Functional Characterization of a Redundant *Plasmodium* TRAP Family Invasin, TRAP-Like Protein, by Aldolase Binding and a Genetic Complementation Test

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Efficient and specific host cell entry is of exquisite importance for intracellular pathogens. Parasites of the phylum *Apicomplexa* are highly motile and actively enter host cells. These functions are mediated by type I transmembrane invasins of the TRAP family that link an extracellular recognition event to the parasite actin-myosin motor machinery. We systematically tested potential parasite invasins for binding to the actin bridging molecule aldolase and complementation of the vital cytoplasmic domain of the sporozoite invasin TRAP. We show that the ookinete invasin CTRP and a novel, structurally related protein, termed TRAP-like protein (TLP), are functional members of the TRAP family. Although *TLP* is expressed in invasive stages, targeted gene disruption revealed a nonvital role during life cycle progression. This is the first genetic analysis of *TLP*, encoding a redundant TRAP family invasin, in the malaria parasite.

The phylum *Apicomplexa* consists of unicellular eukaryotes, such as *Plasmodium* and *Toxoplasma gondii*, that are obligate intracellular parasites in a wide range of vertebrate and invertebrate hosts, including humans. Despite vast differences in host range and target cell specificity, these parasites share a unique mechanism of active actin-dependent motility and host cell entry (18, 27). Gliding locomotion and successful host cell entry through simultaneous formation of a parasitophorous vacuole are rapid processes and are thought to be mechanistically coupled (21). Both functions are mediated by the parasite’s actin-myosin motor machinery (6, 22) and additional proteins, including type I transmembrane proteins of the TRAP/MIC2 family of invasins (12, 29, 30), the invasin bridging protein aldolase (4, 14), and the myosin tail interacting protein MTIP (3). According to the current model (1, 26), an extracellular recognition event results in connection of the transmembrane invasins to short actin polymers that in turn are rapidly pulled backwards by immobilized motor myosins.

So far, only TRAP-like invasins have been shown to act directly in parasite locomotion and invasion. In two invasive stages of the *Plasmodium* parasite, sporozoites and ookinetes, thrombospondin-related anonymous protein (TRAP) and circumsporozoite- and TRAP-related protein (CTRP), respectively, fulfill these functions (5, 29, 30). These proteins share a unifying primary structure, i.e., combinations of two adhesive modules, the von Willebrand factor A-domain (A domain) (37) and the thrombospondin type I repeat (TSR) (33), in their ectodomains, a transmembrane domain (25) and a cytoplasmic tail domain (CTD) (16). Importantly, the CTD of the sporozoite invasin TRAP is essential for gliding motility and cell entry of *Plasmodium* sporozoites, since a carboxy-terminal truncation of *Plasmodium berghei* TRAP resulted in noninvasive sporozoites. This finding also permitted a functional assay, and it was demonstrated that the CTD of the *T. gondii* tachyzoite invasin MIC2 could rescue the loss-of-function mutant (16). This complementation experiment was the first direct proof for a functional TRAP homolog in *T. gondii* tachyzoites. However, this reverse-genetics approach has not been extended to other potential members of the TRAP/MIC2 family yet.

Sporozoites cross various biological barriers and migrate over long distances at a relatively high speed (1 to 3 \(\mu m/s\)) (7, 21). Similarly, ookinetes traverse barriers, such as the peritrophic membrane and the mosquito midgut, albeit at a considerably lower speed (5 \(\mu m/min\)) (35). In contrast, merozoites, the invasive stage of the pathogenic red blood cell phase, do not display active locomotion on substrates but employ their motor machinery exclusively for entry into host erythrocytes. In these stages, two TSR-containing transmembrane proteins, termed *Plasmodium* thrombospondin-related apical merozoite protein (PTRAMP) (31) and merozoite-specific TRAP homolog (MTRAP) (2), have been detected recently. Both proteins are reportedly essential for parasite survival (2, 31). MTRAP contains a CTD and interacts with aldolase in vitro, whereas PTRAMP lacks the characteristics of the TRAP family CTD. A direct function during the merozoite invasion process has not been demonstrated for any protein yet. Additional invasion-related transmembrane proteins exist; they contain...
TSR domains only, namely, secreted protein with altered thrombospondin repeat (SPATR) (17) and thrombospondin-related sporozoite protein (TRSP/S21) (15, 19). Both proteins lack the CTD, and none of them have been linked to the parasite actin/myosin motor.

In this study, we established a systematic approach to functionally identify TRAP family invasins. Employing two complementary assays, in vitro binding to the actin-motor protein actin/myosin and genetic complementation of the TRAP CTD, we tested potential candidates for their capacity to interact with the actomyosin motor. We show that the ookinete invasion CTRP and one of the last uncharacterized TRAP-like proteins (TLP) (PFF0800w) are functional members of the TRAP family while EBA175 is not. TLP is expressed in multiple invasive stages but has a redundant role during Plasmodium life cycle progression.

MATERIALS AND METHODS

Plasmodium life cycle. P. berghei strain NK65 was maintained in NMRI mice and Sprague/Dawley rats. For mosquito transmission, mice were assayed for a high proportion of differentiated gametocytes and microgametocyte-stage parasites capable of egress. Anopheles stephensi mosquitoes were allowed to blood feed for 15 min on anesthetized mice and maintained under a 14-h light/10-h dark cycle at 25°C humidity and 20°C. Mosquitoes were dissected at days 10, 14, and 17 to determine infectivity, midgut sporozoite numbers, and salivary gland sporozoite numbers, respectively. To detect liver-stage parasites in hepatocytes, ~3 x 10^5 Huh7 cells were seeded in eight-well chamber slides and grown to semiconfluence. P. berghei sporozoites were added, incubated for 90 min at 37°C, and washed off. After 42 to 48-h, liver-stage parasites were visualized using a primary antibody against P. berghei heat shock protein 70 (32). For analysis of gliding motility, salivary gland sporozoites were deposited on bovine serum albumin-coated glass slides and incubated at 37°C. After fixation with 4% paraformaldehyde, sporozoites and deposited trails were visualized using an anti-P. berghei CSP antibody. To determine the prepatent period, 10,000 sporozoites were injected intravenously into Sprague/Dawley rats and parasitemia detected by daily examination of Giemsa-stained blood films. For natural transmission experiments, young Sprague/Dawley rats were infected by five mosquito bites and parasitemia was examined daily.

PbTRAP cytoplasmic tail swapping. The insertion plasmid used for the tail swap approach contains the 3′ untranslated region of P. dhfr (PSE02) (7). For targeting of PbTRAP, a 5′- and 3′-truncated sequence of the P. berghei TRAP open reading frame generated by PCR using the primers TRAPf-Pb and TRAPrev-Pb (5′ GGATATCCATAATGGTCAGGAAATTCTTGACG 3′ and 5′ CTAGATCATTTCCATGGAGAATTGTCATTATAATC 3′). After RNase inhibitor was added, cell pellets were frozen immediately at -80°C. Total RNA was isolated using Trizol and a Qiagen RNAeasy kit. cDNAs were synthesized from 2 μg RNA using the poly(A) polymerase (Ambion). Control genomic DNA was isolated from P. falciparum or P. berghei erythrocytic-stage parasites using silica-gel columns (Qiagen). cDNA or genomic DNA (1.0 μl) was used for the PCR amplifications using gene-specific primer sets.

Gradient purification of parasites and quantitative RT-PCR. After 3 days of infection, when the ascending parasitemia averaged between 15% and 25%, blood was drawn from P. yoelii-infected mice and fractionated on Ficol/Radi-Grad density gradients (Amersham Biosciences). Blood was layered on top of the gradient and centrifuged for 10 min at 10°C. The five gradient fractions were removed separately, washed with Hanks balanced salt solution, and treated with HEPES buffer solution, and parasitized cells were collected by centrifugation. After RNase inhibitor was added, cell pellets were frozen immediately at -80°C. Total RNA was isolated using Trizol and a Qiagen RNAasy kit. cDNAs were synthesized from 2 μg of total RNA isolated from each stage using the Omniscript cDNA synthesis reaction kit (Qiagen). A reference pool was made by adding equal amounts of total RNA from each fraction prior to cDNA synthesis. Primer pairs were generated for P. yoelii TLP and MSP1 using the Primer3 software (http://primer3.sourceforge.net; Whitehead Institute for Biomedical Research). All reactions were run in duplicate using an ABI Prism 7700 sequence detection system, and data were analyzed using the Applied Biosystems Sequence Detector (v.1.7) program. Serial dilutions of input reference pool cDNA were used to generate a standard curve for each target gene. The relative expression levels of each target gene in the five different stages were normalized to the expression level of the reference pool for that particular gene. Bar graphs were plotted as log ratios of amounts of cDNA of each stage to the reference pool.

PbTRAP gene targeting. For disruption of PbTRAP, two fragments were amplified using primers TLPpre_for (5′ GGCTGACACAAATTAAGGAAATCG AGGG 3′; KpnI site is underlined) and TLPpre_rev (5′ CCACAGTTGAATGGTCTTTATGCCG 3′; HindIII site is underlined) for the 863-bp 5′ fragment and TLPrev_for (5′ CGAATTCGACGCGCCCTCTTATTATAGATGTGA 3′) and TLPrev_rev (5′ CCCCGCGGTG ACGCTCAGATGACCCGACCGCC 3′; SacI site is underlined) for the 1208-bp 3′ fragment. Both fragments were cloned into the P. berghei transformation vector b3.D.T’.H.D., resulting in the plasmid pKDH2. After transfection, recombinant parasite populations were selected using pyrimethamine (13). Plasmodium parasite strains were obtained by limiting dilution to 15 recipient NMRI mice. Genotyping of recombinant parasite
populations was performed by PCR with the following primer combinations: TgPromrev (5′ CGCATATTACTGCTTCTTTACACPAC 3′) with PbTLPesfC (5′ TTTTGAGAAGTTACCATCATTC 3′) and Tgflor and PbTLPesfrev (5′ TCCCCCGGAACTCCATAAAATAC ATCG 3′) (test1) for successful gene replacement and PbTLfor_1 (5′ CGGG ATCTAGTGTCCCTACAGG 3′) and PbTLPrev_1 (5′ TGGACCTGCAG TCAATTTGTCTTATTAATTTTC 3′) for the PbTL WT signal.

For RT-PCR analysis, poly(A) + RNA from gradient-purified schizonts of WT and knockout parasites was isolated and used for cDNA synthesis. For detection of TRLP transcripts, two different primer sets were used: PbTLfor_2 (5′ CGCG GATCCCTATTTGATAATATCGATACAGACCC 3′) and PbTLPrev_2 (5′ TGCCTGAATACCTATACCTTTGGTACATCCAC 3′) and PbTLfor_2 and PbTLPrev_2. MyoA-specific primer sets were used as transcript controls.

Nucleotide sequence accession number. The nucleotide sequence reported in this paper has been submitted to the GenBank database with the accession number AY484471.

RESULTS

Preselection of parasite invasins by in vitro aldolase binding. We initiated our analysis by prescreening the cytoplasmic CTDs of candidate transmembrane proteins for in vitro binding to aldolase. We coated microtiter plates with recombinant His-tagged peptides that correspond to the CTDs of the sporozoite invasin PbTRAP (29), the ookinete-specific protein PbCTRP (5, 30), the candidate merozoite invasin PfEBA-175 (10), and a previously uncharacterized potential PfTRAP-like protein (PFF0800w), termed TRAP-like protein (PfTLP).

Transmembrane PbTRAP and PbCTRP express aldolase binding activity identical to that of their respective native proteins (Fig. 1). PfEBA175 CTD, which is acidic in nature but lacks the key tryptophan residue (10), failed to bind aldolase. Failure of PfEBA175 to bind to aldolase further substantiates that the observed in vitro interactions are specific and reflect a shared property of TRAP proteins.

To confirm the relevance of the PfTLP/aldolase interaction, we tested a mutant form of TLP that contained an alanine in place of the penultimate tryptophan (Fig. 1). As expected, aldolase binding was reduced to background levels. Together, these findings suggest that CTRP and TLP might interact with the parasite actomyosin motor machinery.

TRAP-like protein. TLP is a type I transmembrane protein (Fig. 2A) that contains in its ectodomain one TSR (Fig. 2B) and an A domain (Fig. 2C) in the reverse order compared to TRAP. Of note, the key residues of A domains are also present in the amino-terminal portion between the signal sequence and the TSR, indicating a potential additional binding motif (data not shown). In its transmembrane domain, TLP contains the signature for a potential rhomboid cleavage site (2, 34), indicating that TLP may be processed similarly to TRAP and MIC2. The carboxy-terminal domain of TLP contains the penultimate tryptophan and scattered negatively charged residues (Fig. 2D). In the case of TLP, both signatures were previously shown to drive sporozoite motility (16). Similarity of the domain architecture and the in vitro aldolase binding suggested that TLP belongs to the TRAP family of parasite invasins.

Genetic complementation of the TRAP tail. We next employed a reverse-genetics approach with the rodent malaria model parasite P. berghei in order to test whether the candidate proteins can be functionally grouped into the class of TRAP family invasins, as has been shown previously for MIC2 (16). Similar to this strategy, we designed a set of TRAP CTD swap mutants based on the corresponding regions that we tested in the aldolase assay (Fig. 3A). We included the CTDs of PfTLP, PfCTRP, PfTLfor, and PfEBA175. In addition, we generated a potential gain-of-function mutant of PfEBA175 that contains a penultimate tryptophan in order to test whether the acidic carboxy-terminal residues of EBA175 can at least partially interact with the motor machinery.

The TRAP tail fusion genes were introduced into WT parasites by insertional replacement (Fig. 3B). The targeting plasmids are predicted to insert by a single-crossover event, resulting in a 5′ functional copy that contains the desired TRAP fusion protein under the control of the endogenous TRAP promoter and a 3′ copy that lacks the promoter and start codon and hence is not expressed. The successful integration events were confirmed in the clonal parasite populations by insertion-specific PCR amplification (Fig. 3C). The clonal parasite populations were transmitted to Anopheles stephensi mosquitoes and assessed for their capacity to form oocysts and viable midgut sporozoites (Table 1). As expected, no differences were observed between the swap parasites. Functional production of midgut sporozoites permitted testing for proper expression of the PbTRAP fusion proteins in Western blot analysis (Fig. 4). All parasites produced comparable amounts of the PbTRAP protein.

Phenotypic analysis of the TRAP swap sporozoites enabled us to identify TRAP family members based on functional criteria (Table 1). As expected, the mutant that contained a CTD truncation lacks the capacities to invade mosquito salivary glands and to induce a patent blood-stage infection in rats (16). Replacement of the P. berghei TRAP CTD with the corre-
sponding *P. falciparum* TRAP region resulted in viable sporozoites that invade salivary glands comparably to the WT. When tested for infectivity of the mammalian host in vitro or in vivo, PfTRAP sporozoites are as infectious as the WT. As predicted, the PfCTRP parasites were infectious in the mammalian host in numbers comparable to those of the PfTRAP parasites. Although infectivity of mosquito salivary glands was reduced in PfCTRP parasites, they were evidently capable of invasion (Table 1). Similarly, PbTLP parasites showed a reduced rate of invasion of salivary glands. While liver-stage development in vitro was intermediate between WT parasites and a negative control with a large deletion of the major portion of the tail (*H9004* tail parasites), inoculation with PbTLP swap mutants consistently resulted in substantial numbers of mature liver-stage parasites and patent animals when injected in vivo (Table 1).

Notably, both versions of the PfEBA175 CTDs failed to complement the PbTRAP deletion. This finding excludes a potential role of EBA175 in a TRAP-related step during invasion by providing a direct link to the parasite motor. Most importantly, the failure of the PfEBA175L/W mutant (containing an additional penultimate tryptophan) to complement the PbTRAP CTD indicates that presence of a penultimate tryptophan and a carboxy-terminal acidic cluster, while necessary, is not sufficient for a direct function in parasite motility. We conclude that the CTDs of CTRP and TLP complement TRAP functions, albeit not as well as TRAP. Therefore, both proteins are functional members of the TRAP family in addition to their overall structural relationship.

**TLP** is expressed in blood-stage parasites and sporozoites. The functional characterization of **TLP** as a third TRAP family member in *Plasmodium* prompted us to determine its expression in the *Plasmodium* life cycle. We first tested expression of *P. berghei* TLP in sporozoites and late-blood-stage parasites by RT-PCR (Fig. 5A). In contrast to PbTRAP (29) and PbCTRP (5, 30), PbTLP is expressed in multiple stages, indicating a shared function between sporozoites and merozoites. We next examined the expression profiling of the *P. yoelii* ortholog during erythrocytic schizogony by quantitative real-time RT-PCR (Fig. 5B). PyTLP is apparently under stage-specific expression control and highly upregulated in schizonts, the stage preceding infectious merozoites.

To exclude potential minor contamination with sexual blood-stage parasites, we extended our expression analysis to *P. falciparum* strains (strains HB3 and 3D7) that both lost their ability to form gametocytes in vitro. We synchronized *P. fal-
ciparum parasites and generated total cDNA of highly purified ring, trophozoite, and schizont stages (Fig. 5C). Similar to that of transcripts that function in merozoite invasion (PfAMA1 and PfMSP7 [23]), PfTLP expression commences in schizonts, corroborating our expression data from the rodent parasites. Together our data demonstrate expression of TLP in multiple invasive stages of rodent and human Plasmodium parasites and specific upregulation prior to merozoite invasion.

TLP is dispensable for Plasmodium life cycle progression. To test whether TLP is important for asexual replication of P. berghei, we first targeted the PbTLP gene with an integration vector that disrupts the gene locus via a single-crossover event (data not shown). Several attempts to disrupt the gene were not successful, while an integration control that recovered the WT TLP copy yielded recombinant parasites (data not shown). To distinguish between an essential function and difficulties in targeting the gene, we constructed a replacement vector containing the PbTLP 5′/H11032 and 3′/H11032 untranslated regions that flank the positive selection marker cassette (Fig. 6A). Upon a double-crossover event, this vector is predicted to delete the entire PbTLP locus. After transfection and continuous selection with the antifolate pyrimethamine, we obtained a parental population that was used for single parasite cloning. Genotyping of two independent clonal parasite lines verified the correct gene replacement event (Fig. 6B). To further confirm the absence of TLP transcripts in the tlp/H11002 parasites, we performed RT-PCR of cDNAs generated from blood-stage mutant and WT parasite poly(A)+ RNA (Fig. 6C). As predicted, no TLP transcripts were detected in the knockout parasites, while it was readily detectable in WT parasites. The successful generation of TLP-deficient parasites demonstrates that this gene is not essential during the pathogenic blood-stage cycle in vivo.

We extended our in vivo analysis to the entire parasite life cycle and tested sporozoite formation and maturation in the
Anopheles vector and transmission to the mammalian host (Table 2). Natural transmission experiments by blood-feeding of tlp−-parasite-infected Anopheles mosquitoes on malaria-naive rats revealed normal completion of the life cycle, indistinguishable from that of WT parasites. Quantification of sporozoite numbers in midgut-associated oocysts and salivary glands, the final target organ in the mosquito, yielded similar numbers for tlp− and WT parasites. Mature, salivary gland-associated sporozoites displayed continuous gliding locomotion, albeit at a lower frequency, and full in vitro and in vivo infectivity in cultured hepatoma cells and when injected intravenously into rats, respectively. Together, these findings show that TLP does not play an essential role at any stage of the malaria parasite life cycle.

**DISCUSSION**

The most important physiological function of TRAP family invasins is to drive parasite motility and host cell entry (16). The identification of the merozoite invasin that links the actomyosin motor machinery to the merozoite surface ligands would set the stage for novel intervention strategies that specifically target merozoite entry into host erythrocytes. Toward the identification of this missing link in the Plasmodium life cycle, we combined two approaches, in vitro binding to aldolase (14) and genetic complementation of the PbTRAP tail (16), which together permit the systematic identification of candidate proteins that link an extracellular recognition event to the intracellular parasite motor complex.

Examination of the P. falciparum genome database (9) revealed the presence of a previously unidentified TRAP-like protein in addition to the sporozoite and ookinete invasins TRAP and CTRP, respectively. While the Plasmodium genomes harbor numerous proteins that contain thrombospondin repeats, such as SPATR (17), TRSP (15, 19), PTRAMP (31), and MTRAP (2), only TLP shares all unifying structural features of TRAP family invasins, i.e., the combined presence of A domains and TSRs in their ectodomain and a conserved cytoplasmic tail domain (Fig. 2A). The TSR contains the typical amino-terminal WSxW tetrapeptide and carboxy-terminal basic residues that are separated by two invariant cysteines (Fig. 2B). A domains contain metal ion-dependent adhesion sites (MIDAS) that are implicated in ligand binding (20, 24). Of the invariant residues that coordinate the central divalent cation, the two flanking aspartates and the central threonine are conserved (Fig. 2C). Notably, only one of generally two serines is present in the amino-terminal DxSxS sequence. We also noticed the presence of a second region between amino acids 48 and 267 of PfTLP that might constitute an unconventional A domain. Biochemical approaches are required to test the adhesive properties of this region in comparison to the conventional A domain. In addition to the two well-characterized adhesion modules, TLP contains a sizeable portion, termed "charged region," between the A domain and the type I transmembrane span that displays an unusually high content of lysine, arginine, glutamic acid, and aspartic acid residues. The carboxy-terminal domain of TLP contains the penultimate tryptophan and a cluster of negatively charged residues (Fig. 2D), which, in the case of TRAP, drive parasite motility (16).

By two independent assays, in vitro aldolase binding of the recombinant CTD and a reverse-genetics strategy, we functionally identified TLP as a third Plasmodium member of this protein family. The CTD of TLP carries key residues that are

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**TABLE 1. Phenotypes of TRAP tail swap parasites**

| Genotype | No. of midgut sporozoites/infected mosquito | No. of salivary gland sporozoites/infected mosquito | No. of liver-stage parasites | No. of animals infected (no. positive/no. injected) | Prepatent period, days |
|----------|--------------------------------------------|----------------------------------------------------|-----------------------------|-----------------------------------------------|------------------------|
| WT       | 24,870 (±17,373)                            | 12,271 (±5,816)                                    | 259 (±48)                   | 6/6                                           | 3.5                    |
| Δtail    | 23,514 (±6,282)                            | 1,353 (±428)                                       | 0.9 (±0.7)                  | 0/6                                          | 4.3                    |
| P'TRAP   | 34,064 (±15,707)                            | 16,037 (±7,114)                                    | 229 (±75)                   | 8/8                                          | 4.2                    |
| P'CTRP   | 23,283 (±9,163)                            | 5,872 (±2,081)                                     | 151 (±92)                   | 8/8                                          | 4.5                    |
| PbTRL    | 24,845 (±14,047)                            | 5,314 (±3,038)                                     | 34.5 (±5)                   | 8/8                                          | 4.6                    |
| P'EB175L | 31,715 (±18,805)                            | 2,943 (±1,256)                                     | 0.2 (±0.4)                  | 1/10                                         | 6.5                    |
| P'EB175LW| 31,645 (±9,622)                             | 3,074 (±1,339)                                     | 0                           | 0/8                                          | 6.5                    |

* Values are means, with SDs in parentheses.

b Liver-stage parasites are total mature exoerythrocytic forms visualized 48 h after incubation of 10,000 salivary gland sporozoites with subconfluent cultured hepatocytes. Values are means ± SDs.

c Prepatent period is the time until the first detection of an erythrocytic-stage parasite in Giemsa-stained blood smears after intravenous injection of 10,000 salivary gland sporozoites.

d Parentheses indicate that a proportion of parasites contained mixed populations.

e One animal became patent and was subsequently shown to have completely reverted to the WT locus.

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**FIG. 4. Western blot analysis of TRAP tail swap parasites.** Midgut sporozoite extracts from 100,000 WT or mutant sporozoites were separated on a 10% SDS gel and probed with polyclonal anti-PbTRAP-repeat serum (α-TRAP) and monoclonal anti-PbCSP antibodies.
shared by members of the TRAP family. This region can partially complement TRAP functions during sporozoite invasion of mosquito salivary glands and hepatocytes. Similarly, the CTD of the ookinete invasin CTRP can functionally replace the TRAP tail. Functional assignment of CTRP to the TRAP family was predicted, since *ctrp*/*H11002* ookinetes no longer traverse the mosquito midgut and do not display productive motility (5, 30). It will be interesting to test whether MTRAP, which was previously shown to likely exert a vital function during in vitro
growth of *P. falciparum* blood stages (2), can complement the TRAP tail. To date, the bridging protein of the motor machinery used by the malaria merozoite to propel itself into the host erythrocyte remains unknown, although biochemical evidence suggests a potential, specific role for MTRAP in this process (2).

In this study, we provide evidence from independent approaches that TLP is a member of the growing family of TRAP invasins. Although the precise role of aldolase in bridging the CTDs of invasins to microfilaments remains to be determined, in vitro binding to aldolase is a hallmark of members of the TRAP family and a valuable predictor for a role in motility and invasion (4, 14). That efficient interaction of the CTD of TLP with aldolase is relevant in vivo is corroborated by our finding in invasion (4, 14). That efficient interaction of the CTD of TLP in vitro binding to aldolase is a hallmark of members of the TRAP tail. To date, the bridging protein of the motor machinery used by the malaria merozoite to propel itself into the host erythrocyte remains unknown, although biochemical evidence suggests a potential, specific role for MTRAP in this process (2).

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Based on our data, we propose that TLP interacts with the actin-bridging molecule aldolase in invading-parasite stages and plays a redundant role in linking target cells and parasite ligands to the actin-myosin motor machinery. In analogy to TRAP, the prototype of this family of proteins, TLP secretion may be precisely regulated and may occur only after initial target cell contact (8). The presence of TLP in all stages tested suggests a conserved, albeit not essential, function that is shared in all life cycle stages. Our expression data are further supported by the presence of *PyTLP* in a sporozoite expressed-sequence-tag library (17) and a cDNA library generated from axenic liver stages (36). Indeed, abundant expression of known merozoite ligands in sporozoites has been described for AMA1 and EBA175 (11, 28), suggesting that merozoites and sporozoites, which both invade via simultaneous formation of a parasitophorous vacuole, share multiple surface ligands.

Our finding that a previously unrecognized TRAP family member performs a redundant role in parasite life cycle progression suggests that parasite motility and host cell entry are driven by a more complex and partially redundant group of transmembrane and surface proteins than previously anticipated.

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