（第３号様式）

学 位 論 文 要 旨

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論　文　名　　マラリア原虫スポロゾイトのロプトリータンパク質（RON11）は
蚊唾液腺侵入および肝細胞侵入に重要な分子である

学位論文要旨

Malarial sporozoite, which invades sequentially mosquito salivary gland cells and mammalian hepatocytes, is one of the key infective forms during *Plasmodium* life cycle regarding parasite transmission via mosquito vector. Identification of several sporozoite-specific secretory proteins involved in invasion processes has revealed that sporozoite motility and specific recognition of target cells are crucial for transmission. It has also demonstrated that some invasion machinery is conserved among infective stage parasites. However, the mechanisms of signal transduction inside sporozoites, such as calcium signaling, remain still largely unknown. To determine the mechanisms of sporozoite invasion machinery, we focused on the rhoptry proteins because it has been suggested that they are involved in erythrocyte invasion by merozoites. Rhoptry neck protein 11 (RON11), one of the rhoptry proteins, is highly conserved in phylum Apicomplexa, suggesting an important role in rhoptry-mediated functions. In addition, the attempts to disrupt the *pbron11* locus failed suggesting that RON11 has a crucial role during blood-stage parasite proliferation. In this study, we intended to elucidate the function of RON11 in the sporozoite, using rodent malaria *Plasmodium berghei*.

RON11 is expressed in Plasmodium sporozoites and localized to rhoptries

PbRON11 (PBANKA_132710) has a unique feature containing an N-terminal signal peptide, 7 transmembrane domains and an EF-hand domain pair, known as a general moiety in Ca^{2+} binding proteins, at C-terminal. Western blot results using anti-PbRON11N antibodies revealed thatPbRON11 band, detected at about 100 kDa bands in sporozoite samples collected from mosquito oocyst, hemocoel and salivary gland, indicating that PbRON11 is synthesized early in sporozoite formation and its protein amount remains until after sporozoite invaded the salivary glands. By Indirect fluorescence microscopy, PbRON11 was detected after sporozoite invaded the salivary glands. By Indirect fluorescence

microscopy, PbRON11 was detected as a punctate pattern in mature schizonts, distributed widely in the anterior part of midgut sporozoites and concentrated in the apical region in salivarysporozoites. Immunoelectron microscopy was performed for precise localization, PbRON11 was demonstrated to localize to the neck portion of rhoptry in merozoites and body portion of rhoptry in salivary gland sporozoites.

RON11 is required for sporozoite attachment followed by continuous gliding.

In order to elucidate the PbRON11 role in sporozoite stage during sporozoite invasion, sporozoite stage-specific *pbron11* silencing transgenic parasites in *P. berghei* (PbRON11-cKD) was generated using promoter-swapping strategy. The 5' UTR of *pbron11* was replaced with a promoter region of *pbmsp9* (PBANKA_144330) using double crossover homologous recombination. Mutant sporozoites collected from hemocoel demonstrated a severe defect of attachment and gliding motility indicating that PbRON11 plays essential role in sporozoite attachment and gliding motility.

RON11 is essential for sporozoite invasion of two different types of target cells.

We investigated the sporozoite formation in the midgut oocysts and their migration ability of PbRON11-cKO sporozoites in mosquitoes. The numbers of sporozoites from the midgut as well as from hemocoel of PbRON11-cKD infected mosquitoes were equal or greater than that of PbRON11 control (PbRON11-cont) infected mosquitoes. This result indicates that sporozoite formation and release occurs normally in PbRON11-cKD. In contrast, the numbers of sporozoites collected from salivary glands of mosquitoes infected with PbRON11-cKD were considerably reduced by about 100-fold compared to that of PbRON11-cont infected mosquitoes, demonstrates that PbRON11 plays a essential role for sporozoites invasion of mosquito salivary glands. We also investigated whether PbRON11 is required for liver infection using real time PCR analyses. C57BL6 mice were injected intravenously with 20,000 hemolymph sporozoites then livers were collected 24 hours later to quantify the amount of infected parasites using detection of 18S rRNA by real-time PCR. Mutant sporozoites showed about 10^4 times less infectivity than control parasite. *In vitro* culture system using human hepatoma cell, HepG2 were performed to investigate whether PbRON11 is crucial for invasion process into hepatocytes. From 10,000 of hemolymph sporozoites of PbRON11-cont, more than 1,000 sporozoites were attached to HepG2 cells and 400 sporozoites invaded intracellarly. In contrast, about 350 sporozoites of mutant sporozoites were attached on the surface of HepG2 with no sporozoite were observed intracellularly. These results indicated that RON11 has a crucial role both in invasion of mosquito salivary glands and mammalian host liver via its attachment and gliding motility.

In summary, PbRON11 is localized to the rhoptry in sporozoites as well as in merozoites. To repress the PbRON11 expression exclusively in sporozoites, we produced transgenic parasites using promoter-swapping strategy. PbRON11-repressed sporozoites showed significant reduction in attachment and motility *in vitro*, consequently failed to invade salivary glands efficiently. Furthermore, PbRON11 is also essential for sporozoite invasion of hepatocytes, the first step during transmission to mammals. RON11 is the first rhoptry protein crucial for sporozoite motility and attachment to the target host cells.

キーワード	*Plasmodium*, sporozoite, rhoptry, invasion, conditional knock-down