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Plasmodium falciparum: Characterization of a late asexual stage Golgi protein containing both ankyrin and DHHC domains

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Abstract

Proteins containing the DHHC motif have been shown to function as palmitoyl transferases. The palmitoylation of proteins has been shown to play an important role in the trafficking of proteins to the proper subcellular location. Herein, we describe a protein containing both ankyrin domains and a DHHC domain that is present in the Golgi of late schizonts of *P. falciparum*. The timing of expression as well as the location of this protein suggests that it may play an important role in the sorting of proteins to the apical organelles during the development of the asexual stage of the parasite.
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Keywords: Malaria; *Plasmodium*; Palmitoylation; Ankyrin

1. Introduction

The Apicomplexan parasites, that include the medically important *Plasmodium* species, are characterized by the presence of three novel asymmetrically oriented organelles. These organelles, located in the region of the parasite that first enters the erythrocyte, are termed apical organelles. The complicated process of erythrocyte invasion is mediated, in part, by proteins that are stored in these three organelles (rhoptries, micronemes, and dense granules) and released at the time of invasion. The organelles are formed late in the asexual erythrocytic cycle, at the time of formation of merozoites, the invasive form of the parasite. The trafficking processes that lead to the sorting of these proteins from the Golgi to the correct apical organelle remain to be defined. Unlike the closely related *Toxoplasma*, where a tyrosine-based cytoplasmic motif for sorting to rhoptries exists (Hoppe et al., 2000), no such motif has been identified in *Plasmodium*. We describe a molecule that, because of its structure, localization, and temporal expression might catalyze key lipid modifications of proteins that could play an important role in the sorting of proteins to apical organelles in *Plasmodium*.

In the course of the preliminary analysis of the *Plasmodium falciparum* genome (Gardner et al., 2002), it was noted that several proteins contained a novel conserved domain of approximately 48 amino acids which contains seven conserved cysteines and a characteristic DHHC (Asp-His-His-Cys) motif (Fig. 1). Subsequent studies on the yeast endoplasmic reticulum protein Erf2, which also contains a DHHC domain, showed that it was required for the appropriate localization of the yeast RAS protein to the internal leaflet of the plasma membrane (Bateman et al., 2000; Putilina et al., 1999). This failure of proper trafficking of RAS was shown to arise due to a lack of palmitoylation of the RAS protein. Further studies on

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                                  Ank
                              Domains   TM

P.falciparum PFC0160w         5         4    CVTCNIIKPPRVHHCAECFHCIVHQDHHCVWVDNCIGIKNQRCFYMFI
P.vivax H14090                5         4    CVTCNIVKPPRVHQRAECFHCIVHQDHHCVWVDNCIGIKNQRAFYLFI
H.sapien HIP14                6         6    CSTCLIRKPVRSKHCGVCNRCIAKFDHHCPWVGNCVGAGNHRYFMGYL
L.major Q9BLUO                0         4    CMTCHIYRPSQSGHCRRCNNCVALFDHHCRLLGCCIGELNRRYFLLFL
D.melanogaster CG6618         6         4    CHSCRCLRPLRAKHCRVCNRCVSYFDHHCPFIYNCVGLRNRMWFFLFV
S.cerevisiae ERF2             0         4    CPSCRIWRPPRSSHCSTCNVCVMHVDHHCIWVNNCIGKRNYRFFLIFL
A.thaliana AF013294           0         4    CHHCSVCKAK-SDLFILCQRCVLKMDHHCVWIVNCVGARNYKFFLLFL
                                               *  *   :.       *  *:   ****  :  *:*  *   *  ::
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Fig. 1. Multiple alignment of DHHC domains. The alignment was constructed using the ClustalW program.

DHHC-containing proteins have shown that many of them function as membrane-associated enzymes that transfer a 16-carbon palmitate moiety to the sulfur on a cysteine residue (Roth et al., 2002). This palmitoylation is widespread in eukaryotes and results in soluble proteins becoming associated with membranes or altering the behavior of transmembrane proteins by modification of their cytoplamic tails (Linder and Deschenes, 2003).

2. Materials and methods

2.1. Parasite culture

The 3D7 strain of *P. falciparum* was cultured essentially as described by Trager and Jensen, with the modification of the addition of Albumax II (Invitrogen) rather than serum (Trager and Jensen, 1976). Parasites were synchronized by the treatment of cultures with 5% sorbitol, resulting in the lysis of trophozoite stages and survival of ring stage parasites.

2.2. Antisera production

An 800 bp portion of the DHHC-containing gene was amplified using the following primers: 5′ GAGAG ATCTAATTCTATAAACATCTTACATTGGGC and 3′ GAGACGCGTATAAACCATATTTAAACAGCC ATAATCACA. The resulting product was cut with *Mlu*I and *Bgl*II and ligated into a modified VR1020 vector (gift of Stephen Hoffman). Five hundred micrograms of this purified vector was injected intradermally into Sprague–Dawley rats three times at three week intervals. Animals were exsanguinated 10 days after the final injection and sera were collected.

2.3. Western blotting

Late stage parasites were saponin lysed and subsequently resuspended in PBS with 2% SDS and protease inhibitors (complete protease inhibitor cocktail (Roche) plus 1 μM pepstatin A). Extract equivalent to 5×10^5 parasites was run on a 3–8% Tris–acetate gel and transferred to PVDF (Invitrogen). The membrane was probed with a 1:500 dilution of primary sera and a 1:25,000 dilution of goat anti-rat sera conjugated to HRPO (Jackson Immunoresearch). Bands were visualized using ECL (Amersham).

2.4. Immunofluorescence

Late stage parasites were smeared and fixed for 10 min in 1% formaldehyde. Slides were subsequently blocked for 1 h at room temperature in blocking buffer (PBS, 1% normal goat serum (Jackson Immunoresearch), 0.1% Triton X-100). Primary antibodies were diluted in blocking buffer at the following concentrations: rat anti-DHHC 1:200, anti-RAP 1:500, anti-subtilisin 2 (a kind gift of Jean-Christophe Barale) 1:250, and rabbit anti-ERD2 (a kind gift of John Adams) 1:200. Slides were allowed to incubate with primary antibodies at room temperature for 1 h and were subsequently washed in PBS and stained with the appropriate secondary (Jackson Immunoresearch) diluted 1:500 for 30 min at room temperature. Slides were then washed in PBS, mounted in anti-fade reagent (Molecular Probes) and visualized using a Leica TSC-NT/SP confocal microscope under 63× oil immersion objective. Images were subsequently deconvoluted using Imaris software.

3. Results and discussion

Completion of the *Plasmodium* genome has revealed the presence of 13 distinct proteins containing the DHHC domain, suggesting an active palmitoylation system in the organism (Aravind et al., 2003). We have analyzed one of

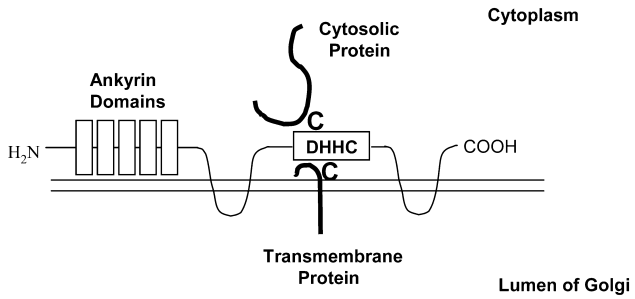

Fig. 2. Proposed model of topology of PfAnkDHHC in the Golgi membrane. The DHHC domain is exposed to the cytosol to serve as a palmitoyl transferase to cytosoloic proteins or transmembrane proteins with cysteines.