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Recent advances in identifying and characterizing secretory proteins of *Toxoplasma gondii* by CRISPR-based screening

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ABSTRACT

The apicomplexan parasite, *Toxoplasma gondii*, develops unique secretory organelles, such as micronemes, rhoptries, and dense granules, which do not exist in other well-studied eukaryotic organisms. These secretory organelles are key features of apicomplexan parasites and discharge various proteins that are essential for invasion, replication, egress, host-parasite interactions, and virulence. Many studies have therefore focused on identifying and characterizing the proteins secreted by *T. gondii* that play essential roles in pathology and that can be targeted for therapeutics and vaccine development. The recent development of functional genetic screens based on CRISPR/Cas9 technology has revolutionized this field and has enabled the identification of genes that contribute to parasite fitness *in vitro* and *in vivo*. Consequently, characterization of genes identified by unbiased CRISPR screens has revealed novel aspects of apicomplexan biology. In this review, we describe the development of CRIPSR-based screening technology for *T. gondii*, and recent advances in our understanding of secretory proteins identified and characterized by CRISPR-based screening.

1. Introduction

The phylum Apicomplexa is a large group of protozoa, mainly consisting of obligate intracellular parasites. Several species, such as *Plasmodium spp.*, *Toxoplasma gondii*, and *Cryptosporidium spp.*, are important pathogens of humans and animals, causing malaria, toxoplasmosis, and cryptosporidiosis, respectively [1]. Apicomplexa have unique properties and complex life cycles; therefore, many aspects of their biology remain to be elucidated.

T. gondii can infect almost any warm-blooded vertebrate. It is estimated that 25 %–30 % of the global human population is infected with *T. gondii*. The route of infection is consumption of undercooked meat containing cysts, or food and water contaminated with oocysts [2]. Toxoplasmosis is usually asymptomatic in healthy people, but ocular toxoplasmosis can occur. Toxoplasmosis can be fatal in immunocompromised patients, such as *Toxoplasma* encephalitis. In pregnant women, primary infection during gestation can lead to congenital toxoplasmosis in newborns [3]. Toxoplasmosis is considered a serious problem in public health, in both developing and developed countries. Currently, no vaccine is available to prevent toxoplasmosis in humans [4].

T. gondii is an established model apicomplexan parasite owing to its ease of continuous *in vitro* culture, its ability to model disease *in vivo* in mice, and the availability of various cell-based assays to investigate biological events. Above all, genetic tractability because of a high transfection rate, a balanced nucleotide composition of the genome, haploidy of the most well-studied life stage (tachyzoite), and non-homologous end joining DNA repair make *T. gondii* ideal to study parasite genetics [5].

T. gondii discharges numerous proteins from three secretory organelles onto the parasite surface, inside the parasitophorous vacuole (PV), a membranous vacuole inside the host cell that parasites proliferate within, onto the PV membrane (PVM), and into the host cytoplasm and nucleus [6,7]. These secreted proteins play pivotal roles in the parasite lytic cycle and host-parasite interactions. *T. gondii* possesses more than 8000 genes. Spatial proteomics data and published studies predict that more than 300 of these genes encode putative secretory proteins; however, the precise functions of most genes are not fully elucidated [8,9]. CRISPR/Cas9-based genome editing technology enables efficient functional genetic screening in *T. gondii*. Numerous key genes have been identified and characterized by CRISPR screens in recent years, shedding

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light on previously unknown biology of this apicomplexan [10].

2. CRISPR screens of *T. gondii*

Methods to identify and characterize secretory proteins of *T. gondii* have been rapidly evolving. Early studies used classical forward genetics approaches and identified polymorphic proteins, such as ROP5, ROP16, ROP18, and GRA15, which determine the different virulence between strains [11–15]. Next, reverse genetics and bioinformatic approaches based on complete genome sequences identified several proteins, such as GRA16, GRA17, GRA23, GRA24, GRA28, TgIST, and HCE1/TEEGR [16–23]. Chemically induced mutagenesis identified important genes, such as MYR1, a component of the MYR translocon [24]. Omics approaches, such as proximity labeling and spatial proteomics, are also powerful tools and have identified numerous secretory proteins [8,25–28]. Notably, the field has increasingly turned to CRSIPR-based functional screening, which possesses aspects of both forward and reverse genetics approaches. CRISPR-based screening is now a widely used, powerful research tool that is used in many fields, including cell biology, cancer research, immunology, and microbiology [29].

When a double-strand break is introduced into the genome, most eukaryotes repair DNA *via* two different pathways known as homologous recombination and non-homologous end-joining [30]. The homologous recombination pathway requires a template DNA, while the non-homologous end-joining pathway does not. Interestingly, the functional non-homologous end-joining pathway is absent in most protozoan parasites [31]. Although CRISPR-based genome editing can be applied to several apicomplexan species, such as *T. gondii*, *Plasmodium falciparum*, and *Cryptosporidium parvum*, *T. gondii* is the only parasite with classical non-homologous end joining among the major apicomplexan species [32–35]. This DNA repair pathway is essential for CRISPR-based gene disruption without the need for homologous template DNA. This feature makes *T. gondii* the optimal parasite for pooled CRISPR screens [36].

In a typical pooled CRISPR screen, a guide RNA (gRNA) library is transfected in *T. gondii* by electroporation, and each gRNA is integrated into the genome, enabling the determination of the introduced knockouts based on the gRNA sequence. Transfected parasites consist of thousands of mutants and are treated with selective pressure such as drug, growth competition, or immune response. Mutants with fitness defects against selection will be eliminated from the pool. The gRNA abundance is determined by next-generation sequencing and is compared between different conditions. The depleted mutants are estimated to be knocked out for a gene that confers the fitness against selective pressure (Fig. 1) [36]. The first CRISPR screen in *T. gondii* cultured in human fibroblasts revealed essential genes for *in vitro* growth throughout the genome [36]. Subsequent studies applied CRISPR screens to reveal essential genes for drug resistance, metabolism, differentiation, oxidative stress resistance, host immune evasion, and host transcriptome perturbation (summarized in Table 1) [37–45]. Two studies reported arrayed CRISPR screens that enabled conditional knockout and conditional knockdown [46,47]. These arrayed screens enabled the direct observation of each mutant phenotype, not gRNA abundance, thereby helping to reveal the function of essential genes [48].

Notably, researchers have been attempting to apply the pooled CRISPR screen approach to an *in vivo* environment (summarized in Table 2) [49–54]. Many aspects of pathogens can only be observed in infected animals, not in cell culture. The genome-wide approach in mice is greatly hindered by infected mice succumbing to the high abundance of parasites necessary for pooled screens. Therefore, *in vivo* screens have employed the targeted approach of making small-scale gRNA sublibraries. Most *in vivo* CRISPR screens of *T. gondii* have focused on identifying secreted virulence proteins, such as rhoptry proteins (ROPs and RONs) and dense granule proteins (GRAs) [49–51]. However, recent studies screened for non-secretory *in vivo* fitness genes [52–54].

A series of *in vivo* CRISPR screens have paved the way for understanding previously unknown host-parasite interactions in infected mice. A promising application of *in vivo* CRISPR screens is using parasites and hosts under various conditions, such as with different strains, genotypes, species, drugs, diets, and immune status. The most studied host factor against *T. gondii* is interferon-gamma (IFNγ). IFNγ-mediated immune response is critical against infection of intracellular pathogens, including T. *gondii* in mice [55]. It is known that *T. gondii* evolved several

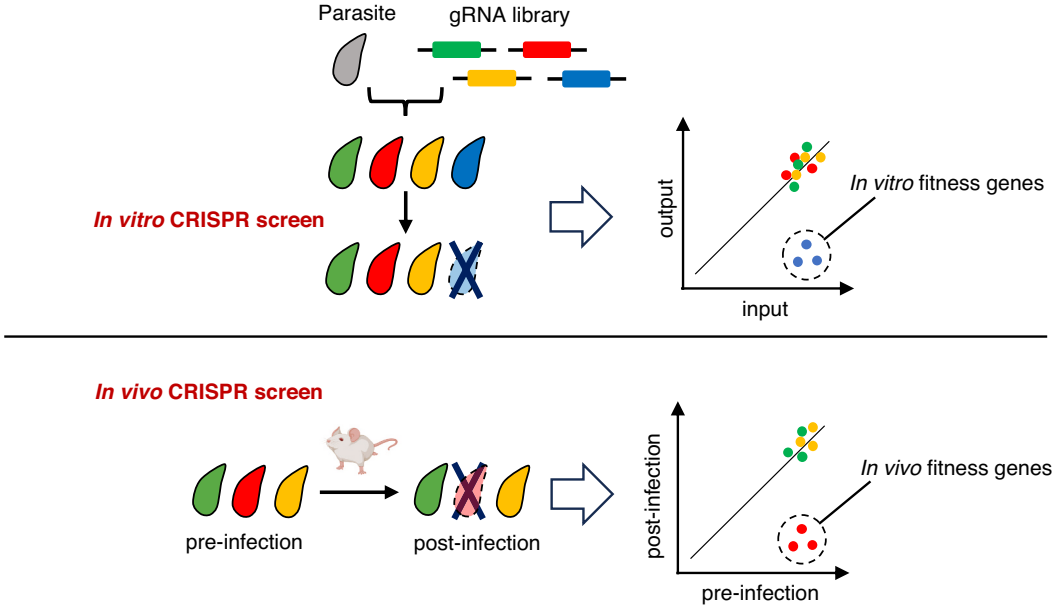

Fig. 1. Schematic of the CRISPR screening in *T. gondii*.
Parasites are transfected with the gRNA library and grown in host cells. A mutant parasite that lacks a gene conferring fitness in *in vitro* growth (blue) is eliminated from the pool. The pool of mutant parasites is injected into mice and retrieved several days post-infection. A mutant parasite that lacks a gene conferring fitness in *in vivo* growth (red) is eliminated from the pool. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Summary of published *in vitro* CRIPSR screens in *T. gondii*.

Parasite strain	Host cell	Aim of screen	Targeted genes	Reference
RH-Cas9	Human fibroblasts	Parasite growth	Genome-wide	Sidik et al. [36]
RH-Cas9	Human fibroblasts	Artemisinin resistance	Genome-wide	Harding et al. [37]
ME49-Cas9	Human fibroblasts	Bradyzoite differentiation	Putative transcription factors	Waldman et al. [39]
RH-Cas9	Human fibroblasts	Purine metabolism	Metabolism related genes	Krishnan et al. [38]
RH-Cas9	Murine macrophages	Resistance against murine IFNγ responses	Genome-wide	Wang et al. [40]
RH-Cas9	Vero cells	Resistance against oxidative stress	Genome-wide	Chen et al. [41]
RH-TIR1	Human fibroblasts	Tagging and conditional knockdown	Kinases	Smith et al. [47]
RH-splitCas9	Human fibroblasts	Conditional knockout	Without signal peptides, apicomplexan-restricted, and fitness-conferring	Li et al. [46]
RH-Cas9	Human fibroblasts	Resistance against human IFNγ responses	Genome-wide	Krishnamurthy et al. [43]
RH, Pru	Human fibroblasts	Resistance against human IFNγ responses	ROPs and GRAs	Lockyer et al. [44]
RH-Cas9ΔGRA17	Human fibroblasts	Synthetic lethality with GRA17	Genome-wide	Paredes-Santos et al. [86]
RH	Human fibroblasts	Host transcriptome perturbation	ROPs and GRAs	Butterworth et al. [42]
RH-Cas9	Lewis rat macrophages	Pyroptosis induction	Genome-wide	Wang et al. [45]
RH-Cas9	Human fibroblasts	Resistance against human IFNγ responses	Genome-wide	Henry et al. [106]

Table 2
Summary of published *in vivo* CRIPSR screens in *T. gondii*.

Parasite strain	Host strain	Infection route	Targeted genes	Reference
Pru	C57BL/6 mice	intraperitoneal	ROPs and GRAs	Young et al. [50]
RH-Cas9	CD-1 mice	intraperitoneal	GRAs	Sangare et al. [49]
Pru	C57BL/6 mice	intraperitoneal	ROPs and GRAs	Butterworth et al. [51]
RH	C57BL/6 mice (WT and IFNγR null)	footpad	ROPs and GRAs, metabolism, endomembrane, nucleus-related genes	Tachibana et al. [52]
RH	C57BL/6 mice	footpad	hyperLOPIT-unassigned localization	Tachibana et al. [54]
RH-Cas9	CD-1 mice	intraperitoneal	Genome-wide	Giuliano et al. [53]

virulence proteins to overcome host IFNγ-mediated immunity [56]. Recent *in vivo* screens in IFNγ receptor (IFNγR) null mice demonstrated that CRISPR screens in hosts with different genotypes can highlight known and novel parasite proteins that are required to overcome specific host bottlenecks (Fig. 2) [52,57].

3. Secretory proteins of *T. gondii*

The apicomplexans possess three distinct secretory organelles, micronemes, rhoptries, and dense granules. Some secretory organelles are restricted to specific species (*e.g.*, exonemes in *Plasmodium* and small granules in *Cryptosporidium*) [58,59]. These secretory organs discharge various proteins at different phases of infection. Micronemes secrete microneme proteins (MICs) onto the parasite surface for invasion, motility, and attachment [60]. Micronemes also play a pivotal role in *T. gondii* egress by secreting PLP1, a parasite perforin that permeabilizes the PVM [61,62]. Rhoptries secrete rhoptry proteins (ROPs and RONs) directly into the host cytosol and play an essential role in invasion (formation of the moving junction) and evasion of host immunity [63]. *T. gondii* also injects rhoptry proteins into non-infected host cells, affecting their signaling and transcription [64]. Dense granules secrete dense granule proteins (GRAs) and play an important role in formation of the PV, interfering with host gene expression, and acquiring small

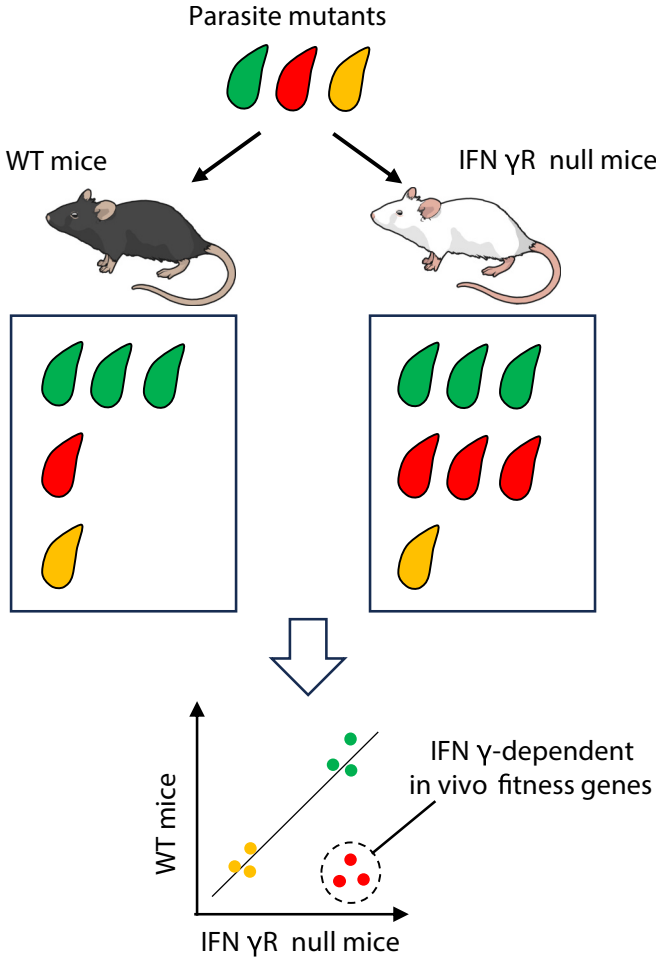

Fig. 2. Schematic of an application of *in vivo* CRISPR screening.
Using hosts with different genetic backgrounds (WT and IFNγR null mice) can detect IFNγ-dependent *in vivo* fitness genes.

molecules [65]. Although the organelles themselves are well conserved, very few secretory proteins are shared among apicomplexan parasites, probably reflecting the importance of secretory proteins as frontline

protection against host defenses [66,67]. Secretory proteins of *T. gondii* show a high ratio of non-synonymous to synonymous substitutions, indicating strong positive pressure in their evolutionary history [8].

Efforts have been made to engineer the secretory machinery of *T. gondii* to generate biological tools, such as drug delivery systems [68]. A recent study has generated engineered parasites in which their secretory machinery was hijacked to deliver large host proteins (>100 kDa) into neurons [69]. These efforts indicate that *T. gondii* has the potential to become a versatile biological tool, like competent cells or viral vectors. Meanwhile, parasite secretory proteins themselves can be used for immunomodulation because some parasite effectors activate or repress host immune signaling [6].

Below, we describe recent advances in our understanding of individual secretory proteins of *T. gondii* that were identified or characterized by a series of CRISPR screens.

4. Rhoptry proteins

4.1. TgWIP

TgWIP (*Toxoplasma gondii* WAVE complex interacting protein, TGGT1_247520) was identified by *in vivo* CRISPR screens as a fitness-conferring gene for dissemination from the site of infection to a distant organ [49]. TgWIP is a rhoptry effector that is secreted into the host cytosol and interacts with host proteins, including the WAVE regulatory complex and the tyrosine phosphatases, Shp1 and Shp2. TgWIP possesses two SH2-binding motifs that interact with Shp1 and Shp2, leading to actin cytoskeletal rearrangement. TgWIP affects the morphology of parasite-infected dendritic cells, induces hypermotility of human and murine infected-dendritic cells, and contributes to parasite dissemination [70]. Other effectors, such as ROP17, GRA6, GRA15, GRA24, and GRA28, are also important for parasite dissemination or hypermigration of infected leukocytes by different mechanisms [71–74]. These parasite effectors can orchestrate a Trojan horse strategy to across the blood–brain barrier [75].

4.2. ROP1

ROP1 (TGGT1_309590) was the first ROP to be identified in *Toxoplasma*, but its function has only recently been discovered [51]. An *in vivo* CRISPR screen in the type II Pru strain revealed that ROP1 is required for *in vivo* fitness. ROP1 localizes to the PVM up to 24 h post-infection (hpi) and is subsequently degraded. The deletion of ROP1 affects rhoptry morphology but not its discharge. Parasites lacking ROP1 show complete loss of virulence in the type II Pru strain. ROP1 also contributes to parasite resistance to IFNγ-mediated responses through an unknown mechanism.

4.3. SOS1

SOS1 (sustainer of STAT signaling 1, TGGT1_222100) was identified as a novel ROP effector influencing the host transcriptome [42]. CRISPR screens combined with host single-cell RNA-sequencing revealed that gene expression patterns of ΔSOS1-infected cells correlate with those of ΔROP16-infected cells. Interestingly, ΔSOS1 parasites have no defects in STAT6 phosphorylation at an early time point (1 h post infection; hpi). However, at 24 hpi, phosphorylated STAT6 was absent in ΔSOS1-infected cells. Therefore, ROP16 may initially induce STAT6 phosphorylation, while SOS1 subsequently sustains STAT6 activation during the infection. ΔSOS1 parasites, as well as ΔROP16 parasites, form fewer cysts in neurons. STAT6 activation induces M2 polarization of *Toxoplasma*-infected macrophages [76]. The alternative activation of macrophages downregulates Th1-immune responses. Therefore, SOS1, in concert with ROP16, can contribute to parasite immune evasion and establishment of chronic persistence. Further investigation is required to determine the precise mode of action and pathophysiological role of SOS1.

4.4. RON11

RON11 (TGGT1_230350) is a rhoptry neck protein with several transmembrane domains and a C-terminal calcium-binding EF-hand domain [77]. RON11 is widely conserved across the Apicomplexa. *In vivo* CRISPR screens in the RH strain revealed that RON11 is required for *in vivo* fitness in an IFNγ-dependent manner [52]. Deletion of RON11 in *T. gondii* causes a minor *in vitro* growth defect and highly attenuated virulence [52,78]. RON11 was further investigated in *Plasmodium*. Knockdown of RON11 in *Plasmodium berghei* indicates that RON11 is required for sporozoite gliding motility and invasion [79], while knockdown of RON11 in *P. falciparum* inhibits merozoite invasion [80]. Interestingly, RON11 knockdown in *P. falciparum* leads to merozoites with only one rhoptry (*Plasmodium spp.* usually possess two rhoptries in the asexual stages, whereas *T. gondii* tachyzoites possess 8–12 rhoptries). RON11 is therefore speculated to be required for rhoptry biogenesis in the *P. falciparum* merozoite stage. Whether RON11 is also involved in rhoptry biogenesis in *T. gondii* is unknown and needs to be further investigated.

5. Dense granule proteins

5.1. GRA45

GRA45 (TGGT1_316250) was originally identified as an interacting partner of two other GRAs, GRA44 and WNG2, and it was shown to be an ASP5 substrate [81]. Subsequently, GRA45 was shown to be a MYR1-interacting protein [82]. A genome-wide *in vitro* CRISPR screen in IFNγ-activated murine macrophages revealed that GRA45 is critical for parasite fitness against IFNγ-dependent clearance [40]. *In vivo* CRISPR screens in IFNγR null mice highlighted GRA45 as a significant IFNγ-dependent *in vivo* fitness gene [52]. Parasites lacking GRA45 showed reduced virulence in the RH strain. GRA45 possesses a chaperone-like domain and is required for the correct localization of other GRAs into the PVM and translocation of some GRA effectors into the host nucleus [40]. The precise mechanism of how GRA45 contributes to IFNγ-dependent fitness in mice is unknown. One possibility is that other critical IFNγ-dependent GRAs are mis-localized in ΔGRA45 parasites. This hypothesis is reasonable because GRA23, which is mis-localized in ΔGRA45 parasites, is important for IFNγ-dependent fitness [40,43,52].

5.2. GRA12

GRA12 (TGGT1_288650) is an intravacuolar network-associated GRA but is dispensable for intravacuolar network formation [83–85]. ΔGRA12 parasites showed reduced virulence in the type I RH strain and complete loss of virulence in the type II Pru strain. GRA12 is essential for parasite resistance to IFNγ responses without affecting the effector immunity-related GTPase (IRG) proteins (Irgb6 or Irga6), which coat the PVM. However, this depends on the regulatory IRG proteins (Irgm1 and Irgm3) [84,85]. *In vivo* CRISPR screens highlighted GRA12 to be a significant IFNγ-dependent *in vivo* fitness gene, consistent with previous results [52]. The mechanisms of how GRA12 protects parasites from host IFNγ-dependent immunity are currently unknown and require further investigation.

5.3. GRA57, GRA70, and GRA71

GRA57 (TGGT1_217680), GRA70 (TGGT1_249990), and GRA71 (TGGT1_309600) were identified as essential genes for parasite fitness in IFNγ-stimulated human fibroblasts [43,44]. These proteins were not identified by *in vitro* CRISPR screens of murine cells, possibly reflecting host species-specific effects [40]. These proteins have homology with GRA32. GRA57 localizes in the PV and likely forms a complex with

GRA70 and GRA71. Deletion of these proteins decreases ubiquitination of PVs by an unknown mechanism, which seems to be beneficial for parasites to evade host clearance [44]. These proteins also inhibit premature egress in IFNγ-stimulated human fibroblasts [43]. It is currently unclear how these GRAs protect parasites from human IFNγ responses.

5.4. GRA72

GRA72 (TGGT1_272460) was first identified as an essential gene for parasite fitness in IFNγ-stimulated human fibroblasts by a genome-wide *in vitro* CRISPR screen [43]. Subsequently, GRA72 was a top hit in an *in vivo* CRISPR screen in mice [52,53]. Independently, GRA72 was identified as synthetically lethal with GRA17 by a genome-wide *in vitro* screen [86]. Therefore, several CRISPR screens independently identified GRA72 as a fitness-conferring gene from different perspectives. Those reports indicate the importance of GRA72, and the power and impartiality of the CRISPR screening technique. GRA72 localizes in the PV and on the PVM. Deletion of GRA72 results in PVs having a bubble-like appearance, which resembles the PV phenotype after deletion of GRA17. Loss of GRA72 results in mis-localization of GRA17 and GRA23 but not of other GRAs. This is different from loss of GRA45, which affects many GRAs. ΔGRA72 parasites lose virulence in the RH strain. Immunoprecipitation of GRA72 identified its interacting partner, GRA47 (TGGT1_254000) [87,88]. Deletion of GRA47 also resulted in an abnormal bubble-like PV appearance. Alphafold predicts that both GRA72 and GRA47 can form pores. The putative pore-lining histidine residues are essential for their function [87]. GRA72, GRA47, GRA17, and GRA23 are estimated to form channels in the PVM and to exchange small molecules between host and parasite, probably to acquire essential nutrients and to excrete waste [18,86,87]. It is not known whether there are other pore-forming GRAs in the PVM. The mechanism of GRA17 and GRA23 mis-localization in ΔGRA72 is unknown [52,86,87]. The mechanism by which GRA72 and GRA23 contribute to fitness in an IFNγ-stimulated environment is also unknown [43,52]. Future studies will reveal host-parasite interaction *via* both known and unidentified PVM-related GRAs.

5.5. GRA59

GRA59 (TGGT1_313440) is a putative component of the export machinery in the PVM [42]. CRISPR screens combined with host single-cell RNA-sequencing determined that genes expressed in ΔGRA59-infected cells correlate with those known to be essential for exporting GRA effectors into the host cell, such as MYR1–4, ROP17, and GRA45 [51,82]. Although GRA59-knockout and MYR1-knockout phenotypes are similar, the export of GRA effectors is not entirely abolished. Further investigation is warranted to understand the detailed mechanisms of parasite protein translocation across the PVM.

5.6. GRA35, GRA42, and GRA43

GRA35 (TGGT1_226380), GRA42 (TGGT1_236870), and GRA43 (TGGT1_237015) were identified by N-ethyl-N-nitrosourea-induced mutagenesis to be important for activating the NLRP1 inflammasome and inducing pyroptosis in Lewis rat macrophages [89]. A subsequent genome-wide *in vitro* CRISPR screen confirmed these three GRAs to be essential for inducing pyroptosis in Lewis rat macrophages [45]. GRA42 and GRA43 are localized in the PV and are required for the correct localization of GRA35. GRA35 recruits the host E3 ubiquitin ligase, ITCH, to the PVM and mediates activation of the NLRP1 inflammasome, leading to pyroptosis in Lewis rat macrophages. The interaction between GRA35 and ITCH is preserved in human fibroblasts and leads to the recruitment of another E3 ubiquitin ligase, RNF213, and ubiquitin to the PVM, resulting in parasite restriction [45]. Although these effects of GRA35 seem harmful to parasites, GRA35 may be important for type II strains in IFNγ-stimulated human fibroblasts [44]. GRA35 may behave differentially depending on parasite strains and host species.

6. Microneme proteins

6.1. CLAMP, CLIP, and SPATR

CLAMP (claudin-like apicomplexan microneme protein, TGGT1_265790) was first identified as an essential MIC for parasite invasion by genome-wide *in vitro* CRISPR screens [36]. A subsequent study revealed that CLAMP forms a complex with two other MICs, CLIP (CLAMP-linked invasion protein, TGGT1_212270) and SPATR (sporozoite protein with an altered thrombospondin repeat, TGGT1_293900) [90,91]. The CLAMP complex is essential for rhoptry discharge, and depletion of any components of the complex reduces rhoptry discharge. The CLAMP complex is conserved in *P. falciparum* and is essential for blood-stage growth. Rhoptry secretion is a tightly regulated event [92]. Other studies have indicated that rhoptry secretion by *T. gondii* is dependent on several secretory and non-secretory proteins, such as MIC7, MIC8, CRMPa, CRMPb, MIC14/RDF2, MIC15/RDF1, RASP2, Nd6, Nd9, ICMAP2, ICMAP3, and RDF3 [93–101]. Some secretory proteins are thought to be bound to specific ligands on the host cell surface (unidentified), triggering signal transduction for rhoptry secretion and subsequent invasion. Future studies will reveal the interactions between the two secretory organelles, micronemes and rhoptries.

7. Conclusions

Parasite secretory proteins have been extensively studied for several decades; however, we still know little about the parasite secretome and host-parasite interactome. Similarly, regulatory networks of the parasite secretome (such as transcriptional and epigenetic control, post-translational modification, processing, and trafficking) are also poorly understood. Therefore, characterizing non-secretory proteins is essential to understand the parasite secretome [102–104]. A detailed analysis of each secretory protein and of the mechanism that regulates the secretome in *T. gondii* will lead to better understanding and better treatment strategies of this globally widespread pathogen.

As a robust technical platform, CRISPR-based screening is expected to yield in-depth knowledge of parasite genes. Technological innovation will enable screening during chronic infection, screens for essential genes of feline-restricted stages such as merozoite or gamete, and CRISPR activation screening [29,105]. Insights from such screenings of *T. gondii* will improve our understanding of other apicomplexan parasites, including *Plasmodium* and *Cryptosporidium*.

CRediT authorship contribution statement

Yuta Tachibana: Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Masahiro Yamamoto:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

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