

EMBO WORKSHOP 2005 "Molecular and Population Biology of Mosquitoes and Other Diseases Vectors"
Kolymbari, Crete, Greece

Robust salivary gland-specific expression in transgenic *Anopheline* mosquito

Shigeto Yoshida & Hiroyuki Watanabe

*Jichi Medical School, Division of Medical Zoology, Department of Infection and Immunity,
Minamikawachimachi, Tochigi329-0498, Japan
e-mail: shigeto@jichi.ac.jp*

Recent advances in the genetically engineering of *Anopheline* mosquitoes have raised hopes for their use as new strategies for malaria control and provided a powerful tool to investigate parasite-mosquito interactions. The interruption of the parasite cycle in the mosquito using transgenic strategies can occur at the midgut level, interfering with ookinete invasion or oocyst differentiation, and at the salivary gland level, blocking invasion of gland cells or the transmission of infective sporozoites from the salivary glands. So far, only the *carboxypeptidase* and *vitellogenine* promoters have been used for driving transgenes in mosquitoes for the gut- and fat body- specific expression, respectively. Therefore, research on transmission blockade using transgenic mosquitoes has been limited to the midgut stage of the parasites.

For the past decades, it has been postulated that establishment of salivary gland-specific gene expression would make it possible to elucidate the parasite-salivary glands interaction and contribute to the biological control of this important infectious disease, for example through the generation of a transgenic mosquito refractory to the parasites. However, robust salivary gland-specific expression in any transgenic mosquitoes has not been achieved yet, and the specific mechanisms by which *Plasmodium* parasites infect the salivary glands remain largely unknown.

Here, we generate transgenic *Anopheles stephensi* mosquitoes that specifically express *Discosoma* sp. red fluorescent protein (DsRed) in their salivary glands (*see photograph*). Using advanced 3D confocal microscopy, we show robust expression of DsRed in the distal-lateral lobes of the salivary glands, where the sporozoites invade preferentially, driven by a newly isolated salivary gland-specific promoter in a living mosquito as well as in dissected salivary

glands. Furthermore, by using GFP-expressing sporozoites, we could observe the parasites passing through the salivary glands from the outer surface. These results open up the possibility of elucidating the process and molecules involved in the salivary glands-parasites interactions, and may lead to the development of transmission-blocking strategies by using genetically modified mosquitoes that express anti-parasitic genes in a salivary gland-specific manner.

Key words. transgenic mosquito, *Anopheles stephensi*, salivary gland, sporozoite, DsRed, GFP

Strong DsRed fluorescence is observed in the thorax of transgenic mosquito.

