

Trafficking of *Plasmodium falciparum* invasion proteins to the parasite micronemes

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Abstract

Malaria continues to be a global health problem. Despite a marked reduction in mortality over the last 15 years, these hard-fought gains are threatened by growing resistance of the *Plasmodium falciparum* malaria parasite to artemisinin, the frontline drug used in treatment of the disease. Clinical symptoms of malaria are caused by the intra-erythrocytic phase of the parasite life cycle. Entry into the erythrocyte is accomplished by several specialised invasion proteins, which are stored in unique apical secretory organelles known as micronemes and rhoptries. Very little is known about the trafficking signals and transport mechanisms of invasion proteins to these organelles.

Three micronemal proteins, Apical Membrane Antigen-1 (*PfAMA-1*), Subtilisin-like protease 2 (*PfSUB2*) and Erythrocyte Binding Antigen 181 (*PfEBA181*) were investigated with the aim of identifying domains responsible for targeting the micronemes. Selected domains were amplified and mini-genes were created by overlap extension PCR. A pARL-mCherry plasmid containing a *Pfama-1* stage-specific promoter that is only active during the schizont stage of parasite development when micronemes are formed, was used to create mCherry-tagged constructs. *P. falciparum* parasites were transfected by electroporation of the plasmid constructs. Transgenic parasites were selected by drug pressure and the expression of red fluorescent mCherry-tagged chimaeric proteins was visualised in live parasites. Co-localisation studies were performed with a microneme marker to assess if the transgenic mini-proteins reached their destination. Interestingly, all three proteins required different domains to target the micronemes: *PfEBA181* required an extended region of a conserved cysteine-rich domain, *PfAMA-1* required the prodomain, and *PfSUB2* required the transmembrane domain. Since no common targeting signal was identified, the possibility of a protein escorter was explored. The *PfAMA-1* prodomain was expressed as a recombinant histidine-tagged protein and immobilised onto Nickel-coated beads, which were exposed to a *P. falciparum* phage display library for four rounds of biopanning. Two novel binding partners were identified: a putative Chaperone Binding Protein and a putative Formin 2.

The identification of the molecular trafficking determinants of three invasion proteins, as well as a potential protein escorter for microneme targeting, represent novel findings that extend our knowledge of a fundamental biological process in the malaria parasite. This pathway may be exploited for drug development and new malaria treatment strategies.