THE CLOËTTA PRIZE 2015
IS AWARDED TO

PROFESSOR

DOMINIQUE SOLDATI-FAVRE

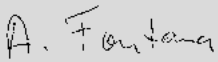
BORN IN 1962 IN CHENIT (VD), SWITZERLAND
PROFESSOR IN THE DEPARTMENT OF MICROBIOLOGY
AND MOLECULAR MEDICINE AND VICE-DEAN OF THE
FACULTY OF MEDICINE AT THE UNIVERSITY
OF GENEVA

MEMBER OF THE SWISS ACADEMY OF MEDICAL SCIENCES
FOR HER OUTSTANDING WORK IN THE DOMAIN OF
MOLECULAR PARASITOLOGY

GENEVA, 13 NOVEMBER 2015

IN THE NAME OF THE FOUNDATION BOARD:

THE PRESIDENT



THE VICE PRESIDENT

MEMBER

Prof. Dr. Dominique Soldati-Favre

BIOGRAPHY

Family name, First name: **Soldati-Favre, Dominique**
Nationality: Swiss
Status: married, four children
Date of birth: June 26th 1962, Switzerland
URL for web site:http://www.unige.ch/medecine/mimo/fr/groupes/773soldati-favre/

Education
1995–2000: Private-Docent in Cell Biology, University of Heidelberg, Germany. 1987–1990: PhD in Molecular biology University of Zurich, Switzerland. 1986 Master in Biochemistry, University of Geneva, Switzerland.

Current Position
2010–: Full Professor Faculty of Medicine, Department of Microbiology and Molecular Medicine, University of Geneva, Switzerland.

Previous Positions
2004–2010: Associate Professor Faculty of Medicine University of Geneva, Switzerland. 2001–2004: Senior Lecturer and Reader Imperial College London, United Kingdom. 1995–2001: Assistant Professor at the ZMBH University of Heidelberg, Germany. 1991–1995: Post-doctoral fellow University of Stanford, Medical School, USA.

Fellowships and Awards
2015: Cloëtta Foundation Award. 2014: Member of the Swiss Academy of the Medical Sciences. 2013: Member of the European Academy of Microbiology. 2012–2017: HHMI Senior International Research Scholar. 2012: Prix Pfizer for basic Research in infection 2012. Laureate: Joana Santos. 2011: EMBO Member. 2009: Prix Pfizer for basic Research in infection 2009. Laureate: Fabienne Plattner. 2009: Prix de la Fondation Gertrude von Meissner together with Dr. Paco Pino. 2000–2005: HHMI International Scholar in infectious diseases. 2005–2010: HHMI Interna-

tional Scholar in infectious diseases. 2001: Rodolfi Medal Deutsche Gesellschaft für Parasitologie. 1993–1994: EMBO long-term fellowship. 1994: Förderungspreis der Schweizerischen Gesellschaft für Mikrobiologie. 1991–1992: SNSF post-doctoral fellowship.

Supervision of Graduate Students and Postdoctoral Fellows
1995–2015: 15 Postdoctoral fellows/25 PhD students/13 Master students.

Selected Mentoring Activities
2014–: Member of the Swiss-French network for mentoring Women careers. 2008–: Member of the Educational board of SystemX.ch. 2012: Cell Biology workshop Bamako Mali. 2011–2013: Instructor at MBL summer course on the Biology of Parasitism Woods Hole. 2010–2014: Coordinator of the SNSF ProDoc training program on Infection & Immunity. 2009–2011: Swiss-German Mentoring Program for Women careers' development. 2008–2012: Helmholtz International Research School for Infection Biology Germany.

Institutional Responsibilities
2011–: Vice-dean for research at the Faculty of Medicine, University of Geneva. 2008–: President of the Graduate School Biology-Medicine, Faculty of Medicine.

Editorial Activities
2011–: Editor at Molecular Microbiology. 2006–: Associate Editor PLoS Pathogens. 2006–: Contributor for F1000 «the Faculty of 1000». 2007–2010: Deputy Editor International Journal for Parasitology.
Editorial board member: eLife, Cell Host & Microbe, Cellular Microbiology, Traffic, Current Opinion in Microbiology, BMC Microbiology
Reviewer for grants: National Science Foundation (USA), DFG (D). ANR, ATIPs (F), BBSRC (UK) NSERC, EMBO fellowships.

Commissions of Trust
2010–: Member of the Swiss National Research Council, Div. III Biology-Medicine. 2012–: Member of the Wellcome Trust's Peer Review College. 2008–: Member of the Friedrich Miescher Award committee. 2008–2015: Expert for the European Research Council.

Member of evaluation committee Helmholtz Research-Programme.
Member of the SAB Sanger Institute Malaria Programme, Wellcome Trust.
Member of the Working Group Malaria Host-Pathogen Interaction Center NIAID & Emory.

Selected Invitations to International Conferences
Molecular Approaches to Malaria Lorne, Australia MAM 2004, 2008, 2012.
Gordon Research Conference on Host-Parasite Interactions GRC 2008, 2010, 2012.
G8 Summit in Sapporo on Women career Development 2008.
10th Anniversary Hunter Cellular Biology Meeting 2009.
36th FEBS Congress, Torino 2011.
International Congress of Parasitology, Australia 2010, Mexico 2014.
The Human Genome Organization, Singapore 2013.
11th International Coccidiosis meeting, Dresden 2014.

Organiser of International Conferences
Gordon Research Conference on Host-Parasite Interactions 2014.
EMBO conference «Subversion of host cellular functions by pathogens» 2010, 2012.
Molecular Parasitology meeting, Woods Hole 2007, 2008, 2009.

Selected Funding
2015–2017: Sinergia-SNSF co-applicant with A Hehl and D. Bumann. 2014–2017: SystemsX.ch-IPhD co-applicant with A. Hehl and V. Hatzimanikatis. 2013–2016: Project grant Division III-SNSF. 2012–2016: HHMI International Senior Scholar. 2009–2015: EVIMalaR-FP7 FP7 Network of Excellence.

Publications
Total number of publications: 129 (84 research articles, 39 reviews, 6 book chapters)
H-index 46

SELECTED PUBLICATIONS

1. Hammoudi PM, Jacot D, Mueller C, Di Cristina M, Dogga SK, Marq JB, Romano J, Tosetti N, Dubrot J, Emre Y, Lunghi M, Coppens I, Yamamoto Y, Sojka D, Pino P and Soldati-Favre D. Fundamental roles of the Golgi-associated Toxoplasma aspartyl protease, ASP5, at the host-parasite interface. PloS Pathog. 2015. ***In press.***

2. Oppenheim RD, Creek DJ, Macrae JI, Modrzynska KK, Pino P, Limenitakis J, Polonais V, Seeber F, Barrett MP, Billker O, McConville MJ, Soldati-Favre D. BCKDH: the missing link in apicomplexan mitochondrial metabolism is required for full virulence of ***Toxoplasma gondii*** and ***Plasmodium berghei.*** PLoS Pathog. 2014 Jul 17;10(7):e1004263.

3. Frénal K, Marq JB, Jacot D, Polonais V, Soldati-Favre D. Plasticity between MyoC- and MyoA-glideosomes: an example of functional compensation in ***Toxoplasma gondii*** invasion. PLoS Pathog. 2014 Nov 13;10(10):e1004504

4. Jacot D, Daher W, Soldati-Favre D. ***Toxoplasma gondii*** myosin F, an essential motor for centrosomes positioning and apicoplast inheritance. EMBO J. 2013 Jun 12;32(12):1702–16.

5. Mueller C, Klages N, Jacot D, Santos JM, Cabrera A, Gilberger TW, Dubremetz JF, Soldati-Favre D. The Toxoplasma protein ARO mediates the apical positioning of rhoptry organelles, a prerequisite for host cell invasion. Cell Host Microbe. 2013 Mar 13;13(3):289–301.

6. Pino P, Sebastian S, Kim EA, Bush E, Brochet M, Volkmann K, Kozlowski E, Llinás M, Billker O, Soldati-Favre D. A tetracycline-repressible transactivator system to study essential genes in malaria parasites. Cell Host Microbe. 2012 Dec 13;12(6):824–34.

7. Frénal K, Polonais V, Marq JB, Stratmann R, Limenitakis J, Soldati-Favre D. Functional dissection of the apicomplexan glideosome molecular architecture. Cell Host Microbe. 2010 Oct 21;8(4):343–57

8. Daher W, Plattner F, Carlier MF, Soldati-Favre D. Concerted action of two formins in gliding motility and host cell invasion by ***Toxoplasma gondii.*** PLoS Pathog. 2010 Oct 7;6(10):e1001132.

9. Plattner F, Yarovinsky F, Romero S, Didry D, Carlier MF, Sher A, Soldati-Favre D. Toxoplasma profilin is essential for host cell invasion and TLR11-dependent induction of an interleukin-12 response. Cell Host Microbe. 2008 Feb 14; 3(2):77–87.

10. Foth BJ, Goedecke MC, Soldati. New insights into myosin evolution and classification. Proc Natl Acad Sci U S A. 2006 Mar 7;103(10):3681–6.

THE INS AND OUTS OF HOST CELL INVASION BY TOXOPLASMA GONDII

Dominique Soldati-Favre

«It is not the most intellectual or the strongest of species that survives; but the species that survives is the one that is able to adapt to and adjust best to the changing environment in which it finds itself»
Charles Darwin

Summary

One of the fundamental challenges in parasitology is to study and understand how a eukaryotic cell can penetrate survive and replicate within another eukaryotic cell. Pathogens have evolved a great variety of mechanisms to evade the host immune system. One of the efficient strategies is to adopt an intracellular life style. While bacterial pathogens can easily enter non-professional phagocytes by induced-phagocytosis, such a task is more complicated for the significantly larger protozoan parasites. *Leishmania* species rely on the host phagocytosis activity to invade the host but its range of host cell types is limited to macrophages and dendritic cells and consequently, this parasite needs to cope with the hostile environment of the phagolysosomes/lysososmes. In contrast, *Trypanosoma* cruzi penetrates host cells by a more active process, involving the recruitment of the lysosomal compartment at the periphery of the host cell[1]. Soon after penetration, the parasite escapes into the cytosol where it replicates safely. Most Apicomplexa have evolved a unique, fast and active mode of host cell entry. This parasite driven process presents numerous advantages i) the parasite can enter almost any cell types including red blood cells as it is the case for *Plasmodium* species ii) this smooth mode of penetration does not trigger the host cell defense mechanisms iii) the process leads to the formation of a parasitopho-

rous vacuole, a membrane bound compartment that is segregated from most cellular trafficking pathways[2]. This compartment is largely devoid of host cell proteins and completely refractory to acidification and lysosomal fusion, enabling the parasite to survive in almost any cell type including macrophages.

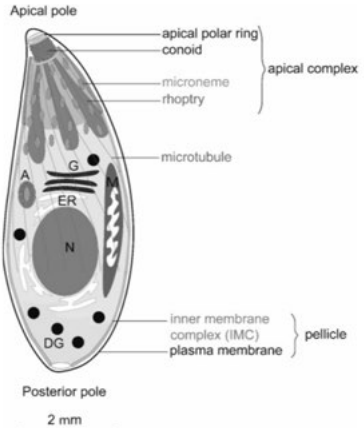
Toxoplasma gondii is an ideal parasite to address some aspects of the vast and fascinating question of host cell entry. This obligate intracellular parasite is one of the most successful invaders, with a remarkable ability to actively penetrate almost any nucleated cells from virtually all warm-blooded animals. This genetically tractable parasite is amenable to biochemical studies and its convenient size combined with its excellent preservation after fixation makes it best suited for high-resolution light and electron microscopy analyses.

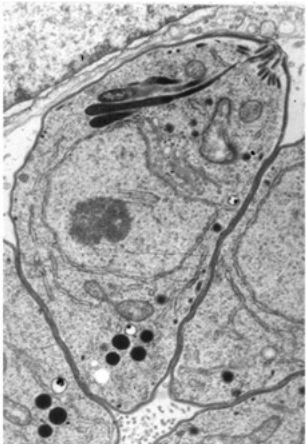
Over the last 25 years, many laboratories including ours have investigated the invasion process in the hope to resolve it in detail at the molecular level. This journey led us to study myosin motors, actin dynamics, adhesins, proteases and more recently lipid signaling mediators. A prerequisite to this work was the establishment of a robust inducible system to dissect the function of essential genes and a better understanding of protein trafficking and organelle biogenesis in this parasite.

Toxoplasma gondii is a member of the Apicomplexa phylum

Protozoan parasites belonging to the phylum of Apicomplexa are of enormous medical and veterinary significance, being responsible for a wide variety of diseases in human and animals, including malaria, toxoplasmosis, coccidiosis and cryptosporidiosis. The life cycle if these of these parasite is complex, alternating between sexual and asexual stages as well as intermediate and definitive hosts[3]. *Toxoplasma T. gondii* is a zoonotic, apicomplexan parasite that belongs to the subclass of coccidians. This protozoan is an important cause for abortion in sheep and responsible for important economic loss. In humans, the global prevalence for *T. gondii* infections is estimated to be approximately 30 %. Infection during pregnancy can lead to congenital toxoplasmosis, which may have serious consequences for the new born or cause abortion. For the majority of in-

fected people, the disease is asymptomatic. Although clinical symptoms can occur in healthy individuals, in particular manifesting as ocular toxoplasmosis and abortion or birth defects, mostly immunosuppressed people are severely affected. The natural life cycle of *T. gondii* takes place in the definitive felid host that sheds up to 10^8 environmentally resistant oocysts. Humans are infected either by ingestion of oocysts shed by felids or by bradyzoites in tissue cysts form infected meat. The high prevalence of latent toxoplasmosis in intermediate hosts such as sheep, pigs, and free-range poultry is responsible for the direct introduction of infectious *T. gondii* tissue cysts into the human food chain. Early during infection, dendritic cells are recruited for rapid dissemination of the fast-replicating tachyzoites[4]. These infected cells are believed to serve as *Trojan horse* for the parasite to cross biological barriers, i.e. into the extravascular space of the brain[5] or across the placenta into the foetus[6] where further dissemination occurs.



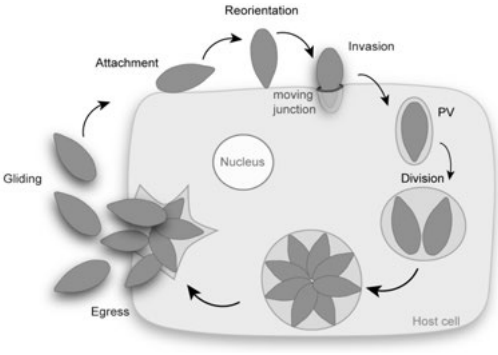


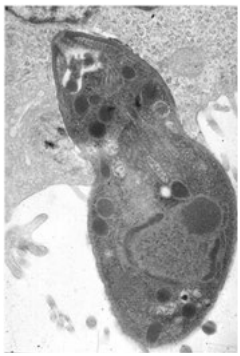
JF Dubremetz

Members of the Apicomplexa are morphologically unified by a common set of apical structures suggesting that invasion is to a large extent mechanistically conserved across the phylum. The apical complex is composed of *rhoptry* and *microneme* organelles that secrete their contents in a regulated fashion during motility and invasion. The coccidian subgroup of

Apicomplexa possesses a dynamic organelle termed the *conoid*. This organelle composed microtubule fibers arranged into a spiral, protrudes in motile and invading parasites.

The actin cytoskeleton has previously been reported to play an essential role in host cell invasion by *T. gondii*[7] *Eimeria* and *Plasmodium*. However, unexpectedly, the parasite actin appears to form short and unstable filaments. As members of the *genus* Alveolata, the Apicomplexa possess a pellicle composed of three membrane layers composed of the plasma membrane and an *inner membrane complex (*IMC) formed by juxtaposed flattened vesicles underneath the plasma membrane and covering a basket of sub-pellicular microtubules[8,9]. The IMC is interconnected with the parasite cytoskeleton and plays a fundamental role in motility and cytokinesis.





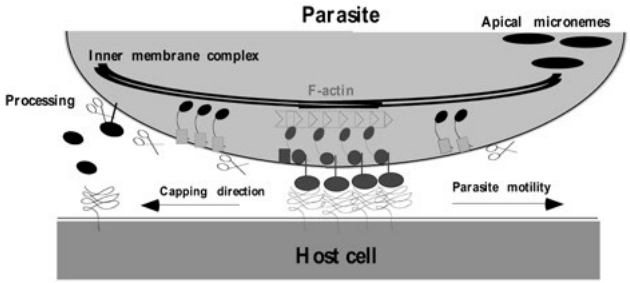
JF Dubremetz

The lytic cycle of *T. gondii* can be divided into several distinct steps that include i) apical attachment to the host cell, ii) parasite apical reorientation, ii) formation of a circular junction (CJ) also referred to as the moving a moving, iii) penetration, iv) sealing of the *parasitophorous vacuolar membrane* (PVM) and host cell plasma membrane. Immediately following invasion, the parasite comes to rest within the PV and vi) at once initiates a program of cell division to produce new daughter cells equipped to egress[10]. Two daughter cells are produced per replication cycle by a mechanism called endodyogeny. Parasites undergo several rounds of rep-

lication prior to vii) egress that causes lysis of the host cell. Finally, viii) the parasites glide and rapidly invade neighboring cells, to initiate new lytic cycles.

Motility and host cell invasion: The «capping model»

In the absence of locomotive organelles such as cilia or flagella, the invasive tachyzoites of *T. gondii* exhibit an unusual form of substrate-dependent motility that is essential for host cell invasion[9]. According to the *«capping model»*[11] gliding motility is driven by the redistribution of adhesins associated to host cell receptors, towards the posterior pole of the parasites. This translocation is powered by the parasite actomyosin system, which is anchored in the inner membrane complex and linked to the cytoplamic tail of the microneme proteins bound to host receptors or extracellular matrix[12]. The model implied the existence of a connector able to couple surface adhesins to the actomyosin system underneath the plasma membrane as well as the action of proteases to release the host-parasite interactions.



Taken together, *T. gondii* utilizes a substrate-dependent motion to propel into host cells and to spread throughout the host organism. The molecular machinery that powers parasite locomotion involves multiple classes of proteins, which are to great extent conserved across the members of the phylum and constitutes the «*glideosome*». To define how structural proteins of the cytoskeleton, the cytoskeletal dynamics and motor proteins are harnessed to generate movement, we have investigated i) regulated exocytosis of apical adhesins, ii) actin polymerization underneath the plasma membrane, iii) recruitment of a connector to link adhesins to

the actomyosin system. iv) myosins implicated in the translocation of adhesins to the rear of the parasite, v) proteolytic processing of the adhesins. Prior to the identification and functional characterization of these various classes of invasion factors, we first established an inducible system to study the function of essential genes in *T. gondiii.*

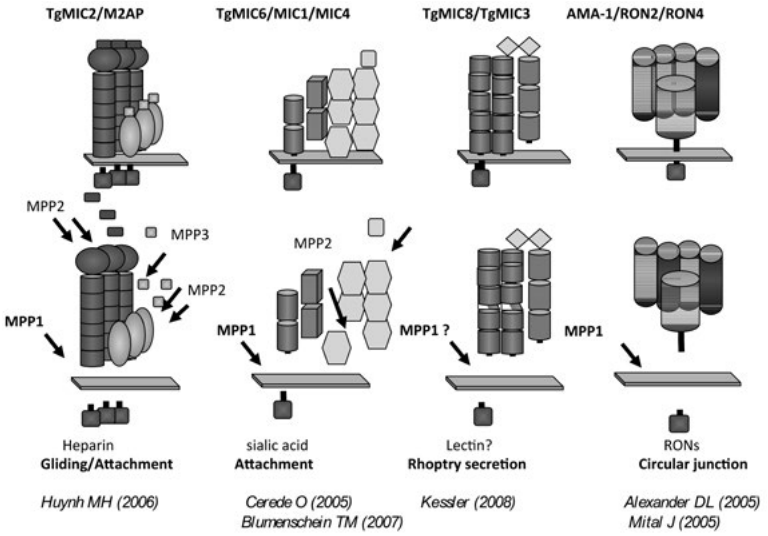
Development of Toxoplasma gondii as a genetically tractable system

Among the Apicomplexa, *T. gondii* was the most attractive model organism with great potentials for genetic tractability. Initially, we developed a transient transfection system[13] and soon after established stable selection protocols for this parasite[14]. We were able to disrupt the first gene in an apicomplexan parasite and also to rescue it[15]. This technology offered the opportunity to study the elements involved in the regulation of gene expression in this primitive eukaryote[16]. Genetic manipulation coupled to expression of green fluorescent protein helped understanding the biogenesis and sorting of secretory proteins[17,18]. We then invested considerable efforts toward the establishment of an inducible control system for gene expression and notably explored the use of Cre recombinase[19]. We first established an inducible gene expression system based on the tetracycline repressor (tetR) of *Escherichia coli*[20]. This system is suitable to conditionally express toxic genes and dominant negative mutants but not appropriate to generate conditional knockouts. In consequence, we developped a genetic screen based on random integration in order to identify a parasite specific transactivator. This approach led us to the establishment of a robust inducible system suitable for the study of essential genes[21].

Regulated secretion of adhesins by the micronemes

The information obtained from *T. gondii* expression sequence tags prior to the era of genome sequencing, uncovered the existence of proteins harboring protein-protein interaction domains and adhesive domains including EGF-like, integrin-like, thrombospondin, and apple domains containing proteins. A comprehensive investigation of these proteins revealed that they corresponded to microneme proteins (MICs), assembled as complexes and contributing to invasion with non-overlapping functions. We established that some transmembrane MICs could act as escorters for targeting of multi-protein complexes to the micronemes[22]. We first charac-

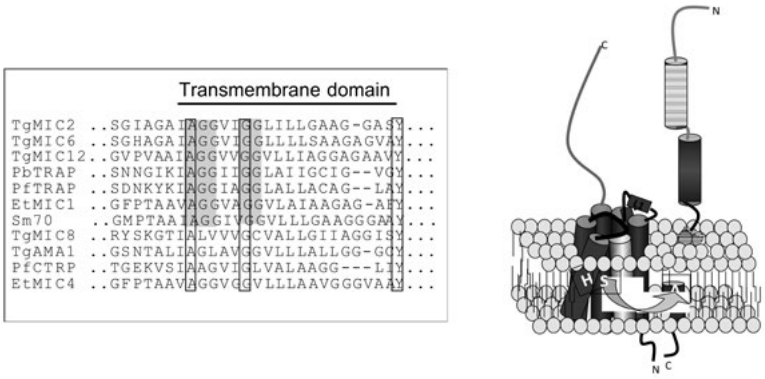
terized the complex composed of TgMIC1, TgMIC4 and TgMIC6[23,24]. MIC6 serves as escorter, is processed within the transmembrane domain during invasion, and contains the conserved C-terminal acidic residues and a tryptophan residue presumed to connect with the actomyosin system. MIC6 contains EGF-like domains, MIC4 contains six apple domains and MIC1 contains two thrombospondin-like domains. Both MIC1 and MIC4 exhibit adhesive properties and bind to host cells. We have built on a fruitful interdisciplinary approach to study the structure/function relationships of the TgMIC1-4-6 complex in collaboration with Dr. S. Matthews at Imperial College. This led to the demonstration that carbohydrate structures on cell surfaces are finely exploited by *T. gondii* for invasion. TgMIC1 is an important adhesin, exhibiting a new structural module termed Microneme Adhesive Repeat (MAR) that recognizes terminally sialylated oligosaccharides on the host cell surface[25,26]. We have elucidated the domains responsible for the interaction between TgMIC1 and its escorter protein TgMIC6, providing new information on the stoichiometry of the complex[27]. Analysis of TgMIC4 led us to postulate that galactose recognition by TgMIC4 may compromise host protection from galectin-mediated activation of the host immune system[28]. Finally, we described the family of MAR containing protein including TgMIC13, which displays distinct preferences to sialylated oligosaccharides on glycoarrays compared to TgMIC1[29]. Importantly, sialic acids that are ubiquitously found on the surface of all vertebrate cells at the extremities of glycan chains were known to be widely exploited by viruses and bacteria to enter host cells and now recognized to critically contribute to selective host cell recognition by the Apicomplexa[30]. We reported an interaction between TgMIC3 and TgMIC8[31] and TgMIC8 was subsequently demonstrated to play a key critical for invasion by contributing to rhoptry secretion[32]. TgAMA1 is an atypical microneme protein that also plays a fundamental role during invasion[33] but forms a large complex with parasite proteins secreted by the rhoptries. This complex uniquely localizes to the circular junction[34] and contains the rhoptry proteins TgRON2, TgRON4, TgRON5 and TgRON8[35] and the TM-MIC TgAMA1, which anchors the complex to the parasite plasma membrane. Others have shown that TgMIC2 forms a multimeric complex with the soluble partner TgM2AP[36,37]. Parasites depleted in TgMIC2 are markedly deficient in host-cell attachment and motility[38].



Proteolytic processing of the microneme proteins by rhomboids

Remarkably, most MICs are proteolytically cleaved during their biogenesis and/or post-exocytosis. We showed that TgAMA1, TgMIC2, TgMIC6, MIC8 and TgMIC12, are all cleaved during invasion within their transmembrane domain (TMD) at the cleavage site IA*GG[39,40] by a protease activity termed microneme protein protease 1 (MPP1) previously described[41]. This shedding was proved to critical for MIC2 function during invasion[42]. Studies have indicated that MPP1 is a member of the rhomboid protease family, a group of broad substrate-specificity serine proteases that recognize helix-destabilizing residues within the TMD of their substrates[43] Among the six rhomboid-like proteins encoded in the *Toxoplasma* genome[44] prime candidates for this shedding activity are TgROM4 and TgROM5[45,46]. ROM4 was recently shown to be responsible for the cleavages of MIC2, AMA1 and most likely MIC8, and to have an important but not essential function during invasion[47,48]. Taken together these results indicate that MPP1 activity primarily contributes to parasite reorientation during invasion by generating a gradient of adhesins that concentrates toward to apical pole rather than causing a disrup-

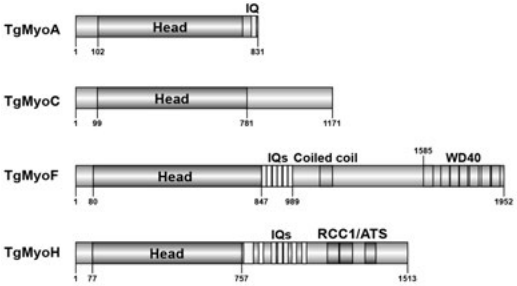
tion of the tight interaction between host cell and parasite at the end of the invasion process. We showed that in addition to TgROM4, TgROM5 is responsible for the processing of a small population of TgAMA1 that is not routing via the micronemes[48]. The significance of that residual cleavage especially in the context of the block in replication observed with a dominant negative catalytically dead mutant of TgROM4 complemented with TgAMA1tail[49] still await further investigations.



The actin-based motors of class XIV in Apicomplexa

The basic engine for this unique type of gliding locomotion is the actin cytoskeleton[7] and involves myosin(s) to generate the mechanochemical force along the actin filaments. In *T. gondii,* gliding is characterized by three distinct forms of motility: circular gliding, upright twirling, and helical rotation. All three forms of motility were disrupted by inhibitors of actin filaments (cytochalasin D) and myosin ATPase (butanedione monoxime), indicating that they rely on the parasite actomyosin system[50]. *T. gondii* possesses eleven myosins that we have been able to assign to various novel classes of myosins based on a comprehensive phylogenic analysis of their conserved heads[51]. The biochemical and biophysical properties of TgMyoA, heroically purified from the parasites, demonstrated that this motor is capable of hydrolysing ATP to track along actin filaments. Motility and microneedle/laser trap assays established that TgMyoA moves in unitary steps of 5.3 nm with a velocity of 5.2 mm/s and toward the plus (barbed) end of actin filaments. TgMyoA is thus likely

powered by novel structural and mechanical element, and is the first example of a fast, single-headed myosin[52]. The inducible system developed from *T. gondii* was exploited to generate a conditional knockout for *TgMyoA,* providing compelling evidence for the critical role of this motor in gliding motility, host cell invasion and egress, and pathogenicity *in vivo*[21]. Demonstration that TgMyoA propels the parasite into host cells validated the capping model[53]. TgMyoB and TgMyoC are encoded by two alternatively spliced mRNA variants and differ only in their C-terminal tails[54]. TgMyoC was demonstrated to play a key role in compensating for the absence of MyoA[55,56]. More recently, we have dissected the function of TgMyoH. This motor possesses 3 alpha tubulin suppressor domains, localizes to the conoid and is associated to microtubules. Despite the presence of MyoA, conditional disruption of TgMyoH revealed the essential nature of this motor for parasite motility, invasion and egress. The detailed phenotypic analyses established that TgMyoH in triggering the first step of the parasite helical motion along the conoid (Graindorge A et al, unpublished). In parallel we have investigated the role of MyoF, which is like MyoA broadly conserved across the phylum. MyoF is a non-dispensable motor implicated in apicoplast inheritance and in the apical positioning of the rhoptries[57]. Importantly, this motor acts in concert with an acylated armadillo repeats containing protein anchored at the surface of the rhoptries[58].

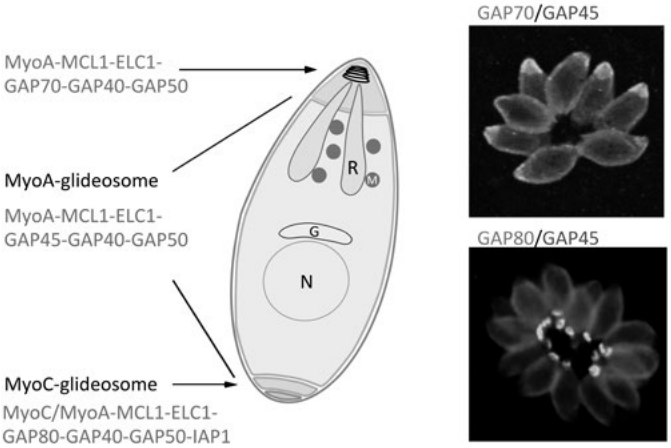


The myosin motor crucial for parasite motility and invasion (MyoA, MyoC and MyoH) and for organelles inheritance (MyoF)

The Components of the glideosome

T. gondii possesses 22 subpellicular microtubules that are covered by an elaborate matrix of articulin-like proteins connected to flattened membranous cisternae composing the IMC, which lies underneath the plasma

membrane. The rigid cytoskeleton formed by these elements is important for maintaining cell shape during parasite motility and invasion and for morphogenesis during cell division. Two gliding associated proteins, TgGAP45 and TgGAP50, have been shown to anchor TgMyoA and its associated light chain TgMLC1 to the IMC[59]. A detailed investigation of GAP45 lead us to establish that the protein is spanning between the plasma membrane and the IMC and plays a key role in preserving the pellicle integrity during invasion. Moreover, GAP70, a coccidian GAP45 homolog GAP70 specifically recruits the glideosome to the apical cap of the parasite[60]. Recently a third member of this family GAP80 was demonstrated to anchor TgMyoC at the posterior pole of the parasite[56].



Actin dynamics

F-actin has been difficult to detect in Apicomplexa with the majority of the actin pool is maintained under its globular form. However, the susceptibility of the parasite to actin-polymerizing and -depolymerizing drugs as well as molecular genetic studies confirmed that actin nucleation and polymerization is critical for motility. Unexpectedly, the Apicomplexans lack a canonical Arp 2/3 complex, which is otherwise widespread among eukaryotes to orchestrate actin assembly by nucleating filaments from the pointed end[61]. Instead these parasites rely on profilin (PRF)[62], two

conserved formins acting as potent actin nucleators[63,64] and a abundantly expressed actin-depolymerizing factor (ADF)[65] to control actin dynamics. We showed that TgPRF is not required for intracellular growth but is indispensable for gliding motility, host cell invasion, active egress from host cells and for virulence in mice[62]. Most interestingly, TgPRF has a separate role as ligand for TLR-11 and the conditional knockout was instrumental in confirming its role in the modulation of the immune response[66]. A study in Plasmodium provided evidence that formin 1 (PfFRM1) is capable of polymerizing actin and guilty by localization, suggestive of a role in motility and invasion[63]. *In T. gondii,* functional analyses of the two formins established that they both play an important role in gliding motility and work in concert during invasion as well as during egress TgFRM1 and TgFRM2 relocalize during invasion to the tip and periphery of the parasite, respectively. Furthermore, the FH2 domains of these fomins have been expressed and shown to exhibit a very potent activity in actin nucleation[64].

Discharge of apical secretory organelles

By successive regulated exocytosis, rhoptries and micronemes release their contents as part of the invasion process. Calcium-mediated signalling is critical for a microneme secretion and invasion[67] and an essential role of TgCDPK1 in the transduction of this calcium signalling was firmly demonstrated[68]. Indeed parasites depleted in CDPK-1 are defective in motility, invasion and egress and treatment with a specific inhibitor of TgCDPK1 recapitulates the phenotype[68]. Recently, TgDOC2.1 was identified as the target gene responsible for a *T. gondii* conditional mutant impaired in host cell invasion and egress[69]. TgDOC2 and other related proteins conserved in Apicomplexa resemble the ferlin/synaptotagmin that trigger many forms of exocytosis by acting on membrane fusion events, in diverse eukaryotic cells. TgDOC2 is the first calcium dependent vesicle fusion mediator implicated in microneme secretion.

The natural triggering factor(s) and the signaling cascade involved are still poorly understood. Changes in extracellular potassium levels and other stimuli trigger a signaling cascade leading to parasite phosphoinositide-phospholipase *C (*PI-PLC) activation, generation of diacylglycerol

(DAG) and inositol triphosphate, and ultimately to microneme secretion. We have recently shown that a delicate balance between DAG and phosphatidic acid (PA) is essential for controlling microneme discharge. Governing this balance is the conserved, apicomplexan-specific DAG-kinase-1, conditional depletion of which leads to parasite death via loss of plasma membrane integrity. Importantly, an acylated pleckstrin homology (PH) domain containing protein (TgAPH) present on the microneme surface, acts as a sensor of PA during microneme secretion. TgAPH is uniquely conserved in Apicomplexa and is critical for microneme secretion, invasion and egress. Overall, this work identifies a key lipid mediator underpinning microneme exocytosis in Apicomplexa (Bullen et al, unpublished). TgAPH presumably contributes to docking of vesicles to the plasma membrane by fostering the close apposition of TgDOC2 and other ferlins to trigger membrane fusion during exocytosis in a Ca2+ dependent manner.

Rhoptry organelles are also viewed as an unusual secretion system, injecting membranous materials to form the PVM as well as effector proteins that contribute to virulence and successful infection by interfering with host cellular functions[70]. Identification of TgARO, an acylated protein implicated in the apical positioning of the rhoptries[58], constitutes a first element to fish the machinery implicated in the discharge of these organelles at the tip of the parasite. The point of discharge might correspond to the *porosome,* a supramolecular lipoprotein structure where the organelle can dock and release its content without substantial fusion[71].

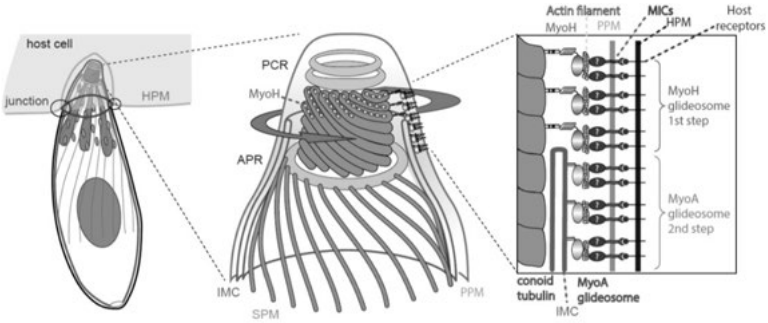
The connector between the actomyosin system and the adhesins

During invasion, transmembrane adhesins discharged by the micronemes on the parasite surface bridge with the targeted host cell. Their reward translocation by the glideosome anchored to the inner membrane complex, propels the parasite inside the host cell. For the last decade, the connection between the adhesins complexes and the glideosome was thought to be established by the glycolytic enzyme *T. gondii* aldolase (TgALD) [72]. However, a recent study demonstrated that depletion of TgALD does not impair motility and invasion but causes the accumulation of toxic me-

tabolites[73]. In consequence, the connection between micronemal adhesins and the glideosome remained an open question. We have identified a glideosome-adhesins connector (TgGAC), which is uniquely conserved across the Apicomplexa phylum. TgGAC binds to rabbit filamentous actin in *in vitro* pull-down assay and predominantly accumulates at the apical pole of intracellular parasites while it progressively re-localizes to the basal end of extracellular gliding parasites. Depletion of TgGAC impairs motility, invasion and egress without affecting microneme or rhoptry secretion. Importantly, *T. gondii* myosin H, a microtubule associated motor confined to the conoid acts as the first translocator of the circular junction complex composed of TgRONs-AMA1 and TgGAC. At the level of the apical polar ring, *T. gondii* myosin. TgMyoA takes the relay and translocates the complex to the posterior pole. This translocation of GAC is necessary for gliding motility, host cell entry and egress from infected cells (Jacot et al, unpublished).

Conclusion

Motion is an intrinsic property of all living organisms and each cell displays a variety of shapes and modes of locomotion. Apicomplexans have



invading/gliding parasite | *protruded parasite* | *MyoH and MyoA glideosomes*

superbly harnessed cytoskeletal structural proteins and molecular motors to generate polarized motion that ensure their survival and infectivity. This closes our current view (opened to future changes) on the molecular composition and mode of action of the glideosome.

Open questions and Future directions

Over the years, the concept of gliding motility and invasion has been consolidated by the identification and characterization of the major actors (regulators of actin dynamics, myosins, microneme adhesins, rhoptry neck proteins, rhomboid proteases, and signalling molecules). Although the critical components of the glideosome have been identified and in large part characterized, the mechanisms allowing its concerted action in a timely controlled fashion remain largely uncharacterized. We have reached a point at which the components of the process have been set on the stage, and reconstructing their interactions is possible. The future challenge will be to understand the mechanistic details of their contributions and how they are orchestrated in time and space. In the next few years, our specific objectives will be to i) *Increase our understanding of the architecture of the glideosome.* If successful, the 3D reconstitution of the glideosome is anticipated to make a seminal contribution to the molecular understanding of a unique cellular mode of motion. Today and despite extensive investigations, its remains a mystery how myosin A, one of the smallest myosin motor, manages to propel the apicomplexan parasites at 5 mm/s. ii) *Elucidate the processes of rhoptry organelle biogenesis and discharge.* This unique club-shaped organelle is at the centre of parasitism by Apicomplexa, contributing to invasion, PVM formation and subversion of host cellular functions. iii) *Assess the function of conoid protrusion.* In intracellular replicating parasites, the conoid remains confined inside the basket of the subpellicular microtubules, which then protrudes in extracellular freshly egressed, gliding or invading parasites. The conoid extends and retracts repeatedly as the parasite moves along the host cell surface but the function if this mysterious, motile organelle is unknown.

The findings that will emerge from our work and others are anticipated to be of significant interest to the fields embracing basic cell biology, microbiology and parasitology. Ultimately, they can lead to the identification of new targets or strategies for intervention against these important human and animal pathogens.

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