

EMBO WORKSHOP "MOLECULAR AND POPULATION BIOLOGY OF MOSQUITOS"
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1) AnoBase, the *Anopheles* database

AnoBase was initiated in the fall of 1995 with the name AnoDB, using a set-up grant from the John D. and Catherine T. MacArthur Foundation; the first version became publicly available on the World Wide Web in May 1996. Since then, the database is continuously supported by TDR, and lately by the NIAID. In its present form AnoBase contains genetic and genomic data concerning all anopheline mosquitoes. The renaming of the database in October 2002 went along with a major change of format, which converted it from a simple text-based one to a relational form modeled on FlyBase, the *Drosophila* database.

In addition to serving as an entry and search site for *Anopheles*-related data (e.g. sequences including a BLAST server, references, nomenclature, general information on anopheline species, links), AnoBase hosts a large collection of images relating to cytogenetics, with an emphasis on *in situ* hybridization results, as well as on genetic markers. Furthermore, within the *Anopheles* genome project, it is responsible for the curation of gene names. The immediate plans of AnoBase based on its transcription to a relational format include, as a first priority, is a curation of the published literature. Given the needs expressed by entomologists in disease-endemic countries (DEC) to include datasets specific to them, AnoBase already started curating and presenting data on insecticide resistance in collaboration with J. Hemingway's group. Moreover, with the support of TDR and NIAID, together with F. Collins, B. Gelbart, E. Birney, K. Kafatos, C. Taylor and their collaborators, it is planned to expand AnoBase to include other disease vector genomes, transforming it, in a sense, to a "portal" dealing with the genomics and genetics of these arthropods (VectorBase). Finally, AnoBase is, and will be actively involved in educational processes aimed at assisting the wider community and in particular scientists in DECs.

2) *Plasmodium*-*Anopheles* interactions

Over the last few years we have focused our attention to the events that take place in the midgut of the mosquito after the uptake of an infected bloodmeal. More specifically, we have tried to identify the presumed receptor(s) that recognise(s) parasite surface proteins and mediates the entry of the protozoa in the epithelial cells. We have identified (and already published) the binding of the mosquito beta2-laminin to at least three *Plasmodium* proteins, P25 and P28, two EGF-repeat containing surface proteins found in all *Plasmodium* species, and SOAP, a protein of, yet, unknown function. We now also know that at least one integrin chain (beta) coats the ookinete soon after the start of the midgut invasion. Moreover, we have also seen that integrin stays in close association to the oocyst, suggesting some role in the development of the parasite in the insect. The initial binding of the ookinete correlates with an increase in mRNA accumulation, and a restructuring of the integrin in the midgut epithelium. These observations suggest an involvement of integrin in the journey of *Plasmodium* through the midgut wall. In contrast to laminin, a direct interplay between integrin and any previously identified component of the parasite surface has not yet been demonstrated. Finally, we recently also identified an additional *Anopheles* protein that binds, with strong affinity, to *in vitro* cultured ookinetes: using a proteomic approach, we found out that the orthologue of Annexin IX is the activity that binds parasites on filter assays.

3) Molecular dissection of parasite invasion

Using an *in vitro* development system for *Plasmodium berghei* mosquito stages that we had developed earlier we performed microarray experiments to examine differential parasite gene expression during ookinete to oocyst transformation and during further oocyst development. The expression patterns of several *P. berghei* genes with a known developmental profile matched published data. Among other genes with known function that were up-regulated, a prominent fraction are genes involved in protein synthesis, hexose metabolism and electron transport, reflecting the changes in metabolic activity that takes place. Other interesting classes of genes showing a dynamic pattern code for RNA binding/splicing factors and cytoskeletal proteins. A large number of genes with unknown function also show a differential pattern of expression, indicating their link to control and execution of active processes during these stages.

The effects of various treatments that interfere with Ca^{2+} signalling and their role in insect cell invasion by *Plasmodium berghei* ookinetes were investigated. Treatment with the Ca^{2+} ionophore A23187 leads to rapid death of the ookinete. This effect can be negated by preincubating the parasites with insect cells, in this case an *Aedes aegypti* cell line, Mos 20. When ookinetes, which have been treated with the intracellular Ca^{2+} chelator BAPTA/AM, are seeded into co-cultures with the Mos20 cell line *in vitro* oocyst formation is increased compared to untreated ookinetes. The effect of Ca^{2+} level perturbation on the micronemal proteins CTRP and SOAP was also studied. A23187 treatment induces secretion of both micronemal proteins, while BAPTA/AM treatment leads to an enhanced localisation of the protein in the micronemes. Finally, incubation of ookinetes with insect cells leads to increased levels of CTRP and SOAP. These results indicate that a signaling pathway that is, at least partly, dependent upon a Ca^{2+} flux is initiated during contact of ookinetes with insect cells. This line of analysis is now supplemented using a post-genomic approach, determining the differential expression of invasion-specific genes under conditions of Ca^{2+} modulation.

4) Other projects (in a "telegraphic" form)

* Molecular systematics: Done in collaboration with the Natural Science Museum of the University of Crete. Our contribution consists in the sequencing of different DNAs to be used in species recognition. The organisms examined include reptilia and snails.

* Molecular analysis of TE-containing elements in *An. gambiae*: A by-product of the genome Project. We are in the process of characterizing the different families of this class of transposable elements.

* Postgenomic analysis of insecticide resistance: In collaboration with J. Hemingway's laboratory we initiated the microarray-based analysis of insecticide resistance. The first phase of the analysis is under way.