Abstract of Dissertation submitted by CHITAMA BEN-YEDDY ABEL

Title: Multiple charged amino acids of *Plasmodium falciparum* SURFIN$_{4.1}$ N-terminal region are important for efficient export to the red blood cell

(熱帯熱マラリア原虫の SURFIN$_{4.1}$ の赤血球への効率的な輸送には N 末端領域の複数の荷電アミノ酸が重要である)

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Introduction:

Plasmodium falciparum, an obligate intracellular protozoan parasite of severe form of malaria, exports numerous proteins important for its survival but of severe pathological effect to its host. These proteins and the export mechanism are candidates for the drug and vaccine development.

One group of the exported proteins contains a conserved motif for export, called the *Plasmodium* Export ELement (PEXEL) or Vacuolar Targeting Sequence (VTS), which consists of a conserved pentameric amino acid, RxLxE/Q/D and has been shown to be essential for export. During transport within the endoplasmic reticulum (ER), this motif is cleaved by the ER-resident protease plasmepsin V. The exposed amino acid residues (xE/Q/D) is often acetylated at the N terminal and then exported to the red blood cell (RBC) cytoplasm.

Another group of exported proteins that lack this PEXEL motif have been identified, and termed as PEXEL-Negative Exported Proteins (PNEPs). These proteins do not contain conserved motif like the former one and it has not yet shown clearly if their N-terminal is being processed or not during their export. SURFIN family proteins are among the members of this group. SURFIN$_{4.1}$ has been shown to localize in the RBC cytoplasm and on the released merozoites. Another member, paralogue SURFIN$_{4.2}$ has been shown to be exported to the surface of infected RBCs.

Previously, N-terminal region with the sequence around transmembrane region of SURFIN$_{4.1}$ was shown to be essential for its export to the Maurer's clefts in the RBC cytoplasm and this region was proposed to be recognized by machinery responsible for the protein translocation across the parasitophorous vacuole membrane surrounding the parasite. However, the role of the N-terminus in this transport was not fully characterized at the amino acid level and its N-terminal processing also was not assessed. Therefore, these subjects are being addressed in

this study.

Materials and Methods:

For analysing the amino acids affecting protein export, I utilized mini-SURFIN$_{4.1}$ that consists of minimum essential components with C-terminus tagging with green fluorescent protein for the export. All charged amino acids in the N-terminal region was replaced to alanine independently and exogenously expressed in *Plasmodium falciparum* MS822 line. After confirming the protein amount, relative fluorescence intensity detected in the infected RBC cytoplasm per total intensity were quantitated by live cell imaging and compared with signal for the wild type mini-SURFIN$_{4.1}$. Substitutions that showed significant reduction in export were further confirmed using *P. falciparum* 3D7S8 line. Signal was analysed by Fiji Image J software and GraphPad PRISM6. Indirect fluorescence assay (IFA) was also conducted to evaluate the location of the exported protein in the RBC cytoplasm. For analysing the N-terminal processing, I N-terminally tag the mini-SURFIN$_{4.1}$ and mini-SURFIN$_{4.2}$ with Myc tag.

Results:

Two pattern of protein export by the transfected mutant parasite was observed in MS822 line parasite. Those that had most signals retained in the parasite (E38A, K42A, E45A, K49A, and E50A substitutions) and those that had signals exported to the infected RBC cytoplasm (D34A, K35A, D37A, and D40A substitutions). Substitutions resulting reduced export were further confirmed using 3D7S8 line parasite. Although detected signal in the RBC cytoplasm was low, IFA revealed that they correctly located at Maurer's clefts. IFA of the N-terminal Myc-tagged mini-SURFIN$_{4.1}$ and mini-SURFIN$_{4.2}$ stained with anti-Myc antibody showed that the signal was colocalized with Maurer’s cleft resident marker SBP1, indicating that N-terminal sequence of SURFIN$_{4.1}$ and mini-SURFIN$_{4.2}$ were not processed.

Discussion:

We were able to probe out five charged amino acids that affect export of SURFIN$_{4.1}$ to the infected RBCs. Both positive and negative charges, together with the correct positioning of these amino acids contributed to the export effect observed. These charged amino acid residues may interact directly with protein translocation machinery or may be responsible for a correct conformation to be recognized by it. Mini-SURFIN$_{4.1}$ and mini-SURFIN$_{4.2}$ proteins containing N-terminal Myc tags were detected at Maurer's clefts, indicating that N-terminal processing of SURFIN sequence is not required for export of these proteins to the RBC cytoplasm.

This is the first report that shows the effect of both positive and negative charges in the export of parasite protein to RBC cytoplasm, also it is the first report showing clearly the uninvolvement of N terminal processing. Our results implicate a conserved nature of SURFIN transport and contribute to understanding of proteins that lack obvious export motif (PNEPs) to Maurer's clefts.