. Acta Pathol Microbiol Scand B 87, 277–283. https://doi.org/10.1111/j.1699-0463.1979.tb02439.x

Ferguson, D.J.P., 2009. Toxoplasma gondii: 1908-2008, homage to Nicolle, Manceaux and Splendore. Mem. Inst. Oswaldo Cruz 104, 133–148. https://doi.org/10.1590/s0074-02762009000200003

Ferguson, D.J.P., 2002. Toxoplasma gondii and sex: essential or optional extra? Trends in Parasitology 18, 351–355. https://doi.org/10.1016/S1471-4922(02)02330-9

Fielding, A., Schonteich, E., Matheson, J., Wilson, G., Yu, X., Hickson, G., Srivastava, S., Baldwin, S., Prekeris, R., Gould, G., 2005. Rab11-FIP3 and FIP4 interact with Arf6 and the Exocyst to control membrane traffic in cytokinesis. The EMBO journal 24, 3389–99. https://doi.org/10.1038/sj.emboj.7600803

Fleckenstein, M.C., Reese, M.L., Könen-Waisman, S., Boothroyd, J.C., Howard, J.C., Steinfeldt, T., 2012. A Toxoplasma gondii Pseudokinase Inhibits Host IRG Resistance Proteins. PLOS Biology 10, e1001358. https://doi.org/10.1371/journal.pbio.1001358

Fletcher, S.J., Rappoport, J.Z., 2010. Moving forward: polarised trafficking in cell migration. Trends in Cell Biology 20, 71–78. https://doi.org/10.1016/j.tcb.2009.11.006

Fomovska, A., Wood, R.D., Mui, E., Dubey, J.P., Ferreira, L.R., Hickman, M.R., Lee, P.J., Leed, S.E., Auschwitz, J.M., Welsh, W.J., Sommerville, C., Woods, S., Roberts, C., McLeod, R., 2012. Salicylanilide Inhibitors of Toxoplasma gondii. J. Med. Chem. 55, 8375–8391. https://doi.org/10.1021/jm3007596

Ford, M.G.J., Mills, I.G., Peter, B.J., Vallis, Y., Praefcke, G.J.K., Evans, P.R., McMahon, H.T., 2002. Curvature of clathrin-coated pits driven by epsin. Nature 419, 361–366. https://doi.org/10.1038/nature01020

Foussard, F., Leriche, M.A., Dubremetz, J.F., 1991. Characterization of the lipid content of Toxoplasma gondii rhoptries. Parasitology 102 Pt 3, 367–370. https://doi.org/10.1017/s0031182000064313

Fox, B.A., Falla, A., Rommereim, L.M., Tomita, T., Gigley, J.P., Mercier, C., Cesbron-Delauw, M.-F., Weiss, L.M., Bzik, D.J., 2011. Type II Toxoplasma gondii KU80 Knockout Strains Enable Functional Analysis of Genes Required for Cyst Development and Latent Infection ▿ . Eukaryot Cell 10, 1193–1206. https://doi.org/10.1128/EC.00297-10

Fox, B.A., Guevara, R.B., Rommereim, L.M., Falla, A., Bellini, V., Pètre, G., Rak, C., Cantillana, V., Dubremetz, J.-F., Cesbron-Delauw, M.-F., Taylor, G.A., Mercier, C., Bzik, D.J., 2019. Toxoplasma gondii Parasitophorous Vacuole Membrane-Associated Dense Granule Proteins Orchestrate Chronic Infection and GRA12 Underpins Resistance to Host Gamma Interferon. mBio 10. https://doi.org/10.1128/mBio.00589-19

Francia, M.E., Jordan, C.N., Patel, J.D., Sheiner, L., Demerly, J.L., Fellows, J.D., Leon, J.C. de, Morrissette, N.S., Dubremetz, J.-F., Striepen, B., 2012. Cell Division in Apicomplexan Parasites Is Organized by a Homolog of the Striated Rootlet Fiber of Algal Flagella. PLOS Biology 10, e1001444. https://doi.org/10.1371/journal.pbio.1001444

Francia, M.E., Striepen, B., 2014. Cell division in apicomplexan parasites. Nat. Rev. Microbiol. 12, 125–136. https://doi.org/10.1038/nrmicro3184

Franco, M., Panas, M.W., Marino, N.D., Lee, M.-C.W., Buchholz, K.R., Kelly, F.D., Bednarski, J.J., Sleckman, B.P., Pourmand, N., Boothroyd, J.C., 2016. A Novel Secreted Protein,

MYR1, Is Central to Toxoplasma's Manipulation of Host Cells. mBio 7. https://doi.org/10.1128/mBio.02231-15

Frénal, K., Dubremetz, J.-F., Lebrun, M., Soldati-Favre, D., 2017a. Gliding motility powers invasion and egress in Apicomplexa. Nat. Rev. Microbiol. 15, 645–660. https://doi.org/10.1038/nrmicro.2017.86

Frénal, K., Jacot, D., Hammoudi, P.-M., Graindorge, A., Maco, B., Soldati-Favre, D., 2017b. Myosin-dependent cell-cell communication controls synchronicity of division in acute and chronic stages of Toxoplasma gondii. Nature Communications 8, 15710. https://doi.org/10.1038/ncomms15710

Frénal, K., Marq, J.-B., Jacot, D., Polonais, V., Soldati-Favre, D., 2014. Plasticity between MyoC- and MyoA-Glideosomes: An Example of Functional Compensation in Toxoplasma gondii Invasion. PLoS Pathog 10. https://doi.org/10.1371/journal.ppat.1004504

Frénal, K., Polonais, V., Marq, J.-B., Stratmann, R., Limenitakis, J., Soldati-Favre, D., 2010. Functional dissection of the apicomplexan glideosome molecular architecture. Cell Host Microbe 8, 343–357. https://doi.org/10.1016/j.chom.2010.09.002

Frenal, K., Soldati-Favre, D., 2013. Un complexe moléculaire unique à l'origine de la motilité et de l'invasion des Apicomplexes. Medecine Sciences 29, 515–22. https://doi.org/10.1051/medsci/2013295015

Frenkel, J.K., 1973. Toxoplasma In and Around Us. BioScience 23, 343–352. https://doi.org/10.2307/1296513

Frenkel, J.K., Dubey, J.P., Miller, N.L., 1970. Toxoplasma gondii in cats: fecal stages identified as coccidian oocysts. Science 167, 893–896. https://doi.org/10.1126/science.167.3919.893

Frenkel, J.K., Escajadillo, A., 1987. Cyst Rupture as a Pathogenic Mechanism of Toxoplasmic Encephalitis. The American Journal of Tropical Medicine and Hygiene 36, 517–522. https://doi.org/10.4269/ajtmh.1987.36.517

Frenkel, J.K., Ruiz, A., Chinchilla, M., 1975. Soil Survival of Toxoplasma Oocysts in Kansas and Costa Rica*. The American Journal of Tropical Medicine and Hygiene 24, 439–443. https://doi.org/10.4269/ajtmh.1975.24.439

Fuchs, E., Haas, A., Spooner, R., Yoshimura, S., Lord, J., Barr, F., 2007. Specific Rab GTPase-activating proteins define the Shiga toxin and epidermal growth factor uptake pathways. The Journal of cell biology 177, 1133–43. https://doi.org/10.1083/jcb.200612068

Fuentes, I., Rubio, J.M., Ramírez, C., Alvar, J., 2001. Genotypic Characterization of Toxoplasma gondii Strains Associated with Human Toxoplasmosis in Spain: Direct Analysis from Clinical Samples. J Clin Microbiol 39, 1566–1570. https://doi.org/10.1128/JCM.39.4.1566-1570.2001

Gadila, S.K.G., Kim, K., 2016. Cargo trafficking from the trans-Golgi network towards the endosome. Biology of the Cell 108, 205–218. https://doi.org/10.1111/boc.201600001

Gaji, R.Y., Johnson, D.E., Treeck, M., Wang, M., Hudmon, A., Arrizabalaga, G., 2015. Phosphorylation of a Myosin Motor by TgCDPK3 Facilitates Rapid Initiation of Motility during Toxoplasma gondii egress. PLoS Pathog 11. https://doi.org/10.1371/journal.ppat.1005268

Gajurel, K., Dhakal, R., Montoya, J.G., 2015. Toxoplasma prophylaxis in haematopoietic cell transplant recipients: a review of the literature and recommendations. Curr. Opin. Infect. Dis. 28, 283–292. https://doi.org/10.1097/QCO.0000000000000169

Gang Dong, 2017. Unravelling the first step of vesicle fusion [WWW Document]. Max Perutz Labs. URL https://www.maxperutzlabs.ac.at/news/latest-news/l/unravelling-the-first-step-of-vesicle-fusion-98 (accessed 1.27.21).

Garcia-Réguet, N., Lebrun, M., Fourmaux, M.-N., Mercereau-Puijalon, O., Mann, T., Beckers, C.J.M., Samyn, B., Beeumen, J.V., Bout, D., Dubremetz, J.-F., 2000. The microneme protein MIC3 of Toxoplasma gondii is a secretory adhesin that binds to both the surface of the host cells and the surface of the parasite. Cellular Microbiology 2, 353–364. https://doi.org/10.1046/j.1462-5822.2000.00064.x

Garg, S., Sharma, V., Ramu, D., Singh, S., 2015. In silico analysis of calcium binding pocket of perforin like protein 1: insights into the regulation of pore formation. Syst Synth Biol 9, 17–21. https://doi.org/10.1007/s11693-015-9166-x

Gaskins, E., Gilk, S., DeVore, N., Mann, T., Ward, G., Beckers, C., 2004. Identification of the membrane receptor of a class XIV myosin in Toxoplasma gondii. J Cell Biol 165, 383–393. https://doi.org/10.1083/jcb.200311137

Gay, G., Braun, L., Brenier-Pinchart, M.-P., Vollaire, J., Josserand, V., Bertini, R.-L., Varesano, A., Touquet, B., De Bock, P.-J., Coute, Y., Tardieux, I., Bougdour, A., Hakimi, M.-A., 2016. Toxoplasma gondii TgIST co-opts host chromatin repressors dampening STAT1-dependent gene regulation and IFN-γ–mediated host defenses. J Exp Med 213, 1779–1798. https://doi.org/10.1084/jem.20160340

Gazzinelli, R.T., Wysocka, M., Hayashi, S., Denkers, E.Y., Hieny, S., Caspar, P., Trinchieri, G., Sher, A., 1994. Parasite-induced IL-12 stimulates early IFN-gamma synthesis and resistance during acute infection with Toxoplasma gondii. The Journal of Immunology 153, 2533–2543.

Gendrin, C., Mercier, C., Braun, L., Musset, K., Dubremetz, J.-F., Cesbron-Delauw, M.-F., 2008. Toxoplasma gondii uses unusual sorting mechanisms to deliver transmembrane proteins into the host-cell vacuole. Traffic 9, 1665–1680. https://doi.org/10.1111/j.1600-0854.2008.00793.x

Gilbert, L.A., Ravindran, S., Turetzky, J.M., Boothroyd, J.C., Bradley, P.J., 2007. Toxoplasma gondii Targets a Protein Phosphatase 2C to the Nuclei of Infected Host Cells. Eukaryot Cell 6, 73–83. https://doi.org/10.1128/EC.00309-06

Girard, P.M., Landman, R., Gaudebout, C., Olivares, R., Saimot, A.G., Jelazko, P., Gaudebout, C., Certain, A., Boué, F., Bouvet, E., 1993. Dapsone-pyrimethamine compared with aerosolized pentamidine as primary prophylaxis against Pneumocystis carinii pneumonia and toxoplasmosis in HIV infection. The PRIO Study Group. N. Engl. J. Med. 328, 1514–1520. https://doi.org/10.1056/NEJM199305273282102

Gisselberg, J.E., Dellibovi-Ragheb, T.A., Matthews, K.A., Bosch, G., Prigge, S.T., 2013. The Suf Iron-Sulfur Cluster Synthesis Pathway Is Required for Apicoplast Maintenance in Malaria Parasites. PLOS Pathogens 9, e1003655. https://doi.org/10.1371/journal.ppat.1003655

Gold, D.A., Kaplan, A.D., Lis, A., Bett, G.C., Rosowski, E.E., Cirelli, K.M., Bougdour, A., Sidik, S.M., Beck, J.R., Lourido, S., Egea, P.F., Bradley, P.J., Hakimi, M.-A., Rasmusson, R.L., Saeij, J.P., 2015. The Toxoplasma dense granule proteins GRA17 and GRA23 mediate the movement of small molecules between the host and the parasitophorous vacuole. Cell Host Microbe 17, 642–652. https://doi.org/10.1016/j.chom.2015.04.003

Goldenring, J., Shen, K., Vaughan, H., Modlin, I., 1993. Goldenring JR, Shen KR, Vaughan HD, Modlin IMIdentification of a small GTP-binding protein, Rab25, expressed in the

gastrointestinal mucosa, kidney and lung. J Biol Chem 268: 18419-18422. The Journal of biological chemistry 268, 18419–22.
Gordon, J.L., Sibley, L.D., 2005. Comparative genome analysis reveals a conserved family of actin-like proteins in apicomplexan parasites. BMC Genomics 6, 179. https://doi.org/10.1186/1471-2164-6-179
Gould, S.B., Tham, W.-H., Cowman, A.F., McFadden, G.I., Waller, R.F., 2008. Alveolins, a new family of cortical proteins that define the protist infrakingdom Alveolata. Mol. Biol. Evol. 25, 1219–1230. https://doi.org/10.1093/molbev/msn070
Grant, B.D., Donaldson, J.G., 2009. Pathways and mechanisms of endocytic recycling. Nature Reviews Molecular Cell Biology 10, 597–608. https://doi.org/10.1038/nrm2755
Gras, S., Jackson, A., Woods, S., Pall, G., Whitelaw, J., Leung, J.M., Ward, G.E., Roberts, C.W., Meissner, M., 2017. Parasites lacking the micronemal protein MIC2 are deficient in surface attachment and host cell egress, but remain virulent in vivo. Wellcome Open Res 2. https://doi.org/10.12688/wellcomeopenres.11594.2
Gras, S., Jimenez-Ruiz, E., Klinger, C.M., Schneider, K., Klingl, A., Lemgruber, L., Meissner, M., 2019. An endocytic-secretory cycle participates in Toxoplasma gondii in motility. PLOS Biology 17, e3000060. https://doi.org/10.1371/journal.pbio.3000060
Grasse, P.P., 1957. [Ultrastructure, polarity and reproduction of Golgi apparatus]. C R Hebd Seances Acad Sci 245, 1278–1281.
Green, E.G., Ramm, E., Riley, N.M., Spiro, D.J., Goldenring, J.R., Wessling-Resnick, M., 1997. Rab11 Is Associated with Transferrin-Containing Recycling Compartments in K562 Cells. Biochemical and Biophysical Research Communications 239, 612–616. https://doi.org/10.1006/bbrc.1997.7520
Greenfield, J.P., Leung, L.W., Cai, D., Kaasik, K., Gross, R.S., Rodriguez-Boulan, E., Greengard, P., Xu, H., 2002. Estrogen lowers Alzheimer beta-amyloid generation by stimulating trans-Golgi network vesicle biogenesis. J Biol Chem 277, 12128–12136. https://doi.org/10.1074/jbc.M110009200
Grigg, M.E., Bonnefoy, S., Hehl, A.B., Suzuki, Y., Boothroyd, J.C., 2001. Success and Virulence in Toxoplasma as the Result of Sexual Recombination Between Two Distinct Ancestries. Science 294, 161–165. https://doi.org/10.1126/science.1061888
Gubbels, M.-J., Vaishnava, S., Boot, N., Dubremetz, J.-F., Striepen, B., 2006. A MORN-repeat protein is a dynamic component of the Toxoplasma gondii cell division apparatus. J. Cell. Sci. 119, 2236–2245. https://doi.org/10.1242/jcs.02949
Gubbels, M.-J., White, M., Szatanek, T., 2008. The cell cycle and Toxoplasma gondii cell division: tightly knit or loosely stitched? Int. J. Parasitol. 38, 1343–1358. https://doi.org/10.1016/j.ijpara.2008.06.004
Guérin, A., Corrales, R.M., Parker, M.L., Lamarque, M.H., Jacot, D., El Hajj, H., Soldati-Favre, D., Boulanger, M.J., Lebrun, M., 2017. Efficient invasion by Toxoplasma depends on the subversion of host protein networks. Nat Microbiol 2, 1358–1366. https://doi.org/10.1038/s41564-017-0018-1
Guerina, N.G., Hsu, H.-W., Meissner, H.C., Maguire, J.H., Lynfield, R., Stechenberg, B., Abroms, I., Pasternack, M.S., Hoff, R., Eaton, R.B., Grady, G.F., 1994. Neonatal Serologic Screening and Early Treatment for Congenital Toxoplasma gondii Infection. New England Journal of Medicine 330, 1858–1863. https://doi.org/10.1056/NEJM199406303302604

Guichard, A., Nizet, V., Bier, E., 2014. RAB11-mediated trafficking in host–pathogen interactions. Nature Reviews Microbiology 12, 624–634. https://doi.org/10.1038/nrmicro3325

Guo, X., Farías, G.G., Mattera, R., Bonifacino, J.S., 2016. Rab5 and its effector FHF contribute to neuronal polarity through dynein-dependent retrieval of somatodendritic proteins from the axon. PNAS 113, E5318–E5327. https://doi.org/10.1073/pnas.1601844113

Guthrie, C.R., Schellenberg, G.D., Kraemer, B.C., 2009. SUT-2 potentiates tau-induced neurotoxicity in Caenorhabditis elegans. Hum Mol Genet 18, 1825–1838. https://doi.org/10.1093/hmg/ddp099

Hajj, H.E., Lebrun, M., Fourmaux, M.N., Vial, H., Dubremetz, J.F., 2007. Inverted topology of the Toxoplasma gondii ROP5 rhoptry protein provides new insights into the association of the ROP2 protein family with the parasitophorous vacuole membrane. Cellular Microbiology 9, 54–64. https://doi.org/10.1111/j.1462-5822.2006.00767.x

Håkansson, S., Charron, A.J., Sibley, L.D., 2001. Toxoplasma evacuoles: a two-step process of secretion and fusion forms the parasitophorous vacuole. EMBO J 20, 3132–3144. https://doi.org/10.1093/emboj/20.12.3132

Håkansson, S., Morisaki, H., Heuser, J., Sibley, L.D., 1999. Time-Lapse Video Microscopy of Gliding Motility in Toxoplasma gondii Reveals a Novel, Biphasic Mechanism of Cell Locomotion. Mol Biol Cell 10, 3539–3547.

Hakimi, M.-A., Bougdour, A., 2015. Toxoplasma's ways of manipulating the host transcriptome via secreted effectors. Curr. Opin. Microbiol. 26, 24–31. https://doi.org/10.1016/j.mib.2015.04.003

Hakimi, M.-A., Olias, P., Sibley, L.D., 2017. Toxoplasma Effectors Targeting Host Signaling and Transcription. Clin Microbiol Rev 30, 615–645. https://doi.org/10.1128/CMR.00005-17

Hales, C., Vaerman, J.-P., Goldenring, J., 2003. Rab11 Family Interacting Protein 2 Associates with Myosin Vb and Regulates Plasma Membrane Recycling. The Journal of biological chemistry 277, 50415–21. https://doi.org/10.1074/jbc.M209270200

Hanson, P.I., Roth, R., Morisaki, H., Jahn, R., Heuser, J.E., 1997. Structure and conformational changes in NSF and its membrane receptor complexes visualized by quick-freeze/deep-etch electron microscopy. Cell 90, 523–535. https://doi.org/10.1016/s0092-8674(00)80512-7

Harper, J.M., Hoff, E.F., Carruthers, V.B., 2004. Multimerization of the Toxoplasma gondii MIC2 integrin-like A-domain is required for binding to heparin and human cells. Mol. Biochem. Parasitol. 134, 201–212. https://doi.org/10.1016/j.molbiopara.2003.12.001

Harper, J.M., Huynh, M.-H., Coppens, I., Parussini, F., Moreno, S., Carruthers, V.B., 2006. A Cleavable Propeptide Influences Toxoplasma Infection by Facilitating the Trafficking and Secretion of the TgMIC2–M2AP Invasion Complex. Mol Biol Cell 17, 4551–4563. https://doi.org/10.1091/mbc.E06-01-0064

Hartmann, J., Hu, K., He, C.Y., Pelletier, L., Roos, D.S., Warren, G., 2006. Golgi and centrosome cycles in Toxoplasma gondii. Mol. Biochem. Parasitol. 145, 125–127. https://doi.org/10.1016/j.molbiopara.2005.09.015

He, X., Grigg, M.E., Boothroyd, J.C., Garcia, K.C., 2002. Structure of the immunodominant surface antigen from the Toxoplasma gondii SRS superfamily. Nat. Struct. Biol. 9, 606–611. https://doi.org/10.1038/nsb819

Heaslip, A.T., Ems-McClung, S.C., Hu, K., 2009. TgICMAP1 Is a Novel Microtubule Binding Protein in Toxoplasma gondii. PLOS ONE 4, e7406. https://doi.org/10.1371/journal.pone.0007406

Heaslip, A.T., Nelson, S.R., Warshaw, D.M., 2016. Dense granule trafficking in Toxoplasma gondii requires a unique class 27 myosin and actin filaments. Mol Biol Cell 27, 2080–2089. https://doi.org/10.1091/mbc.E15-12-0824

Heaslip, A.T., Nishi, M., Stein, B., Hu, K., 2011. The Motility of a Human Parasite, Toxoplasma gondii, Is Regulated by a Novel Lysine Methyltransferase. PLoS Pathog 7. https://doi.org/10.1371/journal.ppat.1002201

Hehl, A.B., Lekutis, C., Grigg, M.E., Bradley, P.J., Dubremetz, J.-F., Ortega-Barria, E., Boothroyd, J.C., 2000. Toxoplasma gondii Homologue of Plasmodium Apical Membrane Antigen 1 Is Involved in Invasion of Host Cells. Infect Immun 68, 7078–7086.

Herda, S., Raczkowski, F., Mittrücker, H.-W., Willimsky, G., Gerlach, K., Kühl, A.A., Breiderhoff, T., Willnow, T.E., Dörken, B., Höpken, U.E., Rehm, A., 2012. The Sorting Receptor Sortilin Exhibits a Dual Function in Exocytic Trafficking of Interferon-γ and Granzyme A in T Cells. Immunity 37, 854–866. https://doi.org/10.1016/j.immuni.2012.07.012

Hermanns, T., Müller, U.B., Könen-Waisman, S., Howard, J.C., Steinfeldt, T., 2016. The Toxoplasma gondii rhoptry protein ROP18 is an Irga6-specific kinase and regulated by the dense granule protein GRA7. Cell Microbiol 18, 244–259. https://doi.org/10.1111/cmi.12499

Herm-Götz, A., Agop-Nersesian, C., Münter, S., Grimley, J., Wandless, T., Frischknecht, F., Meissner, M., 2008. Rapid control of protein level in the apicomplexan Toxoplasma gondii. Nature methods 4, 1003–5. https://doi.org/10.1038/nmeth1134

Herm-Götz, A., Weiss, S., Stratmann, R., Fujita-Becker, S., Ruff, C., Meyhöfer, E., Soldati, T., Manstein, D.J., Geeves, M.A., Soldati, D., 2002. Toxoplasma gondii myosin A and its light chain: a fast, single-headed, plus-end-directed motor. EMBO J 21, 2149–2158. https://doi.org/10.1093/emboj/21.9.2149

Hill, D., Dubey, J.P., 2002. Toxoplasma gondii: transmission, diagnosis and prevention. Clin. Microbiol. Infect. 8, 634–640. https://doi.org/10.1046/j.1469-0691.2002.00485.x

Hirst, J., Robinson, M.S., 1998. Clathrin and adaptors. Biochim Biophys Acta 1404, 173–193. https://doi.org/10.1016/s0167-4889(98)00056-1

Hobdy-Henderson, K.C., Hales, C.M., Lapierre, L.A., Cheney, R.E., Goldenring, J.R., 2003. Dynamics of the apical plasma membrane recycling system during cell division. Traffic 4, 681–693. https://doi.org/10.1034/j.1600-0854.2003.00124.x

Hong, D.-P., Radke, J.B., White, M.W., 2017. Opposing Transcriptional Mechanisms Regulate Toxoplasma Development. mSphere 2. https://doi.org/10.1128/mSphere.00347-16

Hoppe, H., Joiner, K., 2000. Cytoplasmic tail motifs mediate endoplasmic reticulum localization and export of transmembrane reporters in the protozoan parasite Toxoplasma gondii. Cellular Microbiology 2, 569–578. https://doi.org/10.1046/j.1462-5822.2000.00081.x

Horgan, C., 2004. Rab11-FIP3 Localises to a Rab11-positive pericentrosomal compartment during interphase and to the cleavage furrow during cytokinesis. Biochemical and Biophysical Research Communications 319, 83–94. https://doi.org/10.1016/S0006-291X(04)00900-3

Horgan, C., Mccaffrey, M., 2012. Endosomal trafficking in animal cytokinesis. Frontiers in bioscience (Scholar edition) 4, 547–55. https://doi.org/10.2741/284

Horgan, C.P., McCaffrey, M.W., 2009. The dynamic Rab11-FIPs. Biochem Soc Trans 37, 1032–1036. https://doi.org/10.1042/BST0371032

Howe, D.K., Sibley, L.D., 1995. Toxoplasma gondii comprises three clonal lineages: correlation of parasite genotype with human disease. J. Infect. Dis. 172, 1561–1566. https://doi.org/10.1093/infdis/172.6.1561

Hsiao, C.-H.C., Hiller, N.L., Haldar, K., Knoll, L.J., 2013. A HT/PEXEL motif in Toxoplasma dense granule proteins is a signal for protein cleavage but not export into the host cell. Traffic 14, 519–531. https://doi.org/10.1111/tra.12049

Hu, K., 2008. Organizational Changes of the Daughter Basal Complex during the Parasite Replication of Toxoplasma gondii. PLoS Pathog 4. https://doi.org/10.1371/journal.ppat.0040010

Hu, K., Johnson, J., Florens, L., Fraunholz, M., Suravajjala, S., DiLullo, C., Yates, J., Roos, D.S., Murray, J.M., 2006. Cytoskeletal Components of an Invasion Machine—The Apical Complex of Toxoplasma gondii. PLOS Pathogens 2, e13. https://doi.org/10.1371/journal.ppat.0020013

Hu, K., Mann, T., Striepen, B., Beckers, C.J.M., Roos, D.S., Murray, J.M., 2002a. Daughter Cell Assembly in the Protozoan Parasite Toxoplasma gondii. Mol Biol Cell 13, 593–606. https://doi.org/10.1091/mbc.01-06-0309

Hu, K., Roos, D.S., Murray, J.M., 2002b. A novel polymer of tubulin forms the conoid of Toxoplasma gondii. J Cell Biol 156, 1039–1050. https://doi.org/10.1083/jcb.200112086

Hume, A., Collinson, L., Rapak, A., Gomes, A., Hopkins, C., Seabra, M., 2001. Rab27a Regulates the Peripheral Distribution of Melanosomes in Melanocytes. The Journal of cell biology 152, 795–808. https://doi.org/10.1083/jcb.152.4.795

Hunter, C.A., Sibley, L.D., 2012. Modulation of innate immunity by Toxoplasma gondii virulence effectors. Nat Rev Microbiol 10, 766–778. https://doi.org/10.1038/nrmicro2858

Hutagalung, A., Novick, P., 2011. Role of Rab GTPases in Membrane Traffic and Cell Physiology. Physiological reviews 91, 119–49. https://doi.org/10.1152/physrev.00059.2009

Huynh, M.-H., Carruthers, V., 2009. Tagging of Endogenous Genes in a Toxoplasma gondii Strain Lacking Ku80. Eukaryotic cell 8, 530–9. https://doi.org/10.1128/EC.00358-08

Huynh, M.-H., Liu, B., Henry, M., Liew, L., Matthews, S.J., Carruthers, V.B., 2015. Structural Basis of Toxoplasma gondii MIC2-associated Protein Interaction with MIC2. J Biol Chem 290, 1432–1441. https://doi.org/10.1074/jbc.M114.613646

Jacot, D., Daher, W., Soldati-Favre, D., 2013. Toxoplasma gondii myosin F, an essential motor for centrosomes positioning and apicoplast inheritance. EMBO J 32, 1702–1716. https://doi.org/10.1038/emboj.2013.113

Jacot, D., Soldati-Favre, D., 2020. CRISPR/Cas9-Mediated Generation of Tetracycline Repressor-Based Inducible Knockdown in Toxoplasma gondii. Methods Mol Biol 2071, 125–141. https://doi.org/10.1007/978-1-4939-9857-9_7

Jacot, D., Tosetti, N., Pires, I., Stock, J., Graindorge, A., Hung, Y.-F., Han, H., Tewari, R., Kursula, I., Soldati-Favre, D., 2016. An Apicomplexan Actin-Binding Protein Serves as a Connector and Lipid Sensor to Coordinate Motility and Invasion. Cell Host Microbe 20, 731–743. https://doi.org/10.1016/j.chom.2016.10.020

Jahn, R., Scheller, R.H., 2006. SNAREs--engines for membrane fusion. Nat Rev Mol Cell Biol 7, 631–643. https://doi.org/10.1038/nrm2002

Jensen, K.D.C., Wang, Y., Wojno, E.D.T., Shastri, A.J., Hu, K., Cornel, L., Boedec, E., Ong, Y.-C., Chien, Y., Hunter, C.A., Boothroyd, J.C., Saeij, J.P.J., 2011. Toxoplasma Polymorphic Effectors Determine Macrophage Polarization and Intestinal Inflammation. Cell Host Microbe 9, 472–483. https://doi.org/10.1016/j.chom.2011.04.015

Johannes, L., Parton, R., Bassereau, P., Mayor, S., 2015. Building endocytic pits without clathrin. Nature reviews. Molecular cell biology 16. https://doi.org/10.1038/nrm3968

Johansson, M., Rocha, N., Zwart, W., Jordens, I., Janssen, L., Kuijl, C., Olkkonen, V., Neefjes, J., 2007. Activation of endosomal dynein motors by stepwise assembly of Rab7-RILP-p150Glued, ORP1L, and the receptor ??III spectrin. The Journal of cell biology 176, 459–71. https://doi.org/10.1083/jcb.200606077

Johnson, T.M., Rajfur, Z., Jacobson, K., Beckers, C.J., 2007. Immobilization of the Type XIV Myosin Complex in Toxoplasma gondii. Mol Biol Cell 18, 3039–3046. https://doi.org/10.1091/mbc.E07-01-0040

Jordens, I., Fernandez-Borja, M., Marsman, M., Dusseljee, S., Janssen, H., Wubbolts, R., Neefjes, J., 2001. The Rab7 effector protein RILP controlslysosomaltransport by inducingthe recruitment of dynein-dynactin motors 18.

Jordens, I., Marsman, M., Kuijl, C., Neefjes, J., 2006. Rab Proteins, Connecting Transport and Vesicle Fusion. Traffic (Copenhagen, Denmark) 6, 1070–7. https://doi.org/10.1111/j.1600-0854.2005.00336.x

Kaddai, V., Le Marchand-Brustel, Y., Cormont, M., 2008. Rab proteins in endocytosis and Glut4 trafficking. Acta Physiol (Oxf) 192, 75–88. https://doi.org/10.1111/j.1748-1716.2007.01787.x

Kafsack, B.F.C., Pena, J.D.O., Coppens, I., Ravindran, S., Boothroyd, J.C., Carruthers, V.B., 2009. Rapid Membrane Disruption by a Perforin-Like Protein Facilitates Parasite Exit from Host Cells. Science 323, 530–533. https://doi.org/10.1126/science.1165740

Karsten, V., Qi, H., Beckers, C.J.M., Reddy, A., Dubremetz, J.-F., Webster, P., Joiner, K.A., 1998. The Protozoan Parasite Toxoplasma gondii Targets Proteins to Dense Granules and the Vacuolar Space Using Both Conserved and Unusual Mechanisms. J Cell Biol 141, 1323–1333.

Katris, N.J., Ke, H., McFadden, G.I., van Dooren, G.G., Waller, R.F., 2019. Calcium negatively regulates secretion from dense granules in Toxoplasma gondii. Cell Microbiol 21. https://doi.org/10.1111/cmi.13011

Katris, N.J., van Dooren, G.G., McMillan, P.J., Hanssen, E., Tilley, L., Waller, R.F., 2014. The Apical Complex Provides a Regulated Gateway for Secretion of Invasion Factors in Toxoplasma. PLoS Pathog 10. https://doi.org/10.1371/journal.ppat.1004074

Kayhoe, D.E., Jacobs, L., Beye, H.K., McCullough, N.B., 1957. Acquired Toxoplasmosis. New England Journal of Medicine 257, 1247–1254. https://doi.org/10.1056/NEJM195712262572601

Keeley, A., Soldati, D., 2004. The glideosome: a molecular machine powering motility and host-cell invasion by Apicomplexa. Trends Cell Biol. 14, 528–532. https://doi.org/10.1016/j.tcb.2004.08.002

Kelly, E., Horgan, C., Mccaffrey, M., 2012. Rab11 proteins in health and disease. Biochemical Society transactions 40, 1360–7. https://doi.org/10.1042/BST20120157

Kessler, D., Gruen, G.-C., Heider, D., Morgner, J., Reis, H., Schmid, K.W., Jendrossek, V., 2012. The Action of Small GTPases Rab11 and Rab25 in Vesicle Trafficking During Cell Migration. CPB 29, 647–656. https://doi.org/10.1159/000295249

Kessler, H., Herm-Götz, A., Hegge, S., Rauch, M., Soldati-Favre, D., Frischknecht, F., Meissner, M., 2008. Microneme protein 8--a new essential invasion factor in Toxoplasma gondii. J. Cell. Sci. 121, 947–956. https://doi.org/10.1242/jcs.022350

Khan, A., Fux, B., Su, C., Dubey, J.P., Darde, M.L., Ajioka, J.W., Rosenthal, B.M., Sibley, L.D., 2007. Recent transcontinental sweep of Toxoplasma gondii driven by a single monomorphic chromosome. Proc Natl Acad Sci U S A 104, 14872–14877. https://doi.org/10.1073/pnas.0702356104

Khan, A., Taylor, S., Ajioka, J.W., Rosenthal, B.M., Sibley, L.D., 2009. Selection at a Single Locus Leads to Widespread Expansion of Toxoplasma gondii Lineages That Are Virulent in Mice. PLoS Genet 5. https://doi.org/10.1371/journal.pgen.1000404

Khan, A., Taylor, S., Su, C., Mackey, A.J., Boyle, J., Cole, R., Glover, D., Tang, K., Paulsen, I.T., Berriman, M., Boothroyd, J.C., Pfefferkorn, E.R., Dubey, J.P., Ajioka, J.W., Roos, D.S., Wootton, J.C., Sibley, L.D., 2005. Composite genome map and recombination parameters derived from three archetypal lineages of Toxoplasma gondii. Nucleic Acids Res 33, 2980–2992. https://doi.org/10.1093/nar/gki604

Kim, J.Y., Ahn, H.-J., Ryu, K.J., Nam, H.-W., 2008. Interaction between Parasitophorous Vacuolar Membrane-associated GRA3 and Calcium Modulating Ligand of Host Cell Endoplasmic Reticulum in the Parasitism of Toxoplasma gondii. Korean J Parasitol 46, 209–216. https://doi.org/10.3347/kjp.2008.46.4.209

Kim, K., Weiss, L.M., 2004. Toxoplasma gondii: the model apicomplexan. Int J Parasitol 34, 423–432. https://doi.org/10.1016/j.ijpara.2003.12.009

Klinger, C.M., Klute, M.J., Dacks, J.B., 2013. Comparative Genomic Analysis of Multi-Subunit Tethering Complexes Demonstrates an Ancient Pan-Eukaryotic Complement and Sculpting in Apicomplexa. PLOS ONE 8, e76278. https://doi.org/10.1371/journal.pone.0076278

Knödler, A., Feng, S., Zhang, J., Zhang, X., Das, A., Peränen, J., Guo, W., 2010. Coordination of Rab8 and Rab11 in primary ciliogenesis. Proceedings of the National Academy of Sciences of the United States of America 107, 6346–51. https://doi.org/10.1073/pnas.1002401107

Kobayashi, H., Fukuda, M., 2013. Rab35 establishes the EHD1-association site by coordinating two distinct effectors during neurite outgrowth. Journal of cell science 126. https://doi.org/10.1242/jcs.117846

Kouranti, I., Sachse, M., Arouche, N., Goud, B., Echard, A., 2006. Rab35 Regulates an Endocytic Recycling Pathway Essential for the Terminal Steps of Cytokinesis. Current biology : CB 16, 1719–25. https://doi.org/10.1016/j.cub.2006.07.020

Krämer, H., Phistry, M., 1999. Genetic analysis of hook, a gene required for endocytic trafficking in drosophila. Genetics 151, 675–684.

Krämer, H., Phistry, M., 1996. Mutations in the Drosophila hook gene inhibit endocytosis of the boss transmembrane ligand into multivesicular bodies. J Cell Biol 133, 1205–1215. https://doi.org/10.1083/jcb.133.6.1205

Kremer, K., Kamin, D., Rittweger, E., Wilkes, J., Flammer, H., Mahler, S., Heng, J., Tonkin, C.J., Langsley, G., Hell, S.W., Carruthers, V.B., Ferguson, D.J.P., Meissner, M., 2013. An Overexpression Screen of Toxoplasma gondii Rab-GTPases Reveals Distinct Transport Routes to the Micronemes. PLOS Pathogens 9, e1003213. https://doi.org/10.1371/journal.ppat.1003213

Kvaal, C.A., Radke, J.R., Guerini, M.N., White, M.W., 2002. Isolation of a Toxoplasma gondii cyclin by yeast two-hybrid interactive screen. Mol. Biochem. Parasitol. 120, 187–194. https://doi.org/10.1016/s0166-6851(01)00454-6

Labruyere, E., Lingnau, M., Mercier, C., Sibley, L.D., 1999. Differential membrane targeting of the secretory proteins GRA4 and GRA6 within the parasitophorous vacuole formed by Toxoplasma gondii. Mol Biochem Parasitol 102, 311–324. https://doi.org/10.1016/s0166-6851(99)00092-4

Lagal, V., Binder, E.M., Huynh, M.-H., Kafsack, B.FC., Harris, P.K., Diez, R., Chen, D., Cole, R.N., Carruthers, V.B., Kim, K., 2010. Toxoplasma gondii Protease TgSUB1 is Required for Cell Surface Processing of Micronemal Adhesive Complexes and Efficient Adhesion of Tachyzoites. Cell Microbiol 12, 1792–1808. https://doi.org/10.1111/j.1462-5822.2010.01509.x

Lai, F., Stubbs, L., Artzt, K., 1994. Molecular Analysis of Mouse Rab11b: A New Type of Mammalian YPT/Rab Protein. Genomics 22, 610–616. https://doi.org/10.1006/geno.1994.1434

Laliberte, J., Carruthers, V., 2008. Host cell manipulation by the human pathogen Toxoplasma gondii. Cellular and molecular life sciences : CMLS 65, 1900–15. https://doi.org/10.1007/s00018-008-7556-x

Lamarque, M., Besteiro, S., Papoin, J., Roques, M., Vulliez-Le Normand, B., Morlon-Guyot, J., Dubremetz, J.-F., Fauquenoy, S., Tomavo, S., Faber, B.W., Kocken, C.H., Thomas, A.W., Boulanger, M.J., Bentley, G.A., Lebrun, M., 2011. The RON2-AMA1 Interaction is a Critical Step in Moving Junction-Dependent Invasion by Apicomplexan Parasites. PLoS Pathog 7. https://doi.org/10.1371/journal.ppat.1001276

Lamarque, M.H., Roques, M., Kong-Hap, M., Tonkin, M.L., Rugarabamu, G., Marq, J.-B., Penarete-Vargas, D.M., Boulanger, M.J., Soldati-Favre, D., Lebrun, M., 2014. Plasticity and redundancy among AMA-RON pairs ensure host cell entry of Toxoplasma parasites. Nat Commun 5, 4098. https://doi.org/10.1038/ncomms5098

Lapierre, L., Kumar, R., Hales, C., Navarre, J., Bhartur, S., Burnette, J., Provance, J., D., Mercer, J., Bähler, M., Goldenring, J., 2001. Myosin Vb Is Associated with Plasma Membrane Recycling Systems. Molecular Biology of the Cell 12. https://doi.org/10.1091/mbc.12.6.1843

Lebrun, M., Michelin, A., El Hajj, H., Poncet, J., Bradley, P.J., Vial, H., Dubremetz, J.F., 2005. The rhoptry neck protein RON4 re-localizes at the moving junction during Toxoplasma gondii invasion. Cell. Microbiol. 7, 1823–1833. https://doi.org/10.1111/j.1462-5822.2005.00646.x

Lecordier, L., Mercier, C., Sibley, L.D., Cesbron-Delauw, M.-F., 1999. Transmembrane Insertion of the Toxoplasma gondii GRA5 Protein Occurs after Soluble Secretion into the Host Cell. Mol Biol Cell 10, 1277–1287.

Lee, S.-B., Lee, T.-G., 2017. Toxoplasmic Encephalitis in Patient with Acquired Immunodeficiency Syndrome. Brain Tumor Res Treat 5, 34–36. https://doi.org/10.14791/btrt.2017.5.1.34

Lekutis, C., Ferguson, D.J., Grigg, M.E., Camps, M., Boothroyd, J.C., 2001. Surface antigens of Toxoplasma gondii: variations on a theme. Int. J. Parasitol. 31, 1285–1292. https://doi.org/10.1016/s0020-7519(01)00261-2

Lemgruber, L., De Souza, W., Vommaro, R.C., 2008. Freeze-fracture study of the dynamics of Toxoplasma gondii parasitophorous vacuole development. Micron 39, 177–183. https://doi.org/10.1016/j.micron.2007.01.002

Lemgruber, L., Lupetti, P., Martins-Duarte, E.S., De Souza, W., Vommaro, R.C., 2011. The organization of the wall filaments and characterization of the matrix structures of Toxoplasma gondii cyst form. Cell. Microbiol. 13, 1920–1932. https://doi.org/10.1111/j.1462-5822.2011.01681.x

Lentini, G., Dubois, D.J., Maco, B., Soldati-Favre, D., Frénal, K., 2019. The roles of Centrin 2 and Dynein Light Chain 8a in apical secretory organelles discharge of Toxoplasma gondii. Traffic 20, 583–600. https://doi.org/10.1111/tra.12673

Lentini, G., Kong-Hap, M., El Hajj, H., Francia, M., Claudet, C., Striepen, B., Dubremetz, J.-F., Lebrun, M., 2015. Identification and characterization of Toxoplasma SIP, a conserved apicomplexan cytoskeleton protein involved in maintaining the shape, motility and virulence of the parasite. Cell Microbiol 17, 62–78. https://doi.org/10.1111/cmi.12337

Lesche, R., Peetz, A., van der Hoeven, F., Rüther, U., 1997. Ft1, a novel gene related to ubiquitin-conjugating enzymes, is deleted in the Fused toes mouse mutation. Mamm Genome 8, 879–883. https://doi.org/10.1007/s003359900604

Leung, J.M., He, Y., Zhang, F., Hwang, Y.-C., Nagayasu, E., Liu, J., Murray, J.M., Hu, K., 2017. Stability and function of a putative microtubule-organizing center in the human parasite Toxoplasma gondii. Mol Biol Cell 28, 1361–1378. https://doi.org/10.1091/mbc.E17-01-0045

Leung, J.M., Rould, M.A., Konradt, C., Hunter, C.A., Ward, G.E., 2014. Disruption of TgPHIL1 Alters Specific Parameters of Toxoplasma gondii Motility Measured in a Quantitative, Three-Dimensional Live Motility Assay. PLoS One 9. https://doi.org/10.1371/journal.pone.0085763

Li, B.X., Satoh, A.K., Ready, D.F., 2007. Myosin V, Rab11, and dRip11 direct apical secretion and cellular morphogenesis in developing Drosophila photoreceptors. The Journal of Cell Biology 177, 659. https://doi.org/10.1083/jcb.200610157

Li, X., Sapp, E., Chase, K., Comer-Tierney, L., Masso, N., Alexander, J., Reeves, P., Kegel, K., Valencia, A., Esteves, M., Aronin, N., Difiglia, M., 2009. Disruption of Rab11 activity in a knock-in mouse model of Huntington's Disease. Neurobiology of disease 36, 374–83. https://doi.org/10.1016/j.nbd.2009.08.003

Liendo, A., Stedman, T.T., Ngo, H.M., Chaturvedi, S., Hoppe, H.C., Joiner, K.A., 2001. Toxoplasma gondii ADP-ribosylation factor 1 mediates enhanced release of constitutively secreted dense granule proteins. J Biol Chem 276, 18272–18281. https://doi.org/10.1074/jbc.M008352200

Liesenfeld, O., Kosek, J., Remington, J., Suzuki, Y., 1996. Association of CD4 + T Cell-dependent, Interferon-γ-med iated Necrosis of the Small Intestine with Genetic Susceptibility of Mice to Peroral Infection with Toxoplasma gondii. The Journal of experimental medicine 184, 597–607.

Ligeti, E., Welti, S., Scheffzek, K., 2012. Inhibition and Termination of Physiological Responses by GTPase Activating Proteins. Physiological Reviews 92, 237–272. https://doi.org/10.1152/physrev.00045.2010

Lim, L., McFadden, G.I., 2010. The evolution, metabolism and functions of the apicoplast. Philos Trans R Soc Lond B Biol Sci 365, 749–763. https://doi.org/10.1098/rstb.2009.0273

Lipinski, A.R., Heymann, J., Meissner, C., Karlas, A., Brinkmann, V., Meyer, T.F., Heuer, D., 2009. Rab6 and Rab11 Regulate Chlamydia trachomatis Development and Golgin-84-Dependent Golgi Fragmentation. PLOS Pathogens 5, e1000615. https://doi.org/10.1371/journal.ppat.1000615

Liu, Q., Wang, Z.-D., Huang, S.-Y., Zhu, X.-Q., 2015. Diagnosis of toxoplasmosis and typing of Toxoplasma gondii. Parasit Vectors 8. https://doi.org/10.1186/s13071-015-0902-6

Liu, S.-H., Wong, M.L., Craik, C.S., Brodsky, F.M., 1995. Regulation of clathrin assembly and trimerization defined using recombinant triskelion hubs. Cell 83, 257–267. https://doi.org/10.1016/0092-8674(95)90167-1

Lourido, S., Shuman, J., Zhang, C., Shokat, K.M., Hui, R., Sibley, L.D., 2010. Calcium-dependent protein kinase 1 is an essential regulator of exocytosis in Toxoplasma. Nature 465, 359–362. https://doi.org/10.1038/nature09022

Lourido, S., Tang, K., Sibley, L.D., 2012. Distinct signalling pathways control Toxoplasma egress and host-cell invasion. EMBO J 31, 4524–4534. https://doi.org/10.1038/emboj.2012.299

Lovett, J.L., Marchesini, N., Moreno, S.N.J., Sibley, L.D., 2002. Toxoplasma gondii Microneme Secretion Involves Intracellular Ca2+ Release from Inositol 1,4,5-Triphosphate (IP3)/Ryanodine-sensitive Stores. J. Biol. Chem. 277, 25870–25876. https://doi.org/10.1074/jbc.M202553200

Lüder, C.G.K., Rahman, T., 2017. Impact of the host on Toxoplasma stage differentiation. Microb Cell 4, 203–211. https://doi.org/10.15698/mic2017.07.579

Luft, B.J., Hafner, R., Korzun, A.H., Leport, C., Antoniskis, D., Bosler, E.M., Bourland, D.D., Uttamchandani, R., Fuhrer, J., Jacobson, J., Morlat, P., Vilde, J.-L., Remington, J.S., Team, the A. 077p/ANRS 009 S., 2010. Toxoplasmic Encephalitis in Patients with the Acquired Immunodeficiency Syndrome [WWW Document]. http://dx.doi.org/10.1056/NEJM199309303291403. https://doi.org/10.1056/NEJM199309303291403

Luiro, K., Yliannala, K., Ahtiainen, L., Maunu, H., Järvelä, I., Kyttälä, A., Jalanko, A., 2004. Interconnections of CLN3, Hook1 and Rab proteins link Batten disease to defects in the endocytic pathway. Human Molecular Genetics 13, 3017–3027. https://doi.org/10.1093/hmg/ddh321

Lyons, R.E., McLeod, R., Roberts, C.W., 2002. Toxoplasma gondii tachyzoite-bradyzoite interconversion. Trends Parasitol. 18, 198–201. https://doi.org/10.1016/s1471-4922(02)02248-1

Ma, J.S., Sasai, M., Ohshima, J., Lee, Y., Bando, H., Takeda, K., Yamamoto, M., 2014. Selective and strain-specific NFAT4 activation by the Toxoplasma gondii polymorphic dense granule protein GRA6. J Exp Med 211, 2013–2032. https://doi.org/10.1084/jem.20131272

Magno, R.C., Lemgruber, L., Vommaro, R.C., De Souza, W., Attias, M., 2005a. Intravacuolar network may act as a mechanical support for Toxoplasma gondii inside the parasitophorous vacuole. Microsc. Res. Tech. 67, 45–52. https://doi.org/10.1002/jemt.20182

Magno, R.C., Straker, L.C., de Souza, W., Attias, M., 2005b. Interrelations between the parasitophorous vacuole of Toxoplasma gondii and host cell organelles. Microsc Microanal 11, 166–174. https://doi.org/10.1017/S1431927605050129

Maldonado-Báez, L., Cole, N.B., Krämer, H., Donaldson, J.G., 2013. Microtubule-dependent endosomal sorting of clathrin-independent cargo by Hook1. J Cell Biol 201, 233–247. https://doi.org/10.1083/jcb.201208172

Maldonado-Báez, L., Donaldson, J.G., 2013. Hook1, microtubules, and Rab22. Bioarchitecture 3, 141–146. https://doi.org/10.4161/bioa.26638

Manger, I.D., Hehl, A.B., Boothroyd, J.C., 1998. The Surface of Toxoplasma Tachyzoites Is Dominated by a Family of Glycosylphosphatidylinositol-Anchored Antigens Related to SAG1. Infect Immun 66, 2237–2244.

Mann, T., Beckers, C., 2001. Characterization of the subpellicular network, a filamentous membrane skeletal component in the parasite Toxoplasma gondii. Molecular and Biochemical Parasitology 115, 257–268. https://doi.org/10.1016/S0166-6851(01)00289-4

Marino, N.D., Panas, M.W., Franco, M., Theisen, T.C., Naor, A., Rastogi, S., Buchholz, K.R., Lorenzi, H.A., Boothroyd, J.C., 2018. Identification of a novel protein complex essential for effector translocation across the parasitophorous vacuole membrane of Toxoplasma gondii. PLoS Pathog 14. https://doi.org/10.1371/journal.ppat.1006828

Masatani, T., Matsuo, T., Tanaka, T., Terkawi, M.A., Lee, E.-G., Goo, Y.-K., Aboge, G.O., Yamagishi, J., Hayashi, K., Kameyama, K., Cao, S., Nishikawa, Y., Xuan, X., 2013. TgGRA23, a novel Toxoplasma gondii dense granule protein associated with the parasitophorous vacuole membrane and intravacuolar network. Parasitol. Int. 62, 372–379. https://doi.org/10.1016/j.parint.2013.04.003

Matesic, L., Yip, M.L.R., Reuss, A., Swing, D., O'Sullivan, T., Fletcher, C., Copeland, N., Jenkins, N., 2001. Mutations in Mlph, encoding a member of the Rab effector family, cause the melanosome transport defects observed in leaden mice. Proceedings of the National Academy of Sciences of the United States of America 98, 10238–43. https://doi.org/10.1073/pnas.181336698

Mattera, R., Williamson, C.D., Ren, X., Bonifacino, J.S., 2020. The FTS-Hook-FHIP (FHF) complex interacts with AP-4 to mediate perinuclear distribution of AP-4 and its cargo ATG9A. MBoC 31, 963–979. https://doi.org/10.1091/mbc.E19-11-0658

McAuley, J.B., 2014. Congenital Toxoplasmosis. J Pediatric Infect Dis Soc 3, S30–S35. https://doi.org/10.1093/jpids/piu077

McBride, H.M., Rybin, V., Murphy, C., Giner, A., Teasdale, R., Zerial, M., 1999. Oligomeric Complexes Link Rab5 Effectors with NSF and Drive Membrane Fusion via Interactions between EEA1 and Syntaxin 13. Cell 98, 377–386. https://doi.org/10.1016/S0092-8674(00)81966-2

McCoy, J.M., Whitehead, L., Dooren, G.G. van, Tonkin, C.J., 2012. TgCDPK3 Regulates Calcium-Dependent Egress of Toxoplasma gondii from Host Cells. PLOS Pathogens 8, e1003066. https://doi.org/10.1371/journal.ppat.1003066

McFadden, G.I., Waller, R.F., 1997. Plastids in parasites of humans. Bioessays 19, 1033–1040. https://doi.org/10.1002/bies.950191114

McLeod, R., Boyer, K., Karrison, T., Kasza, K., Swisher, C., Roizen, N., Jalbrzikowski, J., Remington, J., Heydemann, P., Noble, A.G., Mets, M., Holfels, E., Withers, S., Latkany, P., Meier, P., Toxoplasmosis Study Group, 2006. Outcome of treatment for congenital toxoplasmosis, 1981-2004: the National Collaborative Chicago-Based, Congenital Toxoplasmosis Study. Clin. Infect. Dis. 42, 1383–1394. https://doi.org/10.1086/501360

McMahon, H.T., Boucrot, E., 2011. Molecular mechanism and physiological functions of clathrin-mediated endocytosis. Nature Reviews Molecular Cell Biology 12, 517–533. https://doi.org/10.1038/nrm3151

McNamara, C.W., Lee, M.C.S., Lim, C.S., Lim, S.H., Roland, J., Nagle, A., Simon, O., Yeung, B.K.S., Chatterjee, A.K., McCormack, S.L., Manary, M.J., Zeeman, A.-M., Dechering, K.J., Kumar, T.R.S., Henrich, P.P., Gagaring, K., Ibanez, M., Kato, N., Kuhen, K.L., Fischli,

C., Rottmann, M., Plouffe, D.M., Bursulaya, B., Meister, S., Rameh, L., Trappe, J., Haasen, D., Timmerman, M., Sauerwein, R.W., Suwanarusk, R., Russell, B., Renia, L., Nosten, F., Tully, D.C., Kocken, C.H.M., Glynne, R.J., Bodenreider, C., Fidock, D.A., Diagana, T.T., Winzeler, E.A., 2013. Targeting Plasmodium PI(4)K to eliminate malaria. Nature 504, 248–253. https://doi.org/10.1038/nature12782

Mehta, S., Sibley, L.D., 2010. Toxoplasma gondii Actin Depolymerizing Factor Acts Primarily to Sequester G-actin. J Biol Chem 285, 6835–6847. https://doi.org/10.1074/jbc.M109.068155

Meissner, M., Reiss, M., Viebig, N., Carruthers, V.B., Toursel, C., Tomavo, S., Ajioka, J.W., Soldati, D., 2002. A family of transmembrane microneme proteins of Toxoplasma gondii contain EGF-like domains and function as escorters. J. Cell. Sci. 115, 563–574.

Mellman, I., Yarden, Y., 2013. Endocytosis and Cancer. Cold Spring Harb Perspect Biol 5, a016949. https://doi.org/10.1101/cshperspect.a016949

Memon, A.R., 2004. The role of ADP-ribosylation factor and SAR1 in vesicular trafficking in plants. Biochim Biophys Acta 1664, 9–30. https://doi.org/10.1016/j.bbamem.2004.04.005

Ménard, R., 2001. Gliding motility and cell invasion by Apicomplexa: insights from the Plasmodium sporozoite. Cell. Microbiol. 3, 63–73. https://doi.org/10.1046/j.1462-5822.2001.00097.x

Mendoza-Lujambio, I., Burfeind, P., Dixkens, C., Meinhardt, A., Hoyer-Fender, S., Engel, W., Neesen, J., 2002. The Hook1 gene is non-functional in the abnormal spermatozoon head shape ( azh ) mutant mouse. Human Molecular Genetics 11, 1647–1658. https://doi.org/10.1093/hmg/11.14.1647

Mercier, C., Adjogble, K.D.Z., Däubener, W., Delauw, M.-F.-C., 2005. Dense granules: are they key organelles to help understand the parasitophorous vacuole of all apicomplexa parasites? Int. J. Parasitol. 35, 829–849. https://doi.org/10.1016/j.ijpara.2005.03.011

Mercier, C., Cesbron-Delauw, M.-F., 2015. Toxoplasma secretory granules: one population or more? Trends Parasitol. 31, 60–71. https://doi.org/10.1016/j.pt.2014.12.002

Mercier, C., Dubremetz, J.-F., Rauscher, B., Lecordier, L., Sibley, L.D., Cesbron-Delauw, M.-F., 2002. Biogenesis of Nanotubular Network in Toxoplasma Parasitophorous Vacuole Induced by Parasite Proteins. Mol Biol Cell 13, 2397–2409. https://doi.org/10.1091/mbc.E02-01-0021

Miller, C.M., Boulter, N.R., Ikin, R.J., Smith, N.C., 2009. The immunobiology of the innate response to Toxoplasma gondii. International Journal for Parasitology 39, 23–39. https://doi.org/10.1016/j.ijpara.2008.08.002

Mills, I.G., Jones, A.T., Clague, M.J., 1998. Involvement of the endosomal autoantigen EEA1 in homotypic fusion of early endosomes. Curr Biol 8, 881–884. https://doi.org/10.1016/s0960-9822(07)00351-x

Mineo, J.R., Kasper, L.H., 1994. Attachment of Toxoplasma gondii to host cells involves major surface protein, SAG-1 (P30). Exp. Parasitol. 79, 11–20. https://doi.org/10.1006/expr.1994.1054

Miranda, K., Pace, D., Cintron, R., Rodrigues, J., Fang, J., Smith, A., Rohloff, P., Coelho, E., de Haas, F., Souza, W., Coppens, I., Sibley, L.D., Moreno, S., 2010. Characterization of a novel organelle in Toxoplasma gondii with similar composition and function to the plant vacuole. Molecular microbiology 76, 1358–75. https://doi.org/10.1111/j.1365-2958.2010.07165.x

Mital, J., Meissner, M., Soldati, D., Ward, G.E., 2005. Conditional Expression of Toxoplasma gondii Apical Membrane Antigen-1 (TgAMA1) Demonstrates That TgAMA1 Plays a Critical Role in Host Cell Invasion. Mol Biol Cell 16, 4341–4349. https://doi.org/10.1091/mbc.E05-04-0281

Monck, J.R., Fernandez, J.M., 1994. The exocytotic fusion pore and neurotransmitter release. Neuron 12, 707–716. https://doi.org/10.1016/0896-6273(94)90325-5

Monteiro, V.G., de Melo, E.J., Attias, M., de Souza, W., 2001. Morphological changes during conoid extrusion in Toxoplasma gondii tachyzoites treated with calcium ionophore. J. Struct. Biol. 136, 181–189. https://doi.org/10.1006/jsbi.2002.4444

Montoya, J.G., Liesenfeld, O., 2004. Toxoplasmosis. The Lancet 363, 1965–1976. https://doi.org/10.1016/S0140-6736(04)16412-X

Moore, H.P., Kelly, R.B., 1985. Secretory protein targeting in a pituitary cell line: differential transport of foreign secretory proteins to distinct secretory pathways. Journal of Cell Biology 101, 1773–1781. https://doi.org/10.1083/jcb.101.5.1773

Moore, H.-P.H., 1987. Factors Controlling Packaging of Peptide Hormones into Secretory Granulesa. Annals of the New York Academy of Sciences 493, 50–61. https://doi.org/10.1111/j.1749-6632.1987.tb27180.x

Mordue, D.G., Håkansson, S., Niesman, I., Sibley, L.D., 1999. Toxoplasma gondii resides in a vacuole that avoids fusion with host cell endocytic and exocytic vesicular trafficking pathways. Exp. Parasitol. 92, 87–99. https://doi.org/10.1006/expr.1999.4412

Moreno, S.N., Zhong, L., 1996. Acidocalcisomes in Toxoplasma gondii tachyzoites. Biochem J 313, 655–659.

Morlon-Guyot, J., Berry, L., Chen, C.-T., Gubbels, M.-J., Lebrun, M., Daher, W., 2014. The Toxoplasma gondii Calcium Dependent Protein Kinase 7 is involved in early steps of parasite division and is crucial for parasite survival. Cell Microbiol 16, 95–114. https://doi.org/10.1111/cmi.12186

Morlon-Guyot J, S, P., Berry, L., M, L., W., D., 2015. Toxoplasma gondii Vps11, a subunit of HOPS and CORVET tethering complexes, is essential for the biogenesis of secretory organelles. Cellular Microbiology.

Morrissette, N., 2015. Targeting Toxoplasma Tubules: Tubulin, Microtubules, and Associated Proteins in a Human Pathogen. Eukaryot Cell 14, 2–12. https://doi.org/10.1128/EC.00225-14

Morrissette, N.S., Murray, J.M., Roos, D.S., 1997. Subpellicular microtubules associate with an intramembranous particle lattice in the protozoan parasite Toxoplasma gondii. Journal of Cell Science 110, 35–42.

Morrissette, N.S., Sibley, L.D., 2002a. Cytoskeleton of Apicomplexan Parasites. Microbiol Mol Biol Rev 66, 21–38. https://doi.org/10.1128/MMBR.66.1.21-38.2002

Morrissette, N.S., Sibley, L.D., 2002b. Disruption of microtubules uncouples budding and nuclear division in Toxoplasma gondii. Journal of Cell Science 115, 1017–1025.

Moudy, R., Manning, T.J., Beckers, C.J., 2001. The Loss of Cytoplasmic Potassium upon Host Cell Breakdown Triggers Egress of Toxoplasma gondii. J. Biol. Chem. 276, 41492–41501. https://doi.org/10.1074/jbc.M106154200

Moynihan, K.L., Pooley, R., Miller, P.M., Kaverina, I., Bader, D.M., 2009. Murine CENP-F Regulates Centrosomal Microtubule Nucleation and Interacts with Hook2 at the Centrosome. Mol Biol Cell 20, 4790–4803. https://doi.org/10.1091/mbc.E09-07-0560

Mueller, C., Klages, N., Jacot, D., Santos, J.M., Cabrera, A., Gilberger, T.W., Dubremetz, J.-F., Soldati-Favre, D., 2013. The Toxoplasma protein ARO mediates the apical positioning

of rhoptry organelles, a prerequisite for host cell invasion. Cell Host Microbe 13, 289–301. https://doi.org/10.1016/j.chom.2013.02.001

Muñiz-Hernández, S., González del Carmen, M., Mondragón, M., Mercier, C., Cesbron, M.F., Mondragón-González, S.L., González, S., Mondragón, R., 2011. Contribution of the Residual Body in the Spatial Organization of Toxoplasma gondii Tachyzoites within the Parasitophorous Vacuole [WWW Document]. Journal of Biomedicine and Biotechnology. https://doi.org/10.1155/2011/473983

Nadipuram, S.M., Kim, E.W., Vashisht, A.A., Lin, A.H., Bell, H.N., Coppens, I., Wohlschlegel, J.A., Bradley, P.J., 2016. In Vivo Biotinylation of the Toxoplasma Parasitophorous Vacuole Reveals Novel Dense Granule Proteins Important for Parasite Growth and Pathogenesis. mBio 7. https://doi.org/10.1128/mBio.00808-16

Nagamune, K., Hicks, L.M., Fux, B., Brossier, F., Chini, E.N., Sibley, L.D., 2008. Abscisic acid controls calcium-dependent egress and development in Toxoplasma gondii. Nature 451, 207–210. https://doi.org/10.1038/nature06478

Nagel, S.D., Boothroyd, J.C., 1989. The major surface antigen, P30, of Toxoplasma gondii is anchored by a glycolipid. J. Biol. Chem. 264, 5569–5574.

Nair, S.C., Brooks, C.F., Goodman, C.D., Strurm, A., McFadden, G.I., Sundriyal, S., Anglin, J.L., Song, Y., Moreno, S.N.J., Striepen, B., 2011. Apicoplast isoprenoid precursor synthesis and the molecular basis of fosmidomycin resistance in Toxoplasma gondii. J Exp Med 208, 1547–1559. https://doi.org/10.1084/jem.20110039

Naor, A., Panas, M.W., Marino, N., Coffey, M.J., Tonkin, C.J., Boothroyd, J.C., 2018. MYR1-Dependent Effectors Are the Major Drivers of a Host Cell's Early Response to Toxoplasma, Including Counteracting MYR1-Independent Effects. mBio 9. https://doi.org/10.1128/mBio.02401-17

Nelson, D.S., Alvarez, C., Gao, Y., García-Mata, R., Fialkowski, E., Sztul, E., 1998. The Membrane Transport Factor TAP/p115 Cycles between the Golgi and Earlier Secretory Compartments and Contains Distinct Domains Required for Its Localization and Function. J Cell Biol 143, 319–331.

Nevin, W., Dacks, J., 2009. Repeated secondary loss of adaptin complex genes in the Apicomplexa. Parasitology international 58, 86–94. https://doi.org/10.1016/j.parint.2008.12.002

Ngô, H.M., Yang, M., Joiner, K.A., 2004. Are rhoptries in Apicomplexan parasites secretory granules or secretory lysosomal granules? Molecular Microbiology 52, 1531–1541. https://doi.org/10.1111/j.1365-2958.2004.04056.x

Ngô, H.M., Yang, M., Paprotka, K., Pypaert, M., Hoppe, H., Joiner, K.A., 2003. AP-1 in Toxoplasma gondii mediates biogenesis of the rhoptry secretory organelle from a post-Golgi compartment. J Biol Chem 278, 5343–5352. https://doi.org/10.1074/jbc.M208291200

Nichols, B.A., Chiappino, M.L., 1987. Cytoskeleton of Toxoplasma gondii. J. Protozool. 34, 217–226. https://doi.org/10.1111/j.1550-7408.1987.tb03162.x

Nichols, B.A., Chiappino, M.L., Pavesio, C.E., 1994. Endocytosis at the micropore of Toxoplasma gondii. Parasitol. Res. 80, 91–98. https://doi.org/10.1007/bf00933773

Nickerson, D.P., Brett, C.L., Merz, A.J., 2009. Vps-C complexes: gatekeepers of endolysosomal traffic. Current Opinion in Cell Biology 21, 543–551. https://doi.org/10.1016/j.ceb.2009.05.007

Nielsen, E., Christoforidis, S., Uttenweiler-Joseph, S., Miaczynska, M., Dewitte, F., Wilm, M., Hoflack, B., Zerial, M., 2000. Rabenosyn-5, a Novel Rab5 Effector, Is Complexed with

hVPS45 and Recruited to Endosomes through a FYVE Finger Domain. The Journal of cell biology 151, 601–12. https://doi.org/10.1083/jcb.151.3.601

Nishi, M., Hu, K., Murray, J.M., Roos, D.S., 2008. Organellar dynamics during the cell cycle of Toxoplasma gondii. J Cell Sci 121, 1559–1568. https://doi.org/10.1242/jcs.021089

Olenick, M.A., Dominguez, R., Holzbaur, E.L.F., 2018. Dynein activator Hook1 is required for trafficking of BDNF-signaling endosomes in neurons. Journal of Cell Biology 218, 220–233. https://doi.org/10.1083/jcb.201805016

Olenick, M.A., Tokito, M., Boczkowska, M., Dominguez, R., Holzbaur, E.L.F., 2016. Hook Adaptors Induce Unidirectional Processive Motility by Enhancing the Dynein-Dynactin Interaction. J Biol Chem 291, 18239–18251. https://doi.org/10.1074/jbc.M116.738211

Ong, Y.-C., Reese, M.L., Boothroyd, J.C., 2010. Toxoplasma Rhoptry Protein 16 (ROP16) Subverts Host Function by Direct Tyrosine Phosphorylation of STAT6. J Biol Chem 285, 28731–28740. https://doi.org/10.1074/jbc.M110.112359

Opitz, C., Soldati, D., 2002. "The glideosome": a dynamic complex powering gliding motion and host cell invasion by Toxoplasma gondii. Mol. Microbiol. 45, 597–604. https://doi.org/10.1046/j.1365-2958.2002.03056.x

Opsteegh, M., Kortbeek, T.M., Havelaar, A.H., van der Giessen, J.W.B., 2015. Intervention strategies to reduce human Toxoplasma gondii disease burden. Clin. Infect. Dis. 60, 101–107. https://doi.org/10.1093/cid/ciu721

Ostrowicz, C., Bröcker, C., Ahnert-Michel, F., Nordmann, M., Lachmann, J., Peplowska, K., Perz, A., Auffarth, K., Engelbrecht-Vandré, S., Ungermann, C., 2010. Defined Subunit Arrangement and Rab Interactions Are Required for Functionality of the HOPS Tethering Complex. Traffic (Copenhagen, Denmark) 11, 1334–46. https://doi.org/10.1111/j.1600-0854.2010.01097.x

O'Sullivan, M.J., Lindsay, A.J., 2020. The Endosomal Recycling Pathway—At the Crossroads of the Cell. International Journal of Molecular Sciences 21, 6074. https://doi.org/10.3390/ijms21176074

Ouologuem, D.T., Roos, D.S., 2014. Dynamics of the Toxoplasma gondii inner membrane complex. J Cell Sci 127, 3320–3330. https://doi.org/10.1242/jcs.147736

Panas, M.W., Naor, A., Cygan, A.M., Boothroyd, J.C., 2019. Toxoplasma Controls Host Cyclin E Expression through the Use of a Novel MYR1-Dependent Effector Protein, HCE1. mBio 10. https://doi.org/10.1128/mBio.00674-19

Paredes-Santos, T.C., de Souza, W., Attias, M., 2012. Dynamics and 3D organization of secretory organelles of Toxoplasma gondii. J Struct Biol 177, 420–430. https://doi.org/10.1016/j.jsb.2011.11.028

Peixoto, L., Chen, F., Harb, O.S., Davis, P.H., Beiting, D.P., Brownback, C.S., Ouologuem, D., Roos, D.S., 2010. Integrative Genomic Approaches Highlight a Family of Parasite-Specific Kinases that Regulate Host Responses. Cell Host Microbe 8, 208–218. https://doi.org/10.1016/j.chom.2010.07.004

Pelham, H.R., 1988. Evidence that luminal ER proteins are sorted from secreted proteins in a post-ER compartment. EMBO J 7, 913–918.

Pelletier, L., Stern, C.A., Pypaert, M., Sheff, D., Ngô, H.M., Roper, N., He, C.Y., Hu, K., Toomre, D., Coppens, I., Roos, D.S., Joiner, K.A., Warren, G., 2002. Golgi biogenesis in Toxoplasma gondii. Nature 418, 548–552. https://doi.org/10.1038/nature00946

Peplowska, K., Markgraf, D.F., Ostrowicz, C.W., Bange, G., Ungermann, C., 2007. The CORVET Tethering Complex Interacts with the Yeast Rab5 Homolog Vps21 and Is Involved in

Endo-Lysosomal Biogenesis. Developmental Cell 12, 739–750. https://doi.org/10.1016/j.devcel.2007.03.006

Periz, J., Del Rosario, M., McStea, A., Gras, S., Loney, C., Wang, L., Martin-Fernandez, M.L., Meissner, M., 2019. A highly dynamic F-actin network regulates transport and recycling of micronemes in Toxoplasma gondii vacuoles. Nature Communications 10, 4183. https://doi.org/10.1038/s41467-019-12136-2

Periz, J., Whitelaw, J., Harding, C., Gras, S., Del Rosario Minina, M.I., Latorre-Barragan, F., Lemgruber, L., Reimer, M.A., Insall, R., Heaslip, A., Meissner, M., 2017. Toxoplasma gondii F-actin forms an extensive filamentous network required for material exchange and parasite maturation. eLife 6, e24119. https://doi.org/10.7554/eLife.24119

Pernas, L., Adomako-Ankomah, Y., Shastri, A.J., Ewald, S.E., Treeck, M., Boyle, J.P., Boothroyd, J.C., 2014. Toxoplasma Effector MAF1 Mediates Recruitment of Host Mitochondria and Impacts the Host Response. PLoS Biol 12. https://doi.org/10.1371/journal.pbio.1001845

Persson, E.K., Agnarson, A.M., Lambert, H., Hitziger, N., Yagita, H., Chambers, B.J., Barragan, A., Grandien, A., 2007. Death receptor ligation or exposure to perforin trigger rapid egress of the intracellular parasite Toxoplasma gondii. J. Immunol. 179, 8357–8365. https://doi.org/10.4049/jimmunol.179.12.8357

Pieperhoff, M.S., Schmitt, M., Ferguson, D.J.P., Meissner, M., 2013. The Role of Clathrin in Post-Golgi Trafficking in Toxoplasma gondii. PLOS ONE 8, e77620. https://doi.org/10.1371/journal.pone.0077620

Pomel, S., Luk, F.C.Y., Beckers, C., 2008. Host Cell Egress and Invasion Induce Marked Relocations of Glycolytic Enzymes in Toxoplasma gondii Tachyzoites. PLoS pathogens. https://doi.org/10.1371/journal.ppat.1000188

Portman, N., Slapeta, J., 2014. The flagellar contribution to the apical complex: a new tool for the eukaryotic Swiss Army knife? Trends Parasitol. 30, 58–64. https://doi.org/10.1016/j.pt.2013.12.006

Poupel, O., Tardieux, I., 1999. Toxoplasma gondii motility and host cell invasiveness are drastically impaired by jasplakinolide, a cyclic peptide stabilizing F-actin. Microbes Infect. 1, 653–662. https://doi.org/10.1016/s1286-4579(99)80066-5

Presley, J.F., Cole, N.B., Schroer, T.A., Hirschberg, K., Zaal, K.J.M., Lippincott-Schwartz, J., 1997. ER-to-Golgi transport visualized in living cells. Nature 389, 81–85. https://doi.org/10.1038/38001

Que, X., Ngô, H., Lawton, J., Gray, M., Liu, Q., Engel, J., Brinen, L., Ghosh, P., Joiner, K.A., Reed, S.L., 2002. The Cathepsin B of Toxoplasma gondii,Toxopain-1, Is Critical for Parasite Invasion and Rhoptry Protein Processing. J. Biol. Chem. 277, 25791–25797. https://doi.org/10.1074/jbc.M202659200

Rabaud, C., May, T., Lucet, J.C., Leport, C., Ambroise-Thomas, P., Canton, P., 1996. Pulmonary toxoplasmosis in patients infected with human immunodeficiency virus: a French National Survey. Clin. Infect. Dis. 23, 1249–1254. https://doi.org/10.1093/clinids/23.6.1249

Ramakrishnan, C., Maier, S., Walker, R.A., Rehrauer, H., Joekel, D.E., Winiger, R.R., Basso, W.U., Grigg, M.E., Hehl, A.B., Deplazes, P., Smith, N.C., 2019. An experimental genetically attenuated live vaccine to prevent transmission of Toxoplasma gondii by cats. Scientific Reports 9, 1–14. https://doi.org/10.1038/s41598-018-37671-8

Ramakrishnan, S., Docampo, M.D., MacRae, J.I., Pujol, F.M., Brooks, C.F., van Dooren, G.G., Hiltunen, J.K., Kastaniotis, A.J., McConville, M.J., Striepen, B., 2012. Apicoplast and

Endoplasmic Reticulum Cooperate in Fatty Acid Biosynthesis in Apicomplexan Parasite Toxoplasma gondii. J Biol Chem 287, 4957–4971. https://doi.org/10.1074/jbc.M111.310144

Reid, A.J., Vermont, S.J., Cotton, J.A., Harris, D., Hill-Cawthorne, G.A., Könen-Waisman, S., Latham, S.M., Mourier, T., Norton, R., Quail, M.A., Sanders, M., Shanmugam, D., Sohal, A., Wasmuth, J.D., Brunk, B., Grigg, M.E., Howard, J.C., Parkinson, J., Roos, D.S., Trees, A.J., Berriman, M., Pain, A., Wastling, J.M., 2012. Comparative Genomics of the Apicomplexan Parasites Toxoplasma gondii and Neospora caninum: Coccidia Differing in Host Range and Transmission Strategy. PLoS Pathog 8. https://doi.org/10.1371/journal.ppat.1002567

Remington, J.S., Gentry, L.O., 1970. Acquired Toxoplasmosis: Infection Versus Disease*. Annals of the New York Academy of Sciences 174, 1006–1017. https://doi.org/10.1111/j.1749-6632.1970.tb45622.x

Remy, I., Michnick, S.W., 2004. Regulation of apoptosis by the Ft1 protein, a new modulator of protein kinase B/Akt. Mol Cell Biol 24, 1493–1504. https://doi.org/10.1128/mcb.24.4.1493-1504.2004

Renard, H.-F., Simunovic, M., Lemière, J., Boucrot, E., Garcia-Castillo, M.D., Arumugam, S., Chambon, V., Lamaze, C., Wunder, C., Kenworthy, A.K., Schmidt, A.A., McMahon, H.T., Sykes, C., Bassereau, P., Johannes, L., 2015. Endophilin-A2 functions in membrane scission in clathrin-independent endocytosis. Nature 517, 493–496. https://doi.org/10.1038/nature14064

Rexach, M., Latterich, M., Schekman, R., 1994. Characteristics of endoplasmic reticulum-derived transport vesicles. The Journal of cell biology 126, 1133–48.

Richardson, S.C.W., Winistorfer, S.C., Poupon, V., Luzio, J.P., Piper, R.C., 2004. Mammalian Late Vacuole Protein Sorting Orthologues Participate in Early Endosomal Fusion and Interact with the Cytoskeleton. Mol Biol Cell 15, 1197–1210. https://doi.org/10.1091/mbc.E03-06-0358

Robert-Gangneux, F., Dardé, M.-L., 2012. Epidemiology of and Diagnostic Strategies for Toxoplasmosis. Clin Microbiol Rev 25, 264–296. https://doi.org/10.1128/CMR.05013-11

Roiko, M.S., Svezhova, N., Carruthers, V.B., 2014. Acidification Activates Toxoplasma gondii Motility and Egress by Enhancing Protein Secretion and Cytolytic Activity. PLoS Pathog 10. https://doi.org/10.1371/journal.ppat.1004488

Romano, J., Beaumont, C., Carrasco Lopez, J.A., Ehrenman, K., Bavoil, P., Coppens, I., 2012. A novel co-infection model with Toxoplasma and Chlamydia trachomatis highlights the importance of host cell manipulation for nutrient scavenging. Cellular microbiology 15. https://doi.org/10.1111/cmi.12060

Rosowski, E.E., Lu, D., Julien, L., Rodda, L., Gaiser, R.A., Jensen, K.D.C., Saeij, J.P.J., 2011. Strain-specific activation of the NF-κB pathway by GRA15, a novel Toxoplasma gondii dense granule protein. J Exp Med 208, 195–212. https://doi.org/10.1084/jem.20100717

Rugarabamu, G., Marq, J.-B., Guérin, A., Lebrun, M., Soldati-Favre, D., 2015. Distinct contribution of Toxoplasma gondii rhomboid proteases 4 and 5 to micronemal protein protease 1 activity during invasion. Mol. Microbiol. 97, 244–262. https://doi.org/10.1111/mmi.13021

Russell, D.G., Burns, R.G., 1984. The polar ring of coccidian sporozoites: a unique microtubule-organizing centre. J. Cell. Sci. 65, 193–207.

Rzomp, K.A., Scholtes, L.D., Briggs, B.J., Whittaker, G.R., Scidmore, M.A., 2003. Rab GTPases Are Recruited to Chlamydial Inclusions in Both a Species-Dependent and Species-Independent Manner. Infect Immun 71, 5855–5870. https://doi.org/10.1128/IAI.71.10.5855-5870.2003

Sacher, M., Barrowman, J., Wang, W., Horecka, J., Zhang, Y., Pypaert, M., Ferro-Novick, S., 2001. TRAPP I implicated in the specificity of tethering in ER-to-Golgi transport. Mol Cell 7, 433–442. https://doi.org/10.1016/s1097-2765(01)00190-3

Sacher, M., Jiang, Y., Barrowman, J., Scarpa, A., Burston, J., Zhang, L., Schieltz, D., Yates, J.R., Abeliovich, H., Ferro-Novick, S., 1998. TRAPP, a highly conserved novel complex on the cis-Golgi that mediates vesicle docking and fusion. EMBO J 17, 2494–2503. https://doi.org/10.1093/emboj/17.9.2494

Saeij, J.P.J., Boyle, J.P., Grigg, M.E., Arrizabalaga, G., Boothroyd, J.C., 2005. Bioluminescence Imaging of Toxoplasma gondii Infection in Living Mice Reveals Dramatic Differences between Strains. Infect Immun 73, 695–702. https://doi.org/10.1128/IAI.73.2.695-702.2005

Sahoo, N., Beatty, W., Heuser, J., Sept, D., Sibley, L.D., 2006. Unusual Kinetic and Structural Properties Control Rapid Assembly and Turnover of Actin in the Parasite Toxoplasma gondii. Mol Biol Cell 17, 895–906. https://doi.org/10.1091/mbc.E05-06-0512

Salogiannis, J., Reck-Peterson, S.L., 2017. Hitchhiking: A Non-Canonical Mode of Microtubule-Based Transport. Trends Cell Biol 27, 141–150. https://doi.org/10.1016/j.tcb.2016.09.005

Sangaré, L.O., Alayi, T.D., Westermann, B., Hovasse, A., Sindikubwabo, F., Callebaut, I., Werkmeister, E., Lafont, F., Slomianny, C., Hakimi, M.-A., van Dorsselaer, A., Schaeffer-Reiss, C., Tomavo, S., 2016. Unconventional endosome-like compartment and retromer complex in Toxoplasma gondii govern parasite integrity and host infection. Nature Communications 7, 11191. https://doi.org/10.1038/ncomms11191

Sangaré, L.O., Yang, N., Konstantinou, E.K., Lu, D., Mukhopadhyay, D., Young, L.H., Saeij, J.P.J., 2019. Toxoplasma GRA15 Activates the NF-κB Pathway through Interactions with TNF Receptor-Associated Factors. mBio 10. https://doi.org/10.1128/mBio.00808-19

Sato, M., Grant, B.D., Harada, A., Sato, K., 2008. Rab11 is required for synchronous secretion of chondroitin proteoglycans after fertilization in Caenorhabditis elegans. J Cell Sci 121, 3177–3186. https://doi.org/10.1242/jcs.034678

Schafer, J.C., Baetz, N.W., Lapierre, L.A., McRae, R.E., Roland, J.T., Goldenring, J.R., 2014. Rab11-FIP2 interaction with MYO5B regulates movement of Rab11a-containing recycling vesicles. Traffic 15, 292–308. https://doi.org/10.1111/tra.12146

Scharton-Kersten, T.M., Yap, G., Magram, J., Sher, A., 1997. Inducible Nitric Oxide Is Essential for Host Control of Persistent but Not Acute Infection with the Intracellular Pathogen Toxoplasma gondii. J Exp Med 185, 1261–1274.

Schatten, H., Sibley, L.D., Ris, H., 2003. Structural evidence for actin-like filaments in Toxoplasma gondii using high-resolution low-voltage field emission scanning electron microscopy. Microsc. Microanal. 9, 330–335. https://doi.org/10.1017/S1431927603030095

Schekman, R., Orci, L., 1996. Coat Proteins and Vesicle Budding. Science 271, 1526–1533. https://doi.org/10.1126/science.271.5255.1526

Scheuring, D., Viotti, C., Krüger, F., Künzl, F., Sturm, S., Bubeck, J., Hillmer, S., Frigerio, L., Robinson, D., Pimpl, P., Schumacher, K., 2011. Multivesicular Bodies Mature from the

Trans-Golgi Network/Early Endosome in Arabidopsis. The Plant cell 23, 3463–81. https://doi.org/10.1105/tpc.111.086918

Schlierf, B., Fey, G., Hauber, J., Hocke, G., Rosorius, O., 2000. Rab11b Is Essential for Recycling of Transferrin to the Plasma Membrane. Experimental cell research 259, 257–65. https://doi.org/10.1006/excr.2000.4947

Schneider, M.M., Hoepelman, A.I., Eeftinck Schattenkerk, J.K., Nielsen, T.L., van der Graaf, Y., Frissen, J.P., van der Ende, I.M., Kolsters, A.F., Borleffs, J.C., 1992. A controlled trial of aerosolized pentamidine or trimethoprim-sulfamethoxazole as primary prophylaxis against Pneumocystis carinii pneumonia in patients with human immunodeficiency virus infection. The Dutch AIDS Treatment Group. N. Engl. J. Med. 327, 1836–1841. https://doi.org/10.1056/NEJM199212243272603

Schreij, A.M.A., Fon, E.A., McPherson, P.S., 2016. Endocytic membrane trafficking and neurodegenerative disease. Cell. Mol. Life Sci. 73, 1529–1545. https://doi.org/10.1007/s00018-015-2105-x

Seals, D., Eitzen, G., Margolis, N., Wickner, W., Price, A., 2000. A Ypt/Rab effector complex containing the Sec1 homolog Vps33p is required for homotypic vacuole fusion. Proceedings of the National Academy of Sciences of the United States of America 97, 9402–7. https://doi.org/10.1073/pnas.97.17.9402

Seaman, M., Marcusson, E., Cereghino, J., Emr, S., 1997. Endosome to Golgi Retrieval of the Vacuolar Protein Sorting Receptor, Vps10p, Requires the Function of the VPS29, VPS30, and VPS35 Gene Products. The Journal of cell biology 137, 79–92. https://doi.org/10.1083/jcb.137.1.79

Seaman, M., McCaffery, J.M., Emr, S., 1998. A Membrane Coat Complex Essential for Endosome-to-Golgi Retrograde Transport in Yeast. The Journal of cell biology. https://doi.org/10.1083/JCB.142.3.665

Seeber, F., Soldati-Favre, D., 2010. Metabolic pathways in the apicoplast of apicomplexa. Int Rev Cell Mol Biol 281, 161–228. https://doi.org/10.1016/S1937-6448(10)81005-6

Segev, N., 2001. Ypt and Rab GTPases: insight into functions through novel interactions. Current Opinion in Cell Biology 13, 500–511. https://doi.org/10.1016/S0955-0674(00)00242-8

Semerdjieva, S., Shortt, B., Maxwell, E., Singh, S., Fonarev, P., Hansen, J., Schiavo, G., Grant, B., Smythe, E., 2008. Coordinated regulation of AP2 uncoating from clathrin-coated vesicles by rab5 and hRME-6. Journal of Cell Biology 183.

Shaib, A.H., 2016. Calcium-Dependent Activator Protein for Secretion Function in Murine Dorsal Root Ganglion Neurons. https://doi.org/10.22028/D291-27279

Shaw, M.K., Tilney, L.G., 1999. Induction of an acrosomal process in Toxoplasma gondii: Visualization of actin filaments in a protozoan parasite. PNAS 96, 9095–9099. https://doi.org/10.1073/pnas.96.16.9095

Shea, M., Jäkle, U., Liu, Q., Berry, C., Joiner, K.A., Soldati-Favre, D., 2007. A family of aspartic proteases and a novel, dynamic and cell-cycle-dependent protease localization in the secretory pathway of Toxoplasma gondii. Traffic 8, 1018–1034. https://doi.org/10.1111/j.1600-0854.2007.00589.x

Sheiner, L., Demerly, J.L., Poulsen, N., Beatty, W.L., Lucas, O., Behnke, M.S., White, M.W., Striepen, B., 2011. A Systematic Screen to Discover and Analyze Apicoplast Proteins Identifies a Conserved and Essential Protein Import Factor. PLOS Pathogens 7, e1002392. https://doi.org/10.1371/journal.ppat.1002392

Sheiner, L., Dowse, T.J., Soldati-Favre, D., 2008. Identification of Trafficking Determinants for Polytopic Rhomboid Proteases in Toxoplasma gondii. Traffic 9, 665–677. https://doi.org/10.1111/j.1600-0854.2008.00736.x

Sheiner, L., Santos, J.M., Klages, N., Parussini, F., Jemmely, N., Friedrich, N., Ward, G.E., Soldati-Favre, D., 2010. Toxoplasma gondii transmembrane microneme proteins and their modular design. Mol Microbiol 77, 912–929. https://doi.org/10.1111/j.1365-2958.2010.07255.x

Shen, B., Brown, K.M., Lee, T.D., Sibley, L.D., 2014a. Efficient Gene Disruption in Diverse Strains of Toxoplasma gondii Using CRISPR/CAS9. mBio 5. https://doi.org/10.1128/mBio.01114-14

Shen, B., Buguliskis, J.S., Lee, T.D., Sibley, L.D., 2014b. Functional Analysis of Rhomboid Proteases during Toxoplasma Invasion. mBio 5. https://doi.org/10.1128/mBio.01795-14

Shen, B., Sibley, L.D., 2014. Toxoplasma aldolase is required for metabolism but dispensable for host-cell invasion. Proc Natl Acad Sci U S A 111, 3567–3572. https://doi.org/10.1073/pnas.1315156111

Shen, B., Sibley, L.D., 2012. The moving junction, a key portal to host cell invasion by apicomplexan parasites. Curr Opin Microbiol 15, 449–455. https://doi.org/10.1016/j.mib.2012.02.007

Shin, H.-W., Hayashi, M., Christoforidis, S., Lacas-Gervais, S., Hoepfner, S., Wenk, M.R., Modregger, J., Uttenweiler-Joseph, S., Wilm, M., Nystuen, A., Frankel, W.N., Solimena, M., De Camilli, P., Zerial, M., 2005. An enzymatic cascade of Rab5 effectors regulates phosphoinositide turnover in the endocytic pathway. Journal of Cell Biology 170, 607–618. https://doi.org/10.1083/jcb.200505128

Sibley, L.D., Adams, L.B., Fukutomi, Y., Krahenbuhl, J.L., 1991. Tumor necrosis factor-alpha triggers antitoxoplasmal activity of IFN-gamma primed macrophages. The Journal of Immunology 147, 2340–2345.

Sibley, L.D., Niesman, I.R., Parmley, S.F., Cesbron-Delauw, M.F., 1995. Regulated secretion of multi-lamellar vesicles leads to formation of a tubulo-vesicular network in host-cell vacuoles occupied by Toxoplasma gondii. J Cell Sci 108 ( Pt 4), 1669–1677.

Sidik, S.M., Hackett, C.G., Tran, F., Westwood, N.J., Lourido, S., 2014. Efficient genome engineering of Toxoplasma gondii using CRISPR/Cas9. PLoS One 9, e100450. https://doi.org/10.1371/journal.pone.0100450

Silveira, C., Vallochi, A.L., Silva, U.R. da, Muccioli, C., Holland, G.N., Nussenblatt, R.B., Belfort, R., Rizzo, L.V., 2011. Toxoplasma gondii in the peripheral blood of patients with acute and chronic toxoplasmosis. British Journal of Ophthalmology 95, 396–400. https://doi.org/10.1136/bjo.2008.148205

Simonsen, A., Gaullier, J.M., D'Arrigo, A., Stenmark, H., 1999. The Rab5 effector EEA1 interacts directly with syntaxin-6. J Biol Chem 274, 28857–28860. https://doi.org/10.1074/jbc.274.41.28857

Sivars, U., Aivazian, D., Pfeffer, S., 2003. Yip3 catalyses the dissociation of endosomal Rab-GDI complexes. Nature 425, 856–9. https://doi.org/10.1038/nature02057

Skariah, S., McIntyre, M.K., Mordue, D.G., 2010. Toxoplasma gondii: determinants of tachyzoite to bradyzoite conversion. Parasitol Res 107, 253–260. https://doi.org/10.1007/s00436-010-1899-6

SKILLMAN, K.M., DAHER, W., Christopher, I., SOLDATI-FAVRE, D., SIBLEY, L.D., 2012. Toxoplasma gondii profilin acts primarily to sequester G-actin while formins efficiently

nucleate actin filament formation in vitro. Biochemistry 51, 2486–2495. https://doi.org/10.1021/bi201704y

Skillman, K.M., Ma, C.I., Fremont, D.H., Diraviyam, K., Cooper, J.A., Sept, D., Sibley, L.D., 2013. The unusual dynamics of parasite actin result from isodesmic polymerization. Nat Commun 4, 2285. https://doi.org/10.1038/ncomms3285

Sloves, P.-J., Delhaye, S., Mouveaux, T., Werkmeister, E., Slomianny, C., Hovasse, A., Dilezitoko Alayi, T., Callebaut, I., Gaji, R.Y., Schaeffer-Reiss, C., Van Dorsselear, A., Carruthers, V.B., Tomavo, S., 2012. Toxoplasma sortilin-like receptor regulates protein transport and is essential for apical secretory organelle biogenesis and host infection. Cell Host Microbe 11, 515–527. https://doi.org/10.1016/j.chom.2012.03.006

Soldati, D., Dubremetz, J.F., Lebrun, M., 2001. Microneme proteins: structural and functional requirements to promote adhesion and invasion by the apicomplexan parasite Toxoplasma gondii. Int. J. Parasitol. 31, 1293–1302. https://doi.org/10.1016/s0020-7519(01)00257-0

Solinger, J.A., Spang, A., 2013. Tethering complexes in the endocytic pathway: CORVET and HOPS. FEBS J 280, 2743–2757. https://doi.org/10.1111/febs.12151

Söllner, T., Bennett, M.K., Whiteheart, S.W., Scheller, R.H., Rothman, J.E., 1993. A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. Cell 75, 409–418. https://doi.org/10.1016/0092-8674(93)90376-2

Sommer, T., Wolf, D., 1998. Endoplasmic reticulum degradation: Reverse protein flow of no return. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 11, 1227–33. https://doi.org/10.1096/fasebj.11.14.9409541

Souza, W. de, 2006. Secretory organelles of pathogenic protozoa. Anais da Academia Brasileira de Ciências 78, 271–292. https://doi.org/10.1590/S0001-37652006000200008

Speer, C.A., Dubey, J.P., 2005. Ultrastructural differentiation of Toxoplasma gondii schizonts (types B to E) and gamonts in the intestines of cats fed bradyzoites. International Journal for Parasitology 35, 193–206. https://doi.org/10.1016/j.ijpara.2004.11.005

Splendore, A., 1908. Un nuovo protozoa parassita deconigli incontrato nelle lesioni anatomiche d'une malattia che ricorda in molti punti il Kalaazar dell'uoma, Nota preliminare pel.

Stedman, T.T., Sussmann, A.R., Joiner, K.A., 2003. Toxoplasma gondii Rab6 mediates a retrograde pathway for sorting of constitutively secreted proteins to the Golgi complex. J Biol Chem 278, 5433–5443. https://doi.org/10.1074/jbc.M209390200

Stenmark, H., 2009. Stenmark, H. Rab GTPases as coordinators of vesicle traffic. Nat Rev Mol Cell Biol 10: 513-525. Nature reviews. Molecular cell biology 10, 513–25. https://doi.org/10.1038/nrm2728

Stommel, E.W., Ely, K.H., Schwartzman, J.D., Kasper, L.H., 1997. Toxoplasma gondii: dithiol-induced Ca2+ flux causes egress of parasites from the parasitophorous vacuole. Exp. Parasitol. 87, 88–97. https://doi.org/10.1006/expr.1997.4187

Strabelli, T.M.V., Siciliano, R.F., Vidal Campos, S., Bianchi Castelli, J., Bacal, F., Bocchi, E.A., Uip, D.E., 2012. Toxoplasma gondii Myocarditis after Adult Heart Transplantation: Successful Prophylaxis with Pyrimethamine. J Trop Med 2012. https://doi.org/10.1155/2012/853562

Straub, K.W., Cheng, S.J., Sohn, C.S., Bradley, P.J., 2009. Novel components of the Apicomplexan moving junction reveal conserved and coccidia-restricted elements. Cell Microbiol 11, 590–603. https://doi.org/10.1111/j.1462-5822.2008.01276.x

Straub, K.W., Peng, E.D., Hajagos, B.E., Tyler, J.S., Bradley, P.J., 2011. The Moving Junction Protein RON8 Facilitates Firm Attachment and Host Cell Invasion in Toxoplasma gondii. PLoS Pathog 7. https://doi.org/10.1371/journal.ppat.1002007

Striepen, B., 2011. The apicoplast: a red alga in human parasites. Essays Biochem. 51, 111–125. https://doi.org/10.1042/bse0510111

Striepen, B., Jordan, C.N., Reiff, S., van Dooren, G.G., 2007. Building the Perfect Parasite: Cell Division in Apicomplexa. PLoS Pathog 3. https://doi.org/10.1371/journal.ppat.0030078

Striepen, B., Yingxin He, C., Matrajt, M., Soldati, D., Roos, D.S., 1998. Expression, selection, and organellar targeting of the green fluorescent protein in Toxoplasma gondii. Molecular and Biochemical Parasitology 92, 325–338. https://doi.org/10.1016/S0166-6851(98)00011-5

Strom, M., Hume, A.N., Tarafder, A.K., Barkagianni, E., Seabra, M.C., 2002. A Family of Rab27-binding Proteins: Melanophilin Links Rab27a and Myosin Va Function in Melanosome Transport 35.

Strom, M., Vollmer, P., Tan, T.J., Gallwitz, D., 1993. A yeast GTPase-activating protein that interacts specifically with a member of the Ypt/Rab family. Nature 361, 736–9. https://doi.org/10.1038/361736a0

Su, C., Evans, D., Cole, R.H., Kissinger, J.C., Ajioka, J.W., Sibley, L.D., 2003. Recent Expansion of Toxoplasma Through Enhanced Oral Transmission. Science 299, 414–416. https://doi.org/10.1126/science.1078035

Suarez, C., Lentini, G., Ramaswamy, R., Maynadier, M., Aquilini, E., Berry-Sterkers, L., Cipriano, M., Chen, A.L., Bradley, P., Striepen, B., Boulanger, M.J., Lebrun, M., 2019. A lipid-binding protein mediates rhoptry discharge and invasion in Plasmodium falciparum and Toxoplasma gondii parasites. Nature Communications 10, 4041. https://doi.org/10.1038/s41467-019-11979-z

Sunio, A., Metcalf, A.B., Krämer, H., 1999. Genetic dissection of endocytic trafficking in Drosophila using a horseradish peroxidase-bride of sevenless chimera: hook is required for normal maturation of multivesicular endosomes. Mol Biol Cell 10, 847–859. https://doi.org/10.1091/mbc.10.4.847

Suss-Toby, E., Zimmerberg, J., Ward, G.E., 1996. Toxoplasma invasion: the parasitophorous vacuole is formed from host cell plasma membrane and pinches off via a fission pore. Proc Natl Acad Sci U S A 93, 8413–8418.

Suvorova, E.S., Duden, R., Lupashin, V.V., 2002. The Sec34/Sec35p complex, a Ypt1p effector required for retrograde intra-Golgi trafficking, interacts with Golgi SNAREs and COPI vesicle coat proteins. J Cell Biol 157, 631–643. https://doi.org/10.1083/jcb.200111081

Suvorova, E.S., Francia, M., Striepen, B., White, M.W., 2015. A Novel Bipartite Centrosome Coordinates the Apicomplexan Cell Cycle. PLoS Biol 13. https://doi.org/10.1371/journal.pbio.1002093

Suzuki, Y., Israelski, D.M., Dannemann, B.R., Stepick-Biek, P., Thulliez, P., Remington, J.S., 1988a. Diagnosis of toxoplasmic encephalitis in patients with acquired immunodeficiency syndrome by using a new serologic method. Journal of Clinical Microbiology 26, 2541–2543.

Suzuki, Y., Orellana, M.A., Schreiber, R.D., Remington, J.S., 1988b. Interferon-gamma: the major mediator of resistance against Toxoplasma gondii. Science 240, 516–518. https://doi.org/10.1126/science.3128869

Szatmari, Z., Kis, V., Lippai, M., Hegedus, K., Faragó, T., Lőrincz, P., Tanaka, T., Juhasz, G., Sass, M., 2013. Rab11 facilitates cross-talk between autophagy and endosomal pathway through regulation of Hook localization. Molecular biology of the cell 25. https://doi.org/10.1091/mbc.E13-10-0574

Szebenyi, G., Hall, B., Yu, R., Hashim, A.I., Krämer, H., 2007. Hook2 localizes to the centrosome, binds directly to centriolin/CEP110 and contributes to centrosomal function. Traffic 8, 32–46. https://doi.org/10.1111/j.1600-0854.2006.00511.x

Sztul, E., Lupashin, V., 2006. Role of tethering factors in secretory membrane traffic. Am J Physiol Cell Physiol 290, C11-26. https://doi.org/10.1152/ajpcell.00293.2005

Taguchi, T., 2013. Emerging roles of recycling endosomes. The Journal of Biochemistry 153, 505–510. https://doi.org/10.1093/jb/mvt034

Takahashi, S., Kubo, K., Waguri, S., Yabashi, A., Shin, H.-W., Katoh, Y., Nakayama, K., 2012. Rab11 regulates exocytosis of recycling vesicles at the plasma membrane. Journal of cell science 125. https://doi.org/10.1242/jcs.102913

Tenter, A.M., Heckeroth, A.R., Weiss, L.M., 2000. Toxoplasma gondii: from animals to humans. Int J Parasitol 30, 1217–1258.

TerBush, D.R., Maurice, T., Roth, D., Novick, P., 1996. The Exocyst is a multiprotein complex required for exocytosis in Saccharomyces cerevisiae. EMBO J 15, 6483–6494.

Tomavo, S., Schwarz, R.T., Dubremetz, J.F., 1989. Evidence for glycosyl-phosphatidylinositol anchoring of Toxoplasma gondii major surface antigens. Mol Cell Biol 9, 4576–4580.

Tomley, F.M., Soldati, D.S., 2001. Mix and match modules: structure and function of microneme proteins in apicomplexan parasites. Trends Parasitol. 17, 81–88. https://doi.org/10.1016/s1471-4922(00)01761-x

Tran, J.Q., de Leon, J.C., Li, C., Huynh, M.-H., Beatty, W., Morrissette, N.S., 2010. RNG1 is a Late Marker of the Apical Polar Ring in Toxoplasma gondii. Cytoskeleton (Hoboken) 67, 586–598. https://doi.org/10.1002/cm.20469

Travier, L., Mondragon, R., Dubremetz, J.-F., Musset, K., Mondragon, M., Gonzalez, S., Cesbron-Delauw, M.-F., Mercier, C., 2008. Functional domains of the Toxoplasma GRA2 protein in the formation of the membranous nanotubular network of the parasitophorous vacuole. International Journal for Parasitology 38, 757–773. https://doi.org/10.1016/j.ijpara.2007.10.010

Trousdale, C., Kim, K., 2015. Retromer: Structure, Function, and Roles in Mammalian Disease. European journal of cell biology 94. https://doi.org/10.1016/j.ejcb.2015.07.002

Tu, V., Yakubu, R., Weiss, L.M., 2018. OBSERVATIONS ON BRADYZOITE BIOLOGY. Microbes Infect 20, 466–476. https://doi.org/10.1016/j.micinf.2017.12.003

Tyler, J.S., Treeck, M., Boothroyd, J.C., 2011. Focus on the ringleader: the role of AMA1 in apicomplexan invasion and replication. Trends Parasitol 27, 410–420. https://doi.org/10.1016/j.pt.2011.04.002

Ullrich, O., Reinsch, S., Urbé, S., Zerial, M., Parton, R., 1996. Rab11 regulates recycling through the pericentriolar recycling endosome. The Journal of cell biology 135, 913–24. https://doi.org/10.1083/jcb.135.4.913

Urbé, S., Huber, L.A., Zerial, M., Tooze, S.A., Parton, R.G., 1993. Rab11, a small GTPase associated with both constitutive and regulated secretory pathways in PC12 cells. FEBS Letters 334, 175–182. https://doi.org/10.1016/0014-5793(93)81707-7

van Dooren, G.G., Kennedy, A.T., McFadden, G.I., 2012. The use and abuse of heme in apicomplexan parasites. Antioxid. Redox Signal. 17, 634–656. https://doi.org/10.1089/ars.2012.4539

van Dooren, G.G., Reiff, S.B., Tomova, C., Meissner, M., Humbel, B.M., Striepen, B., 2009. An novel dynamin-related protein has been recruited for apicoplast fission in Toxoplasma gondii. Curr Biol 19, 267–276. https://doi.org/10.1016/j.cub.2008.12.048

Venugopal, K., Chehade, S., Werkmeister, E., Barois, N., Periz, J., Lafont, F., Tardieux, I., Khalife, J., Langsley, G., Meissner, M., Marion, S., 2020. Rab11A regulates dense granule transport and secretion during Toxoplasma gondii invasion of host cells and parasite replication. PLoS Pathog 16. https://doi.org/10.1371/journal.ppat.1008106

Venugopal, K., Marion, S., 2018. Secretory organelle trafficking in Toxoplasma gondii: A long story for a short travel. International Journal of Medical Microbiology 308, 751–760. https://doi.org/10.1016/j.ijmm.2018.07.007

Venugopal, K., Werkmeister, E., Barois, N., Saliou, J.-M., Poncet, A., Huot, L., Sindikubwabo, F., Hakimi, M.A., Langsley, G., Lafont, F., Marion, S., 2017. Dual role of the Toxoplasma gondii clathrin adaptor AP1 in the sorting of rhoptry and microneme proteins and in parasite division. PLOS Pathogens 13, e1006331. https://doi.org/10.1371/journal.ppat.1006331

Waldman, B.S., Schwarz, D., Wadsworth, M.H., Saeij, J.P., Shalek, A.K., Lourido, S., 2020. Identification of a Master Regulator of Differentiation in Toxoplasma. Cell 180, 359-372.e16. https://doi.org/10.1016/j.cell.2019.12.013

Walenta, J.H., Didier, A.J., Liu, X., Krämer, H., 2001. The Golgi-Associated Hook3 Protein Is a Member of a Novel Family of Microtubule-Binding Proteins. J Cell Biol 152, 923–934.

Waller, R.F., Keeling, P.J., Donald, R.G.K., Striepen, B., Handman, E., Lang-Unnasch, N., Cowman, A.F., Besra, G.S., Roos, D.S., McFadden, G.I., 1998. Nuclear-encoded proteins target to the plastid in Toxoplasma gondii and Plasmodium falciparum. Proc Natl Acad Sci U S A 95, 12352–12357.

Waller, R.F., McFadden, G.I., 2005. The apicoplast: a review of the derived plastid of apicomplexan parasites. Curr Issues Mol Biol 7, 57–79.

Wang, C., Yan, X., Chen, Q., Jiang, N., Fu, W., Ma, B., Liu, J., Li, C., Bednarek, S.Y., Pan, J., 2013. Clathrin light chains regulate clathrin-mediated trafficking, auxin signaling, and development in Arabidopsis. Plant Cell 25, 499–516. https://doi.org/10.1105/tpc.112.108373

Wang, Y., Jeong, Y., Jhiang, S.M., Yu, L., Menq, C.-H., 2014. Quantitative Characterization of Cell Behaviors through Cell Cycle Progression via Automated Cell Tracking. PLOS ONE 9, e98762. https://doi.org/10.1371/journal.pone.0098762

Wang, Z., Edwards, J., Riley, N., Provance, J., D., Karcher, R., Li, X.-D., Davison, I., Ikebe, M., Mercer, J., Kauer, J., Ehlers, M., 2008. Myosin Vb Mobilizes Recycling Endosomes and AMPA Receptors for Postsynaptic Plasticity. Cell 135, 535–48. https://doi.org/10.1016/j.cell.2008.09.057

Weber, T., Zemelman, B.V., McNew, J.A., Westermann, B., Gmachl, M., Parlati, F., Söllner, T.H., Rothman, J.E., 1998. SNAREpins: minimal machinery for membrane fusion. Cell 92, 759–772. https://doi.org/10.1016/s0092-8674(00)81404-x

Weimbs, T., Low, S.H., Chapin, S.J., Mostov, K.E., Bucher, P., Hofmann, K., 1997. A conserved domain is present in different families of vesicular fusion proteins: a new superfamily. Proc Natl Acad Sci U S A 94, 3046–3051. https://doi.org/10.1073/pnas.94.7.3046

Welz, T., Wellbourne-Wood, J., Kerkhoff, E., 2014. Orchestration of cell surface proteins by Rab11. Trends in cell biology 24. https://doi.org/10.1016/j.tcb.2014.02.004

Wetzel, D.M., Chen, L.A., Ruiz, F.A., Moreno, S.N.J., Sibley, L.D., 2004. Calcium-mediated protein secretion potentiates motility in Toxoplasma gondii. J. Cell. Sci. 117, 5739–5748. https://doi.org/10.1242/jcs.01495

Wetzel, D.M., Håkansson, S., Hu, K., Roos, D., Sibley, L.D., 2003. Actin Filament Polymerization Regulates Gliding Motility by Apicomplexan Parasites. Mol Biol Cell 14, 396–406. https://doi.org/10.1091/mbc.E02-08-0458

Whitelaw, J.A., Latorre-Barragan, F., Gras, S., Pall, G.S., Leung, J.M., Heaslip, A., Egarter, S., Andenmatten, N., Nelson, S.R., Warshaw, D.M., Ward, G.E., Meissner, M., 2017. Surface attachment, promoted by the actomyosin system of Toxoplasma gondii is important for efficient gliding motility and invasion. BMC Biology 15, 1. https://doi.org/10.1186/s12915-016-0343-5

Wieland, F., Hartert, C., 1999. Mechanisms of vesicle formation: Insights from the COP system. Current Opinion in Cell Biology 11, 440–446. https://doi.org/10.1016/S0955-0674(99)80063-5

Wilson, G.M., Fielding, A.B., Simon, G.C., Yu, X.Z., Andrews, P.D., Hames, R.S., Frey, A.M., Peden, A.A., Gould, G.W., Prekeris, R., 2005. The FIP3-Rab11 protein complex regulates recycling endosome targeting to the cleavage furrow during late cytokinesis. Molecular Biology of the Cell 16, 849–860.

Wilson, R.J., Denny, P.W., Preiser, P.R., Rangachari, K., Roberts, K., Roy, A., Whyte, A., Strath, M., Moore, D.J., Moore, P.W., Williamson, D.H., 1996. Complete gene map of the plastid-like DNA of the malaria parasite Plasmodium falciparum. J. Mol. Biol. 261, 155–172. https://doi.org/10.1006/jmbi.1996.0449

Withers-Martinez, C., Strath, M., Hackett, F., Haire, L.F., Howell, S.A., Walker, P.A., Evangelos, C., Dodson, G.G., Blackman, M.J., 2014. The malaria parasite egress protease SUB1 is a calcium-dependent redox switch subtilisin. Nat Commun 5. https://doi.org/10.1038/ncomms4726

Wu, S., Mehta, S., Pichaud, F., Bellen, H., Quiocho, F., 2005. Sec15 interacts with Rab11 via a novel domain and affects Rab11 localization in vivo. Nature structural & molecular biology 12, 879–85. https://doi.org/10.1038/nsmb987

wu, X., Bradley, M., Cai, Y., Kuemmel, D., De La Cruz, E., Barr, F., Reinisch, K., 2011. Insights regarding guanine nucleotide exchange from the structure of a DENN-domain protein complexed with its Rab GTPase substrate. Proceedings of the National Academy of Sciences of the United States of America 108, 18672–7. https://doi.org/10.1073/pnas.1110415108

Wu, X., Rao, K., Zhang, H., Wang, F., Sellers, J., Matesic, L., Copeland, N., Jenkins, N., Hammer, J., 2002. Identification of an organelle receptor for myosin-Va. Nature cell biology 4, 271–8. https://doi.org/10.1038/ncb760

X Wu, 2001. Rab27a enables myosin Va-dependent melanosome capture by recruiting the myosin to the organelle.

Xiong, B., Bayat, V., Jaiswal, M., Zhang, K., Sandoval, H., Charng, W.-L., Li, T., David, G., Duraine, L., Lin, Y.-Q., Neely, G.G., Yamamoto, S., Bellen, H.J., 2012. Crag Is a GEF for Rab11 Required for Rhodopsin Trafficking and Maintenance of Adult Photoreceptor Cells. PLOS Biology 10, e1001438. https://doi.org/10.1371/journal.pbio.1001438

Xu, L., Sowa, M.E., Chen, J., Li, X., Gygi, S.P., Harper, J.W., 2008. An FTS/Hook/p107FHIP Complex Interacts with and Promotes Endosomal Clustering by the Homotypic

Vacuolar Protein Sorting Complex. Mol Biol Cell 19, 5059–5071. https://doi.org/10.1091/mbc.E08-05-0473

Yamamoto, M., Ma, J.S., Mueller, C., Kamiyama, N., Saiga, H., Kubo, E., Kimura, T., Okamoto, T., Okuyama, M., Kayama, H., Nagamune, K., Takashima, S., Matsuura, Y., Soldati-Favre, D., Takeda, K., 2011. ATF6β is a host cellular target of the Toxoplasma gondii virulence factor ROP18. J Exp Med 208, 1533–1546. https://doi.org/10.1084/jem.20101660

Yang, M., Coppens, I., Wormsley, S., Baevova, P., Hoppe, H., Joiner, K., 2004. The Plasmodium falciparum Vps4 homolog mediates multivesicular body formation. Journal of cell science 117, 3831–8. https://doi.org/10.1242/jcs.01237

Yang, N., Farrell, A., Niedelman, W., Melo, M., Lu, D., Julien, L., Marth, G.T., Gubbels, M.-J., Saeij, J.P., 2013. Genetic basis for phenotypic differences between different Toxoplasma gondii type I strains. BMC Genomics 14, 467. https://doi.org/10.1186/1471-2164-14-467

Yao, X., Wang, X., Xiang, X., 2014. FHIP and FTS proteins are critical for dynein-mediated transport of early endosomes in Aspergillus. Mol Biol Cell 25, 2181–2189. https://doi.org/10.1091/mbc.E14-04-0873

Yarovinsky, F., 2014. Innate immunity to Toxoplasma gondii infection. Nat. Rev. Immunol. 14, 109–121. https://doi.org/10.1038/nri3598

Yasuda, T., Yagita, K., Nakamura, T., Endo, T., 1988. Immunocytochemical localization of actin in Toxoplasma gondii. Parasitol. Res. 75, 107–113. https://doi.org/10.1007/bf00932709

Yoon, S.-O., Shin, S., Mercurio, A., 2005. Hypoxia Stimulates Carcinoma Invasion by Stabilizing Microtubules and Promoting the Rab11 Trafficking of the α6β4 Integrin. Cancer research 65, 2761–9. https://doi.org/10.1158/0008-5472.CAN-04-4122

Zerial, M., McBride, H., 2001. Rab proteins as membrane organizers. Nature Reviews Molecular Cell Biology 2, 107–117. https://doi.org/10.1038/35052055

Zhang, J., Qiu, R., Arst, H.N., Peñalva, M.A., Xiang, X., 2014. HookA is a novel dynein–early endosome linker critical for cargo movement in vivo. J Cell Biol 204, 1009–1026. https://doi.org/10.1083/jcb.201308009

Zolov, S.N., Lupashin, V.V., 2005. Cog3p depletion blocks vesicle-mediated Golgi retrograde trafficking in HeLa cells. J Cell Biol 168, 747–759. https://doi.org/10.1083/jcb.200412003

# RESUME DETAILLE des TRAVAUX de THESE (FRANÇAIS)

# Caractérisation fonctionnelle de la voie de sécrétion dépendante de Rab11A chez *Toxoplasma gondii*

## I- Introduction :

*Toxoplasma gondii*, un parasite intracellulaire obligatoire appartenant au phylum Apicomlexa, est l'agent causal de la maladie infectieuse « Toxoplasmose ».

Le tachyzoïte, qui représente la forme réplicative et disséminatrice du parasite, présente une organisation très polarisée d'organites sécrétoires spécifiques aux Apicomplexes. Ces organites comprennent des micronèmes et des rhoptries en forme de massue, qui sont ancrés au conoïde au sommet du parasite. En revanche, les granules denses (DG), qui participent à la voie sécrétoire constitutive, sont distribués à travers le cytoplasme du parasite. Ces organites sécrétoires sont essentiels pour compléter le cycle lytique du parasite, ce qui permet l'invasion et la réplication du parasite dans la cellule hôte.

De plus, le parasite possède un complexe membranaire spécial appelé le complexe membranaire interne (IMC), qui se trouve sous la membrane plasmique. L'IMC représente des sacs aplatis articulés qui ne s'interrompent qu'aux pôles apical et basal du parasite (Anderson-White B, 2012), qui sont considérés comme des sites favorables à la libération de DG, en plus des joints entre les sacs. L'IMC prend également en charge la motilité basée sur l'acto-myosine via l'ancrage des composants du glideosome.

Le cycle lytique du tachyzoïte implique une série complexe d'événements séquentiels, qui comprend l'adhésion et la motilité du parasite, l'invasion de la cellule hôte, la réplication intracellulaire et enfin la sortie de la cellule infectée.

L'invasion est assurée par la sécrétion précoce des micronèmes et des protéines rhoptries du cou, qui forment une structure adhésive entre le parasite et la membrane plasmique de la cellule hôte appelée jonction mobile (MJ). Une fois la MJ établie, le contenu du bulbe des rhoptries (protéines ROP) est sécrété dans le cytosol de l'hôte avant que la vacuole ne commence sa formation.

Les granules denses (GRA) sont ensuite massivement sécrétés dans la lumière de la vacuole naissante. Une fois la vacuole formée, les protéines ROP et GRA soit localisées au

niveau de la membrane de la vacuole parasitophore (PVM) soit dans le cytosol et le noyau de l'hôte et jouent un rôle essentiel pour assurer la survie du parasite en bloquant la lyse du PV et en modulant les réponses transcriptionnelles et immunitaires de la cellule hôte (Hunter CA, 2012).

Les granules denses, qui sont également sécrétées de manière constitutive lors de la réplication du parasite, sont des acteurs clés assurant la survie et la dissémination du parasite en modulant les voies de signalisation de l'hôte. Par exemple, il a été démontré que GRA15 régule la transcription du gène pro-inflammatoire médiée par NFκB (Emily E. Rosowski, 2011), tandis que GRA24 module la voie MAPKinase (Braun L, 2013) pour déréguler la réponse inflammatoire des macrophages infectés.

De plus, dans une étude réalisée par Muniz-Hernandez et al, il a également été montré que la déplétion de GRA2 entraîne la formation de rosettes désorganisées et une asynchronie de prolifération corrélée à la perte du réseau nanotubulaire intravacuolaire (IVN) (S. Muniz -Hernandez, 2011). Par conséquent, les protéines GRA participent également à la régulation de la réplication du parasite. Enfin, les protéines GRA sont également essentielles à l'établissement de la toxoplasmose chronique en régulant la formation de la paroi du kyste.

Contrairement à la sécrétion régulée des protéines ROP et MIC lors de l'invasion parasitaire, les mécanismes régulant la voie sécrétoire constitutive chez *T. gondii* sont totalement inconnus, bien qu'étant cruciaux pour la survie et la virulence du parasite. En effet, la voie sécrétoire constitutive pourrait être impliquée dans l'administration transmembranaire de protéines à la membrane plasmique, y compris les transporteurs de nutriments et d'ions, jusqu'à l'administration de l'antigène de surface principal ancré GPI SAG1, ainsi que la libération de protéines GRA dans l'espace vacuolaire, à la PVM et au-delà dans le cytosol de l'hôte. En général, les processus impliqués dans l'échange entre le parasite et l'environnement extérieur (dont l'endocytose du matériel de l'hôte) sont encore très mal compris. Une étude de Kremer K et al, basée sur un criblage de surexpression de *T. gondii* Rabs a révélé que Rab11A pourrait jouer un rôle dans la sécrétion constitutive de SAG1 au niveau de la membrane plasmique (Katrin Kremer, 2013). Rab11A appartient à la famille des petites GTPases, qui représentent des régulateurs clés du trafic vésiculaire intracellulaire chez les eucaryotes. Ils alternent entre

une forme inactive liée au GDP et une forme active liée au GTP (Stenmark, 2009). Les Rab GTPases régulent des étapes distinctes du trafic vésiculaire en s'associant à de nombreux effecteurs différents : bourgeonnement vésiculaire à partir du compartiment donneur, transport vésiculaire en se liant à des moteurs moléculaires (tels que myosine/dynéine/kinésine), ancrage des vésicules en se liant à des complexes d'attache (tels que les composants du complexe exocyste), et la fusion des vésicules par liaison aux protéines SNAREs. Il a été démontré que Rab11 est impliqué dans de nombreux processus biologiques tels que la division cellulaire et la migration cellulaire, notamment en régulant l'exocytose de facteurs régulateurs clés (Takahashi S, 2012).

Par conséquent, le laboratoire a concentré son attention sur Rab11A et l'a identifié comme un régulateur principal de la sécrétion constitutive chez *T. gondii*. En résumé, afin de localiser Rab11A, Kannan Venugopal (KV), un ancien doctorant du laboratoire, a généré un anticorps polyclonal spécifique. Il a découvert qu'au cours de la phase G1, Rab11A est localisé dans les vésicules cytoplasmiques, particulièrement enrichies au pôle apical et basal des parasites, suggérant un rôle dans la sécrétion constitutive. Au cours de la cytokinèse, Rab11A a été observée au niveau du TGN et aux extrémités des parasites filles, suggérant un transport actif dépendant de Rab11A du matériel nouvellement synthétisé ou recyclé du TGN au pôle apical des cellules filles naissantes. De plus, un mutant dominant négatif de la protéine (appelé Rab11ADN), hébergeant une mutation ponctuelle N126I dans le domaine fonctionnel GTPase (Herm-Gotz A, 2007), a été surexprimé afin d'affecter l'activité de Rab11A. En utilisant cette lignée parasitaire, KV a démontré que Rab11A est essentiel pour la libération des protéines GRA dans l'espace vacuolaire et par conséquent dans le cytosol de l'hôte. Il a découvert que Rab11A est également nécessaire pour l'administration de protéines transmembranaires, telles que le transporteur de glucose GT1, suggérant un rôle majeur de Rab11A, non seulement dans l'exocytose DG, mais également dans d'autres voies sécrétoires. Il est important de noter que la biogenèse des DG n'a pas été altérée et que des vésicules de sécrétion contenant GT1 se sont également accumulées dans le cytosol de l'hôte.

De plus, les parasites Rab11ADN ont montré une forte diminution de l'invasion des cellules hôtes causée par un défaut d'adhésion aux cellules hôtes et de motilité du parasite, qui pourrait être corrélé à un défaut des parasites extracellulaires pour sécréter

l'adhésine MIC2, essentielle à la régulation des deux processus. En outre, par imagerie en temps réel, une accumulation intense de vésicules positives Rab11A a été contrôlée au sommet des parasites motiles qui adhèrent et envahit la cellule hôte. Ce résultat a conduit à l'hypothèse que Rab11A pourrait réguler l'adhésion et la motilité des parasites via la sécrétion apicale polarisée de facteurs régulateurs contenus dans les DG, les micronèmes ou les compartiments sécrétoires encore non caractérisés. L'évaluation de cette hypothèse représente le sujet principal de mon projet de thèse.

## II- Objectifs du projet de recherche :

### 1- Caractériser plus en détail le rôle de Rab11A dans la sécrétion constitutive lors de la réplication intracellulaire du parasite.

Cette partie de mon projet vise à approfondir l'étude de la sécrétion dépendante de Rab11A par imagerie en direct afin de compléter les données précédentes obtenues par KV. En particulier, nous nous sommes intéressés à caractériser quelle étape du processus de sécrétion est régulée par Rab11A : ancrage et mouvement des vésicules sur le cytosquelette du parasite ou/et fixation/fusion des vésicules à la membrane plasmique. Cette partie de mon projet a permis de terminer l'étude des fonctions de Rab11A et un article publié, dans lequel je partage la première autrice avec KV.

### 2- Caractériser le rôle du complexe HOOK-FTS-HIP (HFH) dans le transport apical et l'exocytose des micronèmes matures.

Pour disséquer d'avantage les mécanismes moléculaires régulant cette activité sécrétoire dépendante de Rab11A, nous avons cherché à trouver des molécules partenaires. KV a effectué une co-immunoprécipitation (co-IP) avec une version étiquetée de Rab11A et a identifié une protéine contenant un domaine HOOK comme partenaire putatif. La famille de protéines HOOK, se compose de protéines largement conservées qui contribuent au trafic endosomal. La plupart des eucaryotes codent pour une seule isoforme HOOK ; cependant, les mammifères ont trois paralogues, qui semblent avoir des fonctions spécifiques et des tropismes cellulaires ayant également un impact sur la mitose, la cytokinèse jusqu'à la spermatogenèse (Dwivedi D, 2019)

(Olenick MA, 2019). De plus, HOOK joue un rôle dans les organites endocytaires précoces et tardifs, chez les mammifères, par son interaction avec Rab5 ou 7. Nous avons donc décidé d'approfondir et d'étudier en détail cette protéine et son rôle dans la régulation des événements sécrétoires du parasite.

## III- Résultats :

### 1- Caractériser plus en détail le rôle de Rab11A dans la sécrétion constitutive lors de la réplication intracellulaire du parasite.

Pour caractériser d'avantage la localisation dynamique des vésicules positives pour Rab11A, j'ai effectué une imagerie en direct à l'aide d'une microscopie confocale à disque rotatif dans des parasites en réplication intracellulaire exprimant la protéine Rab11A WT-mcherry. Nous avons observé que les vésicules positives pour Rab11A présentaient un mouvement bidirectionnel rapide entre les pôles apical et basal du parasite, qui suivait les côtés latéraux du parasite où les réseaux d'actine et de microtubules sont ancrés à l'IMC. Pour approfondir cet aspect, nous avons utilisé des inhibiteurs spécifiques du réseau actine versus microtubule pour évaluer leur rôle dans le mouvement des vésicules Rab11A. Nous avons traité les parasites avec de la cytochalasine D (CD) qui déclenche la dépolymérisation des filaments d'actine, ou de l'oryzaline (ORYZ) qui affecte le cytosquelette des microtubules, et avons surveillé le déplacement des vésicules en utilisant l'imagerie en direct. Le mouvement des vésicules de Rab11A était totalement inhibé lors du traitement par CD mais pas par ORYZ, suggérant un processus dépendant de l'actine.

De plus, en utilisant l'imagerie en direct, nous avons pu confirmer les données précédemment obtenues par KV montrant que les parasites Rab11ADN présentent un défaut dans la sécrétion de granules denses. Ceci a été contrôlé en co-exprimant la protéine soluble SAG-ΔGPI-GFP, qui se localise dans les DG, avec la protéine Rab11AWT-mcherry ou Rab11ADN-mcherry. Les films, acquis à l'aide d'un microscope confocal à disque rotatif équipé d'un module de super-résolution, ont montré que les DG (vésicules positives SAG-ΔGPI-GFP)

étaient retenus à l'intérieur du cytoplasme des parasites exprimant Rab11ADN, au lieu d'être sécrétées dans l'espace vacuolaire du PV dans des conditions WT. Tout d'abord, l'analyse de colocalisation à l'aide du logiciel Imaris a démontré que les vésicules de Rab11A-mcherrt et les DG co-localisent partiellement et peuvent être associés au déplacement du DG sur une longue distance le long du côté latéral du parasite. Ensemble, ces données suggèrent fortement que Rab11A peut s'associer à DG et peut réguler leur transport le long du cytosquelette d'actine ancré à l'IMC, ainsi que l'étape finale de l'exocytose dans l'espace vacuolaire. Enfin, nous avons examiné l'administration de la protéase rhomboïde transmembranaire ROM4 à la surface du parasite après avoir exprimé de manière transitoire une version étiquetée Ty de la protéine ROM4 chez les parasites Rab11A-WT et Rab11ADN. De la même manière que GT1, ROM4 a été retenu dans les vésicules internes de la souche parasitaire défectueuse pour l'activité de Rab11A (Rab11ADN) au lieu d'être délivré à la membrane plasmique du parasite dans des conditions de contrôle (Rab11AWT), suggérant que Rab11A est impliqué dans la sécrétion non seulement de DG, mais aussi de différentes protéines transmembranaires. Il est à noter que, profitant du défaut d'exocytose des parasites Rab11ADN, nous avons observé que les vésicules cytoplasmiques retenues DG et ROM4 positives étaient mal co-localisées suggérant pour la première fois des voies sécrétoires constitutives distinctes chez *T. gondii*.

De plus, KV a étudié la localisation de Rab11A pendant l'adhésion, la motilité et l'invasion du parasite. Il a observé, en utilisant l'imagerie en direct, une accumulation massive et polarisée inattendue de vésicules positives pour Rab11A au pôle apical des parasites adhérents et envahissants. Ce résultat suggère un rôle putatif de Rab11A dans la livraison polarisée de facteurs parasitaires à l'apex du parasite pendant l'adhésion et la motilité, les deux activités étant altérées chez les parasites Rab11ADN. Ce phénomène rappelle ce qui est observé dans les cellules de mammifères tout au long de la migration cellulaire polarisée, au cours de laquelle la livraison dépendante des microtubules de vésicules au bord d'attaque assure une fixation cellulaire

efficace et une progression vers l'avant. Par conséquent, l'étude de ce processus de sécrétion dépendant de Rab11A peut fournir de nouvelles informations sur la façon dont T. gondii effectue une adhésion et une entrée efficaces dans les cellules hôtes. Nous avons donc décidé de caractériser les mécanismes moléculaires responsables de la sécrétion polarisée médiée par Rab11A et de tenter d'identifier les cargaisons livrées.

**2- Caractériser le rôle du complexe HOOK-FTS-HIP (HFH) dans le transport apical et l'exocytose des micronèmes matures.**

En utilisant des tests de pull-down, KV a identifié la protéine unique contenant le domaine HOOK de *T. gondii*, en tant que partenaire de Rab11A actif lié au GTP. Il a également pu confirmer que *Tg*HOOK n'interagit qu'avec Rab11, pas avec Rab5 et Rab7, suggérant un rôle unique dans les événements sécrétoires dépendants de Rab11A.

Les protéines HOOK possèdent un domaine N-terminal hautement conservé qui médie la fixation aux microtubules, un motif central enroulé en spirale qui médie l'homodimérisation et un domaine C terminal divergent impliqué dans la liaison à des organites spécifiques (domaine de liaison aux organelles) (Walenta JH, 2001). Ils sont cytoplasmiques et présentent dans certains cas une localisation enrichie en organites cellulaires (Walenta J. H., 2001). Il a également été démontré que les protéines HOOK agissent comme des molécules adaptatrices pour la dynéine et jouent un rôle dans le trafic vésiculaire en ancrant les vésicules aux pistes des microtubules via leur interaction avec les protéines Rab (Krämer H, 1996) (Maldonado-Báez L, 2013) (Maldonado -Báez L., 2013). Ainsi, les protéines HOOK sont couplées à la voie endocytaire, mais leurs fonctions biologiques précises sont en fait mal comprises. Richardson SC et al ont démontré que HOOK1 se lie au complexe de tri des protéines vacuolaires homotypiques (HOPS) pour favoriser la fusion homotypique et le regroupement des endosomes/lysosomes précoces et tardifs dans les cellules de mammifères (Richardson SC,2004).

KV a généré une lignée parasitaire stable Knock-In exprimant cette protéine avec une étiquette HA à l'extrémité C terminale afin d'examiner sa localisation.

Il a trouvé que *Tg*HOOK est localisé dans des vésicules réparties dans tout le cytoplasme mais enrichies à l'extrémité apicale du parasite au niveau du conoïde, sous l'anneau polaire apical (APR). *Tg*HOOK affiche un modèle de distribution similaire aux protéines de micronème, donc tout d'abord il a été appelé MIC18. Cependant, nous avons constaté qu'il ne colocalise avec aucune des protéines MIC telles que MIC2 lors de la réplication intracellulaire. De plus, une faible colocalisation a été observée entre les vésicules Rab11A et TgHOOK, limitée uniquement aux vésicules localisées au pôle basal. De plus, nous n'avons vu aucun défaut dans la localisation et la morphologie des compartiments positifs pour Rab5 et Rab7 chez les parasites intracellulaires.

Le rôle de HOOK a été abordé par la génération d'un knock-out (HOOK-KO) à l'aide de la technologie CRISPR/Cas9. In vitro, les parasites dépourvus de HOOK présentaient un grave défaut dans le cycle lytique basé sur le test de plaque. Une dissection phénotypique supplémentaire a révélé un léger défaut de croissance intracellulaire lorsqu'il est mesuré à 30 heures, et des défauts modérés de leur capacité d'invasion et de sortie. Les phénotypes les plus sévères par rapport aux parasites de type sauvage étaient la motilité du parasite et le défaut d'attachement à la cellule hôte. Remarquablement, des tests de sécrétion de micronèmes ont été effectués et aucun défaut d'exocytose discernable n'a été observé en l'absence de HOOK qui pourrait expliquer une altération significative de l'attachement. De plus, aucun changement dans la stabilité ou le trafic des protéines micronèmes n'était détectable.

Pour étudier le rôle fonctionnel de HOOK, nous avons cherché à trouver des molécules partenaires qui interagissent avec *Tg*HOOK pour aider à identifier les mécanismes moléculaires impliqués dans la sécrétion polarisée dépendante de Rab11A. Pour ce fait, des tests de co-immunoprécipitation HOOK-HA ont été réalisés. Étonnamment, seules deux protéines, une protéine de la sous-famille de l'enzyme conjuguée à l'ubiquitine (TGGT1_264050) et une protéine hypothétique à fonction inconnue (TGGT1_306920), ont été identifiées, suggérant la formation d'un complexe stable. TGGT1_264050 et

TGGT1_306920 ont été adressés ici en tant que *Tg*FTS (par homologie avec la protéine Foot Toes décrite chez d'autres eucaryotes) et *Tg*HIP (*Tg*HOOK Interacting Protein), respectivement.

Pour examiner la localisation de ces protéines non caractérisées chez T. gondii, nous avons commencé par générer des souches de parasites exprimant 2Ty étiqueté-APR1 dans des lignées KI exprimant FTS étiqueté cMyc ou HOOK étiqueté HA, en plus du HIP étiqueté 2Ty. Chez les parasites mobiles extracellulaires, TgHOOK, TgFTS et TgHIP sont enrichis à l'extrémité apicale, adjacente ou à l'intérieur du conoïde, sous APR1.

Pour aborder le rôle et l'importance de ces protéines et déchiffrer leur rôle biologique, un système Degron induit par l'auxine (AID) a été appliqué. Une étiquette miniAID C-terminale (mAID) a été ajoutée au locus endogène de FTS et HIP dans une souche exprimant la réponse d'inhibiteur de transport 1 (Tir1) pour cibler la protéine pour la dégradation protéosomique lors de l'ajout d'acide indole-3-acétique auxine (IAA). Les conséquences phénotypiques de la déplétion FTS et HIP ont révélé un défaut sévère d'invasion alors que la sortie était légèrement affectée et uniquement dans le cas de la déplétion HIP. Les deux mutants présentent une altération dans l'exocytose des micronème lors d'un test de sécrétion induite en présence d'EtOH à 2 %. En revanche, les deux mutants présentaient une croissance intracellulaire normale et une protrusion conoïde. De plus, la biogenèse et le positionnement des organites apicaux tels que visualisés par IFA et EM n'ont pas été affectés. Pris ensemble, la délétion de FTS et de HIP présentent des phénotypes très comparables suggérant que les deux protéines pourraient contribuer au même processus biologique. En revanche, la délétion de HOOK a affiché un phénotype plus doux.

Les données obtenues jusqu'à présent suggèrent l'existence d'un complexe HOOK-FTS-HIP à l'extrémité apicale du parasite pertinent pour l'approvisionnement et la reconstitution des protéines du micronème pour assurer l'attachement, l'invasion et la sortie de la cellule hôte. En général, le retrait d'un composant d'un complexe peut déstabiliser et mal cibler le reste du complexe. Pour décrypter l'arrangement hiérarchique de HOOK-FTS-HIP,

une co-immunoprécipitation utilisant des billes d'agarose anti-HA et anti-cMyc, suivie d'un Western Blot, a été réalisée trois fois avec la souche double KI et la souche cMyc HOOK-iKD comme contrôle négatif. Nous avons obtenu deux bandes des deux protéines à la taille attendue, suggérant que *Tg*HOOK interagit parfaitement avec *Tg*FTS.

Comme nous avons utilisé la lignée Tet inductible knockdown cMyc HOOK-iKd afin d'obtenir le double KI, nous avons pensé pouvoir l'utiliser afin d'obtenir un pseudo-double Knockout (KO). Donc en gros, quand on ajoute de l'anhydrotétracycline (ATc) à la souche double KI, on peut la considérer comme un pseudo double KO.

Afin de vérifier en outre l'interaction entre les protéines HOOK et FTS, nous avons effectué une WB en utilisant cMyc HOOK-iKD/FTS-HA -/+ATc et RHTatiΔKU80 comme contrôle négatif. En comparant les résultats obtenus pour la pseudo-double KO à la double lignée KI, on peut voir que l'expression de HOOK était totalement réprimée et l'expression de FTS était réduite de 2 fois, 48 heures après l'ajout d'ATc. Ces résultats suggèrent que les deux protéines interagissent non seulement ensemble mais aussi que l'expression de l'une dépend de l'expression de l'autre, ce qui est en accord avec un complexe HFH stable. Il convient de noter que des résultats similaires ont déjà été décrits dans (Xu et al., 2008), où ils ont découvert que la délétion de HOOK entraîne la dégradation de la protéine FTS.

## IV- Conclusion :

Puisqu'il n'y a pas de défaut évident dans l'abondance des protéines MIC2 dans la région apicale du parasite dans les souches intracellulaires mutantes HFH, nous pourrions suggérer deux rôles pour les membres du complexe HFH. Dans le premier modèle, nous supposons que *Tg*HOOK peut interagir avec les moteurs ancrés dans les microtubules (tels que la dynéine), tandis que *Tg*FTS et *Tg*HIP sont liés à *Tg*HOOK et facilitent le transport des protéines du micronème vers la pointe apicale à proximité de la membrane plasmique où l'exocytose aura lieu. Dans le deuxième modèle, nous supposons que le complexe HFH permet d'ancrer les protéines du micronème à l'anneau polaire apical (APR1) avant qu'elles ne se

déplacent dans le conoïde à la suite d'un signal de sécrétion par un processus ne dépendant pas du complexe HFH.