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RESEARCH NOTE

Trafficking of *Plasmodium falciparum* chimeric rhoptry protein with Brefeldin A

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Abstract: Trafficking of the rhoptry chimeric protein RhopH2-GFP, which contains RhopH2 signal peptide plus the downstream five amino acids, was dissected by treating parasites with Brefeldin A at three different time points. Twenty eight hrs-stage trophozoites accumulated the chimera within the parasite endoplasmic reticulum. In 32 hrs-stage schizonts, the chimera was distributed in the parasite cytoplasm but not in the parasitophorous vacuole. In 36 hrs stage-schizonts, the chimera was detected in individual structures around the developing merozoites and, in contrary to non-treated parasites, no immature rhoptry vesicles could be detected in the cytoplasm of immature merozoites. These data show that this chimera is trafficked to the rhoptries via Brefeldin A-sensitive pathway indicating that this trafficking is similar to that of the endogenous rhoptry proteins, and that the five amino acids downstream of the signal peptide cleavage site may contain the sorting signal required for rhoptry targeting.

Keywords: green fluorescent protein, apical secretions, targeting, organelles, merozoite, Apicomplexa

Invasion of erythrocytes by *Plasmodium* merozoites is a tightly regulated multi-step process. This rapid process begins with the release of proteins from the micronemes that are believed to be involved in the attachment of extracellular parasites to the host membrane, which is then followed by secretion of rhoptry proteins. Secretion of rhoptry proteins is initiated very rapidly after intimate contact of the parasite and the host cell, and is completed within a few minutes of invasion, implying that the contents of rhoptries are vital in the establishment of the parasitophorous vacuole. In comparison to microneme proteins, the role of rhoptry proteins is less well understood in apicomplexan parasites (Dowse and Soldati. 2005, Bradley and Sibley. 2007, Kaneko 2007, Kats et al. 2008, Tufet-Bayona et al. 2009).

Rhoptry proteins are found either in the rhoptry neck or in the rhoptry bulb, and no protein has been observed in both, suggesting that these two locations are discrete compartments (Kawase et al. 2007). In *Plasmodium*, it was suggested that the rhoptries fuse with the micronemes to facilitate release of their contents (Zhao and Satir1998).

A total of 20 proteins have been identified in the rhoptries of *Plasmodium* merozoites (Kaneko 2007, Haase et al. 2008, Wickramarachchi et al. 2008). These include, among others, the high molecular mass RhopH complex, which consists of three proteins: RhopH1, RhopH2, and RhopH3 (Campbell et al. 1984, Holder et al. 1985, Cooper et al. 1988, Hienne et al. 1998). The RhopH complex is conserved across *Plasmodium* species and seems to be secreted from the rhoptry bulb of mature merozoites. After invasion of erythrocytes, RhopH complex is retained in the newly formed ring stage parasites (Ling et al. 2003). The exact function of the complex has not been determined yet but the complex is thought to be involved in erythrocyte binding during or after invasion (Sam-Yellowe 1992, Rungruang et al. 2005), and perhaps participates in the formation of the parasitophorous vacuolar membrane (Ling et al. 2003, 2004).

Unlike proteins targeted to the apicoplast (Foth et al. 2003) or exported into the host erythrocyte (Hiller et al. 2004, Marti et al. 2004), there is no common targeting signal for rhoptry proteins. The accurate targeting of rhoptry proteins to their final destination is an essential process for the invasion of erythrocytes and the growth of parasites. In a previous study (Ghoneim et al. 2007), we determined that the N-terminal 24 amino acids of RhopH2, including signal peptide sequence, are sufficient to target green fluorescent protein (GFP) to the rhoptries and we proposed that this targeting is likely mediated by a unique mechanism that depends on the interaction with N-terminal 24 amino acids of RhopH2 early in the secretory pathway. In this report, we dissected the trafficking of RhopH2-GFP to the rhoptry organelles under the effect of Brefeldin A.

In the RhopH2-GFP chimera, GFP is expressed under the control of the *rhoph2* promoter together with the sequence encoding RhopH2 signal peptide plus the downstream 5 amino acids (amino acid sequence: MIKVTIFLLLSIFSFNLYG\LELNE). This chimera has been previously shown by Ghoneim et al. (2007) to be targeted to the rhoptry efficiently. To dissect the trafficking of this chimera, parasites cultures expressing this chimera were synchronized with 5% Sorbitol twice after 26 hrs window and divided into four cultures. One was maintained as a control and the other three were treated at three different schizont stages with Brefeldin A (Sigma) at 5 μM final concentration. Brefeldin A (BFA) was applied in the new parasite cycle after culture synchronization. Small aliquots of parasite culture were washed and incubated with PBS solution containing the nuclear stain 4′,6-diamidino-2-phenylindole (DAPI) for

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