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Toward the understanding of function and complexity of the *Anopheles gambiae* salivary glands.

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The mosquito salivary glands are an interesting target of molecular entomology studies both for their involvement in the transmission of pathogens and for the pharmacological activities carried by the salivary secretions (anti-hemostatics, immunomodulators, etc.). Using a cloning strategy based on the selective trapping of cDNAs encoding signal peptide-containing proteins we identified, in the last few years, twenty-two novel genes whose expression is either tissue-specific or highly enriched in the *Anopheles gambiae* salivary glands. Among these are the platelet inhibitor apyrase (*AgApy*), a group of D7-related genes (*D7r*) and several polypeptides whose functions still need to be clarified. Thereafter we mainly focused our attention on (i) the identification of regulatory sequences capable to drive tissue-specific expression in the salivary glands and on (ii) the production of recombinant proteins to be employed for functional studies.

Salivary gland promoter analysis. Promoters of the *An. gambiae* female salivary gland-specific genes *AgApy*, *D7r2* and *D7r4* were analyzed in the fruitfly *D. melanogaster* and in the asian malaria mosquito *An. stephensi* using the *E. coli LacZ* as reporter gene. The *AgApy* promoter could drive transcription of *LacZ* in adult transgenic *An. stephensi*. However, β-galactosidase activity could be detected only in one line carrying multiple copies of the transgene and the expression pattern only in part overlapped the expression profile of *AgApy* in *An. gambiae*. We also analyzed the ~1 kb intergenic regions located immediately upstream of the *D7r2* and *D7r4* genes first in *D. melanogaster*, where they worked very nicely, and then in *An. stephensi*. *D7r4*-driven *LacZ* expression could be detected by RT-PCR in adult males and females of all *An. stephensi* transgenic lines; moreover, in two lines (Bα and Eδ) transcription appeared to be restricted to salivary glands. However, histochemical stainings failed to reveal β-galactosidase activity on dissected glands indicating low levels of expression and/or translation of the transgene. These results suggest that salivary gland expression in mosquitoes may require some tissue-specific enhancer or other essential elements and that its regulation is apparently more complex than in the fruitfly. The frequent cluster organization adopted by several salivary gland genes, as revealed by the recently completed *An. gambiae* genome sequence, may be connected to a more complex regulation. Finally, sequence comparison of regulatory regions located immediately upstream of several salivary gland genes shows the presense of consensus binding sites for factors of the *fkh/HNF-3* family suggesting that they may play a role in salivary gland-specific gene expression in mosquitoes.

Functional analysis. A remarkable outcome of studies on mosquito salivary glands is the inability to assign functions to several salivary proteins. As a first step toward functional and structural studies we started the expression of recombinant salivary proteins in the yeast *Pichia pastoris*. gSG6 and gSG7 were recently obtained and purified in small amounts. The first may be a serine-proteinase inhibitor and is distantly related to a family of anticoagulants from the parasitic nematode *Ancylostoma caninum*, the second shows a weak similarity to phospholipase A_2 from snake venom. Expression of additional salivary proteins is underway. The recombinant proteins will be assayed for their ability to affect the hemostatic and/or inflammatory/immune response of the host and evaluated as possible markers of exposure to mosquito bites.

Receptor studies. In the attempt to get insights into the molecular interactions underlying mosquito salivary gland invasion by *plasmodium* sporozoites we are planning to display on the surface of lambda phages putative adhesive domains of a few selected *plasmodium* proteins which may be involved in the invasion (TRAP, CS, MAEBL, CRMPs). This mini phage display library may be

useful for the development of binding assays to whole salivary glands and for additional analyses targeted to the identification of salivary gland receptors.

Isolation of sex determining genes in *Anopheles* and *Aedes* mosquitoes.

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The identification and molecular isolation of the key genetic functions controlling sex determination in insects of economical and medical importance can be relevant for the development of novel strategies of insect control such as the safe and effective sterile insect technique. The *Drosophila* sex determining pathway (*X:A>Sxl>tra+tra-2>dsx*) is partly conserved in other diptera as the agricultural pest *Ceratitis capitata* (Y-linked Male determining factor>tra>dsx). Indeed, partial conservation in mosquito species is also expected since the *Anopheles gambiae doublesex* (*dsx*) has been identified a few years ago and showed an apparently conserved sex-specific splicing regulation (Pannuti and Lucchesi, pers. comm.). The availability of the complete sequence of the *An. gambiae* genome can be exploited for the identification of sex determining genes both in *Anopheles* and in the dengue and yellow fever vector *Aedes aegypti*. As a first step in this direction we focused our attention on the isolation of the *Anopheles* homologue of the *Drosophila transformer (tra)* because of its crucial role of main regulator of the *dsx* sex-specific splicing. We have recently started a PCR-based sex-specific screening of putative *tra* candidates. We analyzed up to now 14 out of approximately 50 putative *tra* genes and found two cases of sex-specific splicing. Further investigations are in progress.