ZIBI Graduate School

Project description

Project Title: Identification and characterization of ML metabolic pathways in *Cooperia oncophora*

Research Group: Institute for Parasitology and Tropical Veterinary Medicine
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Project Description:

Anthelmintic resistance is a widespread phenomenone in parasitic nematodes infecting small and large ruminants and horses but is expected to become increasingly important for human parasites as well. In several areas of the world, presence of anthelmintic resistance is a substantial limitation to the production of livestock and thus represents a threat for human nutrition and economic development. The resistance to the frequently used macrocyclic lactones (MLs) such as ivermectin (Nobel prize in 2015) and moxidectin is not understood so far. The main hypothesis of the project is that metabolism of MLs via pathways used for detoxification of xenobiotics contributes to the overall resistance of parasitic nematodes to this drug class. Experiments with inhibitors of cytochrome P450 enzymes (Cyps) and P-glycoproteins (Pgps) suggest such effects. In addition to Cyps and Pgps, other enzymes involved in metabolism of xenobiotics such as FAD-dependent monooxygenases (FMOs) in phase I and glutathione S- or UDP-glycosyl transferases (GSTs and UGTs) in phase II reactions might be involved. However, it is unclear which of these enzymes is involved in resistance to the individual drug classes.
Using *Cooperia oncophora* isolates differing in their resistance status, we will compare basal mRNA levels and levels after exposure to IVM using RNAseq. Adult worms will be collected from the duodenum of infected calves, *in vitro* cultivated and exposed to a sublethal concentration of IVM for different time periods. RNAseq will be used to identify genes with IVM-inducible expression or constitutively elevated expression in resistant isolates. The results will provide a set of candidate genes that might be responsible for IVM or even multi-drug resistance. We will also compare the primary structure and splice variants of potential IVM targets, in particular glutamate-gated chloride channels (GluCls), ionotropic $GABA_A$ receptors, and the recently identified candidate resistance gene *lgc-54*, a ligand-gated ion channel which is presumably activated by biogenic amines.
Functional analysis of candidate genes will start with previously identified candidates and continues with candidates from the RNAseq experiment. Functional experiments will focus on rescue experiments in *C. elegans*. To achieve comparable levels of gene expression for different transgenes, integration of all genes will be targeted to the same genomic locus using the mos-1 integration system and they will all be under the control of the gut-specific *ges-1* promoter since metabolism of xenobiotics is localized in the gut tissue of *C. elegans*. We will then analyze the effects of IVM and MOX on the development of transgenic *C. elegans* and on pharynx pumping activity by recoding concentration-response-curves.
The PhD student will be trained in bioinformatics, transgenic *Caenorhabditis elegans*, assays to determine drug activity in nematodes including recording of electropharyngiograms.