of these bacteria. We have developed in situ hybridization assays to study *Wolbachia* gene expression in filarial worms. Digoxygenin-labeled RNA probes of 300-500 bp length were used to detect ribosomal and specific messenger RNAs of *Wolbachia* in frozen sections of adult *Brugia malayi*. A probe for 16s rRNA produced intense signals corresponding to Wolbachia in parts of the lateral chord of male worms and in the hypodermis, the lateral chords and in developing embryos in females. This probe is a sensitive marker for *Wolbachia* that demonstrates the abundant but uneven distribution of the bacteria in filarial worms. A probe for a *Wolbachia* surface protein (wsp1) produced weaker signals in the same areas as the 16s probe. This localization pattern was also seen when wsp-1 protein was stained with a monoclonal antibody against wsp1. We conclude that *Wolbachia* endobacteria and their gene products can be sensitively detected by in situ hybridization. Expression signals vary by gene, developmental stage, and tissue type. Messenger RNA is likely to be more labile than DNA. Quantitative gene expression assays should be more useful than DNA assays for studying changes in metabolic activity of *Wolbachia* in different developmental stages and for assessing changes in bacterial viability after chemotherapy.

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COULD SNPS IN *MDR-1* GENE CONTRIBUTE TO OCCASIONAL SEVERE ADVERSE EFFECTS, FOLLOWING IVERMECTIN TREATMENT IN ONCHOCERCIASIS PATIENTS, FROM CAMEROON, THAT WERE CO-INFECTED WITH *LOA LOA*?

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Mass distribution of ivermectin (IVM) to human populations infected with *Onchocerca volvulus* is the mainstay of programs for onchocerciasis control. In Africa, lymphatic filariasis control is conducted using IVM and albendazole combination. IVM is exceptionally safe in humans. However, cases of encephalopathy, which can be fatal, have been reported in a small number of individuals (mostly in Cameroon and Democratic Republic of Congo) who harbored large numbers of *Loa loa* microfilariae (mf) in the blood. The pathophysiological and/or pharmacological basis for these rare serious adverse events (SAEs) is not fully understood. It is critical to clarify the mechanisms associated with the SAEs, in order to identify, if possible, those individuals who are at risk for SAEs, and to identify the most appropriate treatment to manage them. The SAEs could be the result of the effect of IVM on the *Loa* microfilariae, that would lead, in case of high microfilaremias, to embolisms of the mfs in the brain micro-circulation. One possible alternative explanation could involve the pharmacology of IVM. The drug is safe in humans because it is excluded from the CNS by the drug-transporting P-glycoprotein (PgP) of the blood-brain barrier. An absence of a functional PgP can lead to the penetration of the drug into the brain, and cause coma and sometimes death. This is the case in some dog breeds and in mice harboring a loss-of-function mutation in the PgP gene, *mdr-1*. We have investigated the possibility that a similar alteration in the *mdr-1* gene exists in humans who experience a post-IVM SAE. Blood samples from 4 patients who recovered from a SAE and from 8 individuals that never experienced a SAE following IVM (matched on sex, age and village of residence), were collected in Cameroon. RNA from leukocytes was extracted. The full length of *mdr-1* cDNA for each patient was amplified. SNPs were identified. While some of the SNPs led to amino acid changes other were silent. These SNPs are compared to known SNPs in *mdr-1*.

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IDENTIFICATION OF PROTEINS BINDING TO THE ESSENTIAL PROMOTER DOMAIN OF *BRUGIA MALAYI* 12 KDA SMALL SUBUNIT RIBOSOMAL PROTEIN (BMRPS12) GENE USING PHAGE-DISPLAY

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There is little information available about gene regulation in the human filarial parasite *Brugia malayi*. Only two *B. malayi* promoters have been mapped in detail so far, BmHSP70 and BmRPS12. The BmRPS12 promoter contains a 44- nucleotide tandem repeat sequence, the deletion of which results in 80% loss in promoter activity in the transient tranfection assays. This essential promoter domain lacks the binding sites for most general transcription factors present in other eukaryotic promoters but contains several GATA transcription factor binding sites encoded within this repeat. In the present study, we employed the T7 phage display technique to identify putative transcription factors that interact with this repeat domain. Using a *B. malayi* adult female T7 phage cDNA library, we have identified 5 different candidate proteins that were represented more than or equal to 5 times out of total 100 clones sequenced after final round of biopanning. Two of these proteins contain the RNA recognition motif (RRM) and constituted the most abundant group when equal number of phages displaying all five proteins was subjected to selection using stringent conditions. RRM-domain containing proteins have been shown previously to bind to single stranded as well as double stranded DNA, and have been found in several transcription factors. The RRM containing proteins that we have identified could thus constitute a new class of transcription factors in *B. malayi*.

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THE *BRUGIA MALAYI* ANKYRIN DOMAIN CONTAINING *WOLBACHIA* PROTEINS AS POTENTIAL MEDIATORS OF ENDOSYMBIOSIS

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Most human filarial parasites harbor an endosymbiotic bacterium of the genus *Wolbachia*, which appear to be essential for their survival as elimination of *Wolbachia* results in irreversible sterilization of the adult female worm. *Wolbachia* provides therefore an attractive new chemotherapeutic target for the treatment of human filarial infections by exploiting the vulnerability of the filarial parasites to the elimination of the *Wolbachia* endosymbiont. Our aim is to identify and characterize *Brugia malayi* (Bm) and *Wolbachia* (wBm) proteins that are essential for the endosymbiotic relationship. Bioinformatic analysis of the *Wolbachia* genome has identified a number of putatively secreted proteins, some of which contain ankyrin domains. The ankyrin domain is known to mediate protein-protein interactions and has been implicated in host-pathogen interactions in other bacteria. To study the potential role of wBm ankyrins in host-bacterium interaction and to identify their binding partners in Bm, we expressed and purified three wBm recombinant proteins corresponding to the Wolbachia ankyrin domain containing proteins Wbm0287, Wbm0394 and Wbm0447.We used the recombinant ankyrin domain of Wbm0394 with varying concentrations of Bm crude extracts in a modified ELISA assay to first verify whether it binds specifically to Bm extracts in comparison to extracts from *A. viteae* adult worms, which lack the *Wolbachia* endosymbiont. Our preliminary data indicate that the recombinant ankyrin domain of Wbm0394 binds to the Bm extract in a concentration-dependent manner. Moreover, the binding of the recombinant ankyrin protein levels off at higher concentrations suggesting a possible saturation, a characteristic of specific interaction. We are in the process of identifying its specific interacting partner(s) in the filarial host by using panning of a Bm cDNA phage display library and proteomic