

### **The *Anopheles gambiae* salivary gland transcriptome: toward a functional analysis.**

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Mosquito salivary secretions carry a large number of bioactive molecules (i.e. anti-hemostatics, immunomodulators, etc) that are injected into the vertebrate host skin during feeding and perform important functions in blood-feeding and parasite transmission. A few years ago we started investigating the *Anopheles gambiae* salivary gland transcript repertoire and, more recently, we expanded our analysis in the attempt to achieve a comprehensive description of the salivary transcriptome of this most important vector of malaria transmission. The analysis of a large set of salivary gland cDNA sequences allowed for the discovery of 33 novel salivary gland cDNAs and for the assembly of a non redundant catalogue including 71 transcripts coding for proteins of a putative secretory nature. To further obtain an insight onto their possible function we analyzed by RT-PCR their tissue and sex specificity and, in addition to the ones already described in previous studies, we found 27 novel genes which are either enriched or specifically expressed in the salivary glands. Many of the salivary proteins included in the catalogue represent novel protein families of unknown function that are often organized in clusters (D7, Antigen-5, SG1) and potentially code for pharmacologically or microbiologically active substances. Interestingly, 2 gene products appeared to be differentially spliced in the adult female salivary glands whereas 13 contigs matched predicted intronic regions and may include additional alternatively spliced transcripts. Overall, forty seven transcripts encode proteins which may play essential physiological roles as indicated by their exclusive or preferential expression in the female and/or male salivary glands. This catalogue probably represent the most complete salivary transcriptome available among arthropod disease-vectors. However, it should be pointed out that the fraction of genes included in this list for which we know or we can postulate a function is surprisingly small, further emphasizing how much we still have to learn about bioactive molecules from the saliva of blood-feeding arthropods.

In our continuing effort of understanding the evolution of blood sucking in vector arthropods and in the attempt to discover novel pharmacologically active compounds we have started expression of recombinant *An. gambiae* salivary proteins. Using the *Pichia pastoris*-based expression system we could obtain and purify gSG6 and gSG7, two small proteins with similarity to anticoagulants from distantly related species. They are similar respectively to the *Ancylostoma caninum* anti-coagulant AcAP6 (24% identity, 65% similarity) and to a secreted phospholipase A2 from the venom of the cobra *Naja naja atra* (19% identity, 45% similarity). Classical Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) tests indicated that recombinant gSG6 and gSG7 do not affect either the extrinsic or the intrinsic pathway of the coagulation cascade (R. De Cristofaro, Catholic University, Rome). Instead, preliminary analysis of immuno-modulatory activity suggested that they may affect differentiation and maturation of human dendritic cells (V. Barnaba, University La Sapienza, Rome). Expression of additional salivary proteins and analysis of their effects on mouse dendritic cells are presently in progress (ongoing collaboration with J. Anderson, NIAID-NIH, Rockville, MD, USA). [Supported from MIUR-COFIN and BioMalPar LSHP-CT-2004-503578 funds].