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A var specific nuclear factor is involved in transcriptional activation of virulence genes in the malaria parasite *Plasmodium falciparum*

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The major antigenic ligand of *P. falciparum* infected red blood cells is protein encoded by the multi-copy var gene family called PfEMP1. Each individual parasite expresses a single var gene at a time, maintaining the remaining ~60 var genes found in its genome in a transcriptionally silent state. In the present study, *P. falciparum* parasites were transfected with concatameric episomes carrying var promoters that had been disabled in silencing and were thus constitutively active and not recognized by the mechanism that controls mutually exclusive expression. By using increasing levels of drug pressure, it was possible to select parasite populations that carried similarly increasing numbers of active, episomal var promoters. We show that forcing parasites to express increasing numbers of transgenic var promoters resulted in gradual down regulation of transcription of all endogenous var genes, ultimately resulting in silencing of the entire family. The observed down regulation was var specific and did not affect parasite growth or the expression of housekeeping genes. Transient transfection assays using constructs containing heterologous promoters further confirmed that the down regulation was specific to var promoters. Interestingly, when drug pressure was removed and the episomes were shed by the parasites, they did not return to their previous var gene expression pattern, but rather displayed random var gene activation, indicating that the epigenetic memory marks that had previously regulated var gene expression had been completely erased. The data are consistent with the existence of a limiting, titratable var specific nuclear factor that is required for var gene activation.

Characterization of a DNA methylated binding complex in the protozoan parasite *Entamoeba histolytica.*

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DNA methylation and histone modifications are epigenetic modifications involved in the regulation of genomic functions such as differential control of gene expression. Classical methyl-CpG binding proteins (MBDs) mediate histone deacetylase-dependent transcriptional silencing at methylated CpG islands. Previously, we have reported EhMLBP, an *Entamoeba histolytica* nuclear protein that serves as a sensor of methylated repetitive DNA in the parasite. To better understand the role of EhMLBP we have
knocked-down its expression using antisense vector. We observed a significant interference in the growth rate and cytopathic activity of the trophozoites transfected with the antisense vector. EhMLBP is unique to *Entamoeba* species and it does not share homology with human proteins. This feature makes EhMLBP a perfect target for new drugs against this parasite. A screen for inhibitor molecules using the phage-display method has been performed. Several peptides that compete with the binding of EhMLBP to methylated LINE retrotransposon (RT-LINE) are actually studied. In addition, we have identified with the Two-Hybrid screen analysis two proteins P1 and P2 that interact with EhMLBP. P1 shows some homology with a yeast protein involves in gene silencing. Like EhMLBP, P1 appears to bind with high affinity to methylated RT-LINE. P2 has no homology to proteins of known structure and it does not bind to methylated RT-LINE. We are currently examining the cellular localization of P1 and P2, their binding domains to EhMLBP and their roles in the parasite.

**Polymorphism of the single copy BV80 gene in Babesia bovis isolates from distinct geographical regions**

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Effective control of bovine babesiosis is achieved by vaccination with attenuated live parasites of *Babesia bovis*. Disease outbreaks sporadically occur among vaccinated cattle, however tools to discriminate between virulent field and attenuated vaccine strains of the parasite are not yet available. Conventional PCR, RT-PCR and sequence analysis of the single copy BV80 gene were applied to characterize and differentiate the vaccine isolates derived from cattle blood or culture, and field virulent isolates from Israel and Uzbekistan. Two different sets of primers amplified multiple amplicons ranging between 450-800 bp in all field or vaccine isolates, except for a single fragment obtained with the DNA from a calf-derived vaccine MH strain. Sequence analyses of the Israeli and Uzbek strains confirmed presence of mixed parasite subpopulations and extensive polymorphism in attenuated and virulent isolates. It appears that the BV80 is not a suitable marker for discrimination between virulent and attenuated parasites. The selection pressure and mechanism(s) that lead to preferential growth of the attenuated parasite population which comprise the vaccines strain are not yet elucidated.

**Predator-released kairomones repels oviposition by the mosquito Culiseta longiareolata**

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Previous work showed that *Culiseta longiareolata* (Diptera: Culicidae), a potential vector of West Nile Virus, are strongly repelled by one or more kairomones released by the aquatic predator, *Notonecta maculata* (Hemiptera: Notonectidae) when ovipositing. Isolating the kairomone can potentially provide an environmentally friendly method of mosquito population reduction. Using a behavioral assay, we found that the mosquito is repelled without having to touch the water indicating that at least one component of the kairomone is highly volatile. Volatile compounds were extracted from *Notonecta*-conditioned water using Solid Phase Microextraction (SPME). Two hydrocarbons, heneicosane (C\textsubscript{21}H\textsubscript{44}) and tricosane (C\textsubscript{23}H\textsubscript{48}), which were found in *Notonecta*-conditioned water, but not in control water, were tested as potential oviposition repellents in outdoor artificial pool experiments using synthetic compounds of these hydrocarbons. Our preliminary results indicate that both compounds cause oviposition repellency and that the combined effect is additive.

**Mosquito oviposition and the aging effect**

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We studied the effect of physiological age on the oviposition behavior of *Aedes aegypti* following two successive gonotrophic cycles using a choice experiment under laboratory conditions. Blood-engorged mosquitoes (n=10) were introduced into individual cages that contained four oviposition sites (choices) with different salinities: Distilled water (DW), 0.33% NaCl (T1), 0.67% NaCl (T2) and 1% NaCl (T3). The oviposition substrate (seed germination paper) was changed daily and the number of oviposited eggs (the response variable) was recorded. The initial oviposition activity ceased on the tenth day of the experiment and two days later a second blood-meal was offered to the original cohort of mosquitoes; only five became visibly blood-engorged. The oviposition activity was again followed for 12 additional days. During the first oviposition period (days 1-10 of the experiment), the mosquitoes oviposited predominately in the DW and T1 treatments and completely avoided the T3 treatment (p<0.001). In contrast, the oviposition pattern during the second gonotrophic cycle (days 12-24 of the experiment)