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Plasmodium falciparum erythrocyte invasion: A conserved myosin associated complex

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Abstract

Host cell invasion by apicomplexan parasites is powered by an actin/myosin motor complex that has been most thoroughly described in *Toxoplasma gondii* tachyzoites. In *T. gondii*, two inner membrane complex (IMC) proteins, the peripheral protein TgGAP45 and the transmembrane protein TgGAP50, form a complex with the myosin, TgMyoA, and its light chain, TgMLC1. This complex, referred to as the glideosome, anchors the invasion motor to the IMC. We have identified and characterized orthologues of TgMLC1, TgGAP45 and TgGAP50 in blood-stages of the major human pathogen *Plasmodium falciparum*, supporting the idea that the same basic complex drives host cell invasion across the apicomplexan phylum. The *P. falciparum* glideosome proteins are transcribed, expressed and localized in a manner consistent with a role in erythrocyte invasion. Furthermore, PfMyoA interacts with PfMTIP through broadly conserved mechanisms described in other eukaryotes, and forms a complex with PfGAP45 and PfGAP50 in late schizonts and merozoites. *P. falciparum* is known to use multiple alternative invasion pathways to enter erythrocytes, hampering vaccine development efforts targeting erythrocyte invasion. Our data suggests that the same invasion motor underpins all alternative invasion pathways, making it an attractive target for the development of novel intervention strategies.

Keywords: Apicomplexan; Erythrocyte invasion; Glideosome; Merozoite

1. Introduction

Plasmodium parasites cause between 300 and 500 million cases of malaria each year, with 1–3 million of those being fatal. The life cycle of these parasites is complex, involving development within both human and insect hosts, but it is development within the human erythrocyte that results in all malaria-associated pathology. Development within the erythrocyte is initiated by a parasite-driven active invasion process that is followed by subsequent differentiation and multiplication, finally culminating in the release of multiple invasive merozoites upon rupture of the infected erythrocyte membrane. As the entire intra-erythrocytic developmental stage depends on the initial invasive step, a better understanding of the molecular mechanisms that catalyze invasion could lead to the development of new malaria therapies.

Erythrocyte invasion is a complex process, involving an initial reversible interaction between the merozoite and the erythrocyte, followed by a reorientation step that brings the apical end of the merozoite into juxtaposition with the erythrocyte. Upon reorientation, the merozoite forms an irreversible tight junction between its apical end and the erythrocyte membrane, which subsequently splits and is moved backward along the periphery of the merozoite as it enters the erythrocyte [1]. The initial recognition and junction forming steps have been extensively studied, and are mediated by multiple receptor/ligand interactions that, to some degree, are redundant and interchangeable [2–4]. In contrast, the molecular details of erythrocyte entry are poorly understood.

Unlike the merozoite, which appears to only be actively motile during invasion, other apicomplexan zoite stages (sporozoites, tachyzoites) also exhibit a substrate dependent form of motility termed gliding motility [5,6]. Both invasion and gliding motility are sensitive to cytochalasins and myosin inhibitors, suggesting that an actin/myosin motor – located necessarily between the plasma membrane (PM) and the underlying inner membrane complex (IMC), and linked both to transmembrane

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surface receptors and the cytoskeleton – drives both processes [7–10]. This actin/myosin motor complex has been well described in *Toxoplasma* tachyzoites where a cell surface receptor, TgMIC2, has been shown to link to filamentous actin through the glycolytic enzyme aldolase [11]. Actin is in turn bound by an unconventional class XIV myosin, Myosin A, which is essential for gliding motility and invasion [12]. TgMyoA forms a complex with a light chain, TgMLC1 [13], a peripheral IMC protein, TgGAP45, and an IMC integral membrane protein, TgGAP50 that apparently anchors the myosin complex to the IMC [14]. This complex of TgMyoA, TgMLC1, TgGAP45 and TgGAP50 is collectively referred to as the glideosome, and while the regulatory elements controlling this complex remain undefined, these proteins represent the essential link between extracellular ligand/receptor interactions and the IMC and are likely required for providing the mechanical force driving gliding motility and host cell invasion. Similarly, an interaction between an orthologue of TgMIC2 (Thrombospondin Related Anonymous Protein, TRAP) and aldolase has been described in rodent *Plasmodium* sporozoites [15]. TgMyoA homologues have also been characterized in *Plasmodium berghei* and *Plasmodium yoelii* sporozoites [16], and a homologue of TgMLC1, *P. yoelii* Myosin Tail Interacting Protein (MTIP), was identified as interacting with PyMyoA in a yeast two-hybrid system [17].

In contrast, our understanding of *Plasmodium falciparum* motility and host cell invasion is severely limited. *P. falciparum* causes almost all human malaria mortality, and erythrocyte entry is clearly central to malarial pathogenesis. However, PfMyoA, an orthologue of TgMyoA, is the only motility associated protein characterized thus far in *P. falciparum* merozoites [10]. Moreover, orthologues of the cytoskeletal anchoring components, TgGAP45 and TgGAP50, have yet to be described in any *Plasmodium* zoite stage. To establish whether the motility/invasion apparatus defined in *Toxoplasma gondii* tachyzoites is present in *P. falciparum* merozoites, we have characterized orthologues of TgMLC1, TgGAP45 and TgGAP50 in *P. falciparum* blood-stage parasites. We show that these motility-associated proteins are transcribed, expressed and localized in a manner consistent with a role in erythrocyte invasion. Furthermore, we show that PfMyoA interacts with PfMTIP in a manner analogous to the interaction between conventional eukaryotic myosins and their associated regulatory light chains, and that PfMTIP is in a complex with PfGAP45 and PfGAP50—even in *P. falciparum* strains that differ in their receptor/ligand erythrocyte invasion pathways. Taken together our results confirm the idea that gliding motility and host cell invasion in apicomplexan zoite stages rely on the same basic motor components and imply that, in *P. falciparum* merozoites, these components power invasion independent of the receptor/ligand pathway being used.

2. Materials and methods

2.1. P. falciparum culture and strain selection

P. falciparum strains were maintained in culture in human O^+ erythrocytes as described previously [18] and synchronized by treatment with 5% sorbitol. For selection of alternate invasion pathways, strain Dd2 was incubated in the presence of neuraminidase treated O^+ erythrocytes for 14 days before parasites appeared in Giemsa stained thin smears. These parasites were subsequently cloned by limiting dilution, and the ability of individual clones to invade neuraminidase treated erythrocytes compared with the parent strain. One clone, Dd2/Nm D3, was used for subsequent analysis, and could invade neuraminidase treated erythrocytes with a similar efficiency as untreated erythrocytes, unlike the parental strain Dd2 which was completely unable to invade neuraminidase treated erythrocytes (data not shown). Strains 3D7 and Dd2 were obtained from the Malaria Research and Reference Reagent Resource Center (www.mr4.org).

2.2. Quantitative reverse transcriptase PCR

Total RNA was extracted (using ToTALLY RNA Isolation kit–Ambion) from tightly synchronized *P. falciparum* culture at five time points representing predominantly ring, early trophozoite, late trophozoite, early schizont and late schizont stages. RNA samples were treated with RNase-free DNase (Ambion) and used for cDNA synthesis with a 5′-RACE kit (Invitrogen). These cDNA samples – along with primers that amplified 100–400 bp regions from each glideosome component, *PfLSA-1*, and *13P184* – were used in QRTPCR experiments. QRTPCR experiments were performed in duplicate on 96-well plates using SYBR green supermix (Biorad). Annealing temperatures were optimized for each primer set by performing temperature gradient QPCR with gDNA, and a standard curve was generated using a gDNA concentration gradient of 5-fold dilutions (100 ng/rxn–6.4 pg/rxn) that was related to the reaction cycle at which the concentration of amplified product crossed a set threshold (Ct). Standard curves were generated for each experiment and used to calculate the starting quantity of cDNA in each reaction. These starting quantity values, initially in units of nanograms, were converted to picomoles to account for the size of the amplified product, and then normalized by dividing each value with the corresponding value from QRTPCR with *13P184* primers. Each value reported in Fig. 1A therefore represents a relative measure of gene transcription that is controlled for size of the QRTPCR amplification product and for efficiency of cDNA synthesis.

2.3. Preparation of PfMyoA, PfMTIP, PfGAP45 and PfGAP50 antisera and affinity purification

Partial sequences of *PfMyoA* (forward primer: 5′-TGGGTACCGCAAATCCAGTTCTTGAAGCT-3′, reverse primer: 5′-TGGAATTCTTAAAATACTTCAAAACCAAAAATATC-3′) *PfMTIP* (forward primer: 5′-TGGGTACCATGAAACAAGAATGCAATG-3′, reverse primer: 5′-TGGAATTCTTATTGTAATATATCTTCACAG-3′), *PfGAP45* (forward primer: 5′-TGGGTACCATGGGAAATAAATGTTCAAG-3′, reverse primer: 5′-TGGAATTCTTAGCTCAATAAAGGTG-3′) and *PfGAP50* (forward primer: 5′-CGGGATCCACTACGCTTTGCGTCTTTGGG-3′, reverse primer: 5′-CGGAATTCTTATTTATTTCCCATGGGTCCAC-3′) were cloned into the expression plasmid pRSETB (Invitrogen) and transformed into *Escherichia coli*