Abstract of Dissertation submitted by CHAIYAWONG Nattawat

Title: Distinct effects on the secretion of MTRAP and AMA1 in *Plasmodium yoelii* following deletion of acylated pleckstrin homology domain-containing protein

ネズミマラリア原虫 *Plasmodium yoelii* の acylated pleckstrin homology domain-containing protein を欠損させると MTRAP と AMA1 の分泌に異なった影響を与える

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Introduction:

Plasmodium protozoan parasites, the causative agents of malaria, are obligate intracellular organisms. In humans, pathogenesis is caused by the blood stage parasite, which multiplies within erythrocytes, thus erythrocyte invasion is an essential developmental step. Merozoite form parasites released into the blood stream coordinately secrets a panel of proteins from the microneme secretory organelles for gliding motility, establishment of a tight junction with a target naive erythrocyte, and subsequent internalization. A protein identified in *Toxoplasma gondii* facilitates microneme fusion with the plasma membrane for exocytosis; namely, acylated pleckstrin homology domain-containing protein (APH). Conditional knockdown of TgAPH resulted in pronounced defects in both parasite egress and invasion. Specific interaction with PA was shown for recombinant TgAPH as well as recombinant *P. falciparum* APH. Because APH is conserved across Apicomplexa, a similar role is expected for malaria parasites. However, the erythrocyte invasive merozoite stage of malaria parasites has unique features absent in *T. gondii*; for example, only *Plasmodium* merozoites possess EBL and RBL protein families, and *Plasmodium* egress is not controlled by microneme proteins. To obtain insight into the differential microneme discharge by malaria parasites, the consequences of APH deletion in the rodent malaria model, *Plasmodium yoelii* (*P. yoelii*), were analyzed.

Materials and Methods:

To evaluate the expression and function of APH in *P. yoelii*, transgenic *P. yoelii* lines were created using a DiCre-based inducible knockout method, which *aph* gene locus could be excised by rapamycin (RAP) treatment. The effect of RAP administration to PyAPH-iKO parasites on the APH expression level was evaluated by western blot analysis. Indirect immunofluorescence assay (IFA) was performed to evaluate the PyAPH cellular localization Next, the effect of the excision of the *aph* gene locus on parasite asexual growth was examined. The consequence of *aph* gene locus excision in *P. yoelii* on the secretion of

invasion-related molecules was examined by secretion assay. Additionally, Time-lapse image analysis was performed to describe the invasion steps affected by the absence of APH in *P. yoelii*.

Results:

This study demonstrated that IFA signals of APH in *P. yoelii* merozoites were highly overlapped with PyMTRAP signals, but less overlapped with PyAMA1 signals. APH deletion resulted in a reduction in parasite asexual growth and erythrocyte invasion, with some parasites retaining the ability to invade and grow without APH. APH deletion also impaired the secretion of microneme proteins, MTRAP and AMA1, and upon contact with erythrocytes the secretion of MTRAP, but not AMA1, was observed. APH-deleted merozoites were able to attach to and deform erythrocytes, consistent with the observed MTRAP secretion. Tight junctions were formed, but echinocytosis after merozoite internalization into erythrocytes was significantly reduced, consistent with the observed absence of AMA1 secretion. Together with my observation that APH largely colocalized with MTRAP, but less with AMA1, I propose that APH is directly involved in MTRAP secretion; whereas any role of APH in AMA1 secretion is indirect in *Plasmodium*.

Discussion:

Transgenic parasite lines for which PyAPH can be conditionally knocked out were generated using the DiCre conditional recombinase system. I demonstrated that PyAPH has an important role for the secretion of PyMTRAP and PyAMA1, thus excision of the *aph* gene locus severely impaired erythrocyte invasion and parasite asexual growth. I showed that PyAPH colocalized with PyMTRAP, but not with PyAMA1 and proposed that different direct signals exist to trigger the secretion of PyMTRAP versus PyAMA1. I also proposed that erythrocyte-contact may trigger PyMTRAP secretion. Further investigation of these new findings will provide a better understanding of the molecular mechanism of erythrocyte invasion by malaria parasites and may lead to the discovery of novel drug targets.

Notes: Summarize your dissertation with 2 pages of A4 (using 12 point, Times New Roman font, single space. Total number of words should not exceed 1000)