

Display of *plasmodium* adhesive domains on the surface of lambda phage

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With the aim to better understand the molecular interactions underlying mosquito salivary gland invasion by *plasmodium* sporozoites, we are displaying on the surface of lambda phages putative adhesive domains from few selected *plasmodium* proteins which may be involved in the invasion of mosquito salivary glands (domains from TRAP, CS, MAEBL, CRMPs). The lambda system is particularly suitable for our purpose since it makes possible to display a variety of relatively large protein domains (up to 400 aminoacids) as fusions to the C-terminus of the abundant viral capsid protein gpD. Thus, a phage library displaying a collection of *plasmodium* adhesive domains could be employed for the development of affinity selection experiments targeted to the identification of salivary gland receptors. As a first step toward this direction, adhesive domains from *P. falciparum* and *P. berghei* surface proteins putatively involved in recognition/invasion of mosquito salivary glands have been fused to the C-terminus of the lambda phage capsid protein gpD. In order to create the mini-library each domain was amplified by PCR, cloned in a plasmid vector, sequenced and transferred into the lambda vector λD4 (kindly provided by Dr. S. Nasi, University La Sapienza, Rome). After packaging, the resulting libraries were screened for the isolation of recombinant lambda clones. Pilot western blot analysis indicates that, as expected, all the lambda clones identified and isolated to date correctly express the fusion proteins. Screening and verification of correct display of all the selected domains is in progress. The collection of lambda phages will be used for the development of binding assays and used for the affinity selection of salivary gland ligands.

Anopheles gambiae salivary gland promoter analysis

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Regulatory regions driving gene expression in specific target organs of the African malaria vector *Anopheles gambiae* are of critical relevance for basic research in mosquito biology and mosquito-parasite interaction and for the development of new vector control strategies. Recently, we described the transactivation properties of genomic fragments located just upstream of the *An. gambiae* female salivary gland-specific genes *AgApy* and *D7r4* in transgenic *An. stephensi*. An 800 bp fragment from the *AgApy* gene directed specific expression of the LacZ reporter gene in the salivary glands of transgenic *An. stephensi*. However, expression levels were lower than expected and the transgene was expressed in the proximal-lateral rather than in the distal-lateral lobes of female glands. We report here the establishment of transgenic *An. gambiae* lines carrying a larger portion of the *AgApy* promoter (~2,4 kb) driving the *LacZ* reporter gene. A transformation vector based on the *piggyBac* transposon and marked with the *dsRed* gene under control of the 3xP3 promoter was used for the microinjection experiments. A number of different transgenic *An. gambiae* G1 founders were obtained: the progeny was analyzed by Southern blot revealing the presence of single as well as multiple copies of the transgene. Three independent lines containing one (E9), three (D6) and four copies (D4) of the transgene were selected and established for further analysis. RT-PCR expression analysis with *LacZ*-specific primers showed different expression profiles in the three transgenic lines suggesting that position effect and copy number affect reporter gene expression. In fact, *LacZ* mRNA was ubiquitously detectable in transgenic mosquitoes from the D4 line whereas individuals from the D6 line exhibited an expression profile restricted to female salivary glands and adult males. Finally, transgene expression in E9 mosquitoes appeared strong in larval and pupal stages, absent in female carcasses and rather weak in salivary glands and males. Histochemical assays on whole glands and western blot analysis on salivary protein extracts failed so far to reveal β-galactosidase protein in transgenic mosquitoes of the different lines.

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