

MOLECULAR AND CELLULAR BIOLOGY OF HELMINTH PARASITES V



BRATSERIA HOTEL, HYDRA, GREECE
12-17 SEPTEMBER 2008

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This meeting was made possible through the generous support of:

**Burroughs Wellcome Fund**

## SCIENTIFIC PROGRAMME

All timings include minimum of 5 minutes for discussion.

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~ Friday 12 September ~

- 15:00 - 18:00 Registration, Bratsera Hotel
18:15 Introduction: Rick Maizels, University of Edinburgh
18:45 Plenary Lecture: Paul Sternberg, California Institute of Technology Nematode genomics and informatics: leveraging *C. elegans*
20:00 Welcome Reception, Bratsera Hotel

Day 1 ~Saturday 13 September ~ GENOMES AND GENETICS

Session 1: GENOMICS AND EVOLUTION OF PARASITISM

Chair - Murray Selkirk, Imperial College London

- 9:00 Matthew Berriman, Wellcome Trust Sanger Institute. Parasitic helminth genome sequencing
9:40 Juan Pedro Lacleto, Universidad Nacional Autonoma. The genome project of *Taenia solium*
10:00 David Bird, NC State University: Sequence of *Meloidogyne hapla*. A compact nematode genome for plant parasitism
10:20 Sommer Ralf, Max-Planck Institute. The genome of *Pristionchus pacificus* and implications for the evolution of parasitism

Coffee Break

Session 2: CROSSOVER FROM C ELEGANS TO PARASITES

Chair - Paul Sternberg, California Institute of Technology

- 11:10 Adrain Streit, Tübingen. Genetics in the parasitic nematode genus *Strongyloides*
11:30 John Gilleard, Faculty of Veterinary Medicine. Developing *Haemonchus contortus* as a model parasite for forward genetic studies of anthelmintic resistance
11:50 Collette Britton, University of Glasgow. Expression of potential control targets of parasitic nematodes using *Caenorhabditis elegans*
12:10 Peter Geldhof, Ghent University. Functional analysis of *Ostertagia ostertagi* vaccine candidates in *Caenorhabditis elegans*
12:30 Claudia Welz, Univ of Vet Med Hannover. Functional expression of parasitic SLO-1 in *Caenorhabditis elegans slo-1* knockout mutants

Lunch/Free Time

Session 3: TRANSFECTION IN PARASITIC HELMINTHS

Chair - Eileen Devaney, University of Glasgow

- 16:30 James Lok, University of Pennsylvania. Transgenesis in *Strongyloides stercoralis* and *S. ratti*: prospects for transposon-mediated gene transfer and applications in studies of gene function, immunology and sensory neurobiology
17:10 Michelle Castelletto, University of Pennsylvania. Regulation and function of *fttf-1* in transgenic *Strongyloides stercoralis*
17:30 Thomas Unnasch, University of South Florida. Analysis of transcription and mRNA processing in *Brugia malayi* using a homologous transient transfection system
17:50 Paul Brindley, George Wash Med Center. Integration of reporter transgenes into *Schistosoma mansoni* chromosomes mediated by pseudotyped murine leukemia virus

18:10 - 20:00 Poster Session 1

Day 2 ~ Sunday 14 September ~ MOLECULAR AND DEVELOPMENTAL BIOLOGY

Session 4 DEVELOPMENT AND DIFFERENTIATION

Chair - Ed Pearce, University of Pennsylvania

- 9:00 Klaus Brehm, University of Würzburg. Cestode stem cells: their role in host-induced larval development and their utilization to achieve parasite transgenesis
- 9:40 Christoph Grevelding, Institute for Parasitology. Signaling molecules involved in gonad differentiation of *Schistosoma mansoni*
- 10:00 Olson Peter, Nat History Museum. Developmental genes in the life cycle of a parasitic flatworm
- 10:20 Svenja Beckmann, Justus-Liebig-University. The Syk-kinase SmTK4 from *Schistosoma mansoni*: upstream interaction partners and functional aspects in oogenesis and spermatogenesis

Coffee Break

Session 5 HELMINTH GENE FAMILIES AND SIGNALLING

Chair - Charles Shoemaker, Tufts University

- 11:10 Cecilia Fernández, Universidad de la República. A diverse family of Kunitz inhibitors from *Echinococcus granulosus* involved in host-parasite cross-talk in echinococcosis
- 11:30 Bernadette Connolly, University of Aberdeen. TGF-beta and hedgehog signalling pathways in *Trichinella spiralis*
- 11:50 Ed Pearce, University of Pennsylvania. TGFbeta signaling in schistosomes
- 12:10 Sally Williamson, University of Bath. The nicotinic acetylcholine receptors of *Ascaris suum*
- 12:30 Niki Gounaris, Imperial College London. Secreted proteins of *Trichinella spiralis* modulate purinergic signalling in immune cells

Lunch/Free Time

(note earlier start time for session 6 to allow for sunset dinner)

Session 6 MOLECULAR MECHANISMS IN HELMINTHS

Chair - Tom Unnasch, University of South Florida

- 15:30 Alan Scott, Johns Hopkins University. The *Brugia malayi* genome
- 16:10 Larry McReynolds, New England Biolabs. miRNAs in *Brugia malayi*
- 16:30 Murray Selkirk, Imperial College London. RNAi in parasitic nematodes
- 16:50 Richard Davis, Univ of Colorado. RNA metabolism and molecular adaptation in *Ascaris* embryos

18:30 ~ Boats depart from Hydra port for Vlychos

19:00 Dinner, Vlychos Taverna

Day 3 ~ Monday 15 September ~ METABOLISM AND DRUG DISCOVERY

Session 7 INSIGHTS FROM C ELEGANS

Chair - Niki Gounaris, Imperial College London

- 9:00 Nektarios Tavernarakis, Foundation for Research and Technology - Hellas. Cellular energy metabolism and ageing in *Caenorhabditis elegans*
- 9:40 Richard Martin, Iowa State University. Levamisole channel currents and resistance in adult *Caenorhabditis elegans* muscle
- 10:00 Carolyn Behm, Australian National University. MITR-1: a conserved novel nematode-specific protein required for mitochondrial respiration in *C. elegans*
- 10:20 Anthony Page, University of Glasgow. A hypodermally expressed Haem peroxidase is critical for viability and cuticle formation in *C. elegans*

Coffee Break

Session 8 NEW DRUGS AND DRUG TARGETS

Chair - Carolyn Behm, Australian National University

- 11:10 Pascal Mäser, University Of Bern. Molecular mechanisms of drug resistance in *Haemonchus contortus*
- 11:30 Achim Hoerauf, Univ Bonn Med Center. Anti-wolbachial chemotherapy of onchocerciasis - a macrofilaricide at last?
- 11:50 Mark Taylor, Liverpool School of Tropical Med. Filarial *Wolbachia* lipoprotein stimulates innate and adaptive inflammatory responses
- 12:10 Robert Greenberg, University of Pennsylvania. Praziquantel-dependent effects on expression of a P-glycoprotein homolog in *Schistosoma mansoni*
- 12:30 David Williams, Illinois State University. Identification of new drug leads targeting redox biochemistry for the control of schistosomiasis

Lunch/Free Time

Session 9 MOLECULAR DISCOVERY

Chair - Alex Loukas, Queensland Inst of Med Research

- 16:30 Charles Shoemaker, Tufts University. Probing the helminth host-parasite interface with phage-displayed antibodies
- 17:10 Patrick Skelly, Tufts Cummings School of Vet Med. Functional characterization of the schistosome surface
- 17:30 Eileen Devaney, University of Glasgow. Hsp90 and the biology of parasitism
- 17:50 Karl Hoffmann, Aberystwyth Univ. Transcriptomic analyses of schistosome biology: what we have learned and where we are headed
- 18:10 Richard Komuniecki, University of Toledo. Use of the *Caenorhabditis elegans* model system to identify the receptors in parasitic helminths modulating serotonin-stimulated paralysis

18:30 - 20:00 Poster Session 2

Day 4 ~ Tuesday 16 September ~ IMMUNOMODULATION AND IMMUNITY

Session 10 MECHANISMS OF HOST IMMUNITY

Chair - Rick Maizels, University of Edinburgh

- 9:00 Jonathon Ewbank, Centre d'Immunologie de Marseille-. Innate immunity in *C. elegans*
9:40 Rachel Lawrence, Royal Vet College. The mechanism by which mannose-binding lectin deficiency prevents parasite clearance and development of anti-parasite IgM
10:00 Nicola Harris, Swiss Federal Institute of Technology. Polyclonal and specific antibodies mediate protective immunity against enteric helminth infection
10:20 Graham LeGros, Malaghan Inst of Med Research The lung is a key site for protective immunity against gastrointestinal helminth parasites

Coffee Break

Session 11 HELMINTH IMMUNOMODULATION

Chair - Achim Hoerauf, Univ Bonn Med Center

- 11:10 Helmut Haas, Borstel. *S. mansoni* egg excretory/secretory proteins - major modulators of the host's immune response
11:30 Corinna Schnöller, Humboldt University. Gastrointestinal nematode infection inhibits experimental allergic airway inflammation but not atopic dermatitis
11:50 Matthew Taylor, University of Edinburgh. Induction and maintenance of effector and regulatory T cell responses during filarial infection
12:10 Sabine Specht, Univ Bonn Med Center. Murine filarial infection induces protection against malaria and inhibits allergic asthma
12:30 Rick Maizels, University of Edinburgh. Immunomodulation by *H polygyrus* products

Lunch/Free Time

Session 12 IMMUNE RECOGNITION AND VACCINES

Chair - Graham LeGros, Malaghan Inst of Medical Research

- 16:30 Dave Knox, Moredun Research Institute. Proteomics and vaccine candidate discovery – the old and the new
16:50 Lustigman Sara, NY Blood Center. The immunopotentiating properties of the *Onchocerca volvulus* recombinant Ov-ASP-1 protein
17:10 Jan Bradley, University of Nottingham. The immunomodulatory properties of hOv-FAR 1; the fatty acid and retinol binding protein of *Onchocerca volvulus*
17:30 David Dunne, University of Cambridge. Human sensitization and desensitization to allergen-like proteins from *Schistosoma mansoni* depends on their expression patterns through the parasite life-cycle
17:50 Alex Loukas, Queensland Inst of Med Research. Apical membrane proteins as recombinant vaccines against *Schistosoma mansoni* - from men to mice then back to men

20:00 Farewell Dinner, Douskos Taverna, Hydra

POSTER SESSION 1: SATURDAY 13 SEPTEMBER, 18:10 - 20:00

- Bamber, Bruce Chimeric nematodes as a new platform for anthelmintic drug screening
- Bethony, Jeffrey Type 1 hypersensitivity reactions in a phase 1 trial of the Na-ASP-2 hookworm vaccine in previously-infected Brazilian adults
- Chalmers, Iain Immune recognition of *Schistosoma mansoni* venom allergen-like (Sm-VAL) proteins in infected humans and identification of novel VAL homologs across the Platyhelminthes
- Chaudhary, Kshitiz Purine and pyrimidine metabolism in *Brugia malayi*
- deWalick, Saskia The proteome of the core matrix of the *Schistosoma mansoni* eggshell
- Dvorak, Jan RNAi - its usefulness as a tool for schistosomiasis drug discovery
- Eleftheriou, Emily *Trichinella spiralis* secreted proteins modulate immune cell effector functions induced by extracellular nucleotides
- Ford, Louise A-WOL drug discovery – from target discovery to in vitro drug screening of novel drugs with efficacy against *Wolbachia*
- Freitas, Tori Bacteriophage fC31 integrase-mediated transgenesis of *Schistosoma mansoni*
- Fritz, Julie-Anne pWormgateMulti: Tissue-specific RNAi using Gateway® hpRNAi vectors
- Galanti, Sarah The cellular basis for reproductive regression in female schistosomes following separation from their male partners
- Gillan, Vicky Comparative biology of Hsp90 in *C. elegans* and *Brugia*
- Jolly, Emmitt Identification of Transcriptional Activators in *Schistosoma mansoni*
- Klotz, Christian Role of macrophages in the cystatin-induced modulation of inflammatory responses
- Knight, Alison VHA-19, a protein predicted to associate with the vacuolar ATPase, has a crucial role in *C. elegans* reproduction
- Korten, Simone A novel and divergent role of granzyme A and B in resistance to filariasis
- Krücken, Jürgen Nematode latrophilin repertoire: mapping and comparative analysis of latrotoxin binding sites
- Laha, Thewarach Digestive proteases of the carcinogenic liver fluke, *Opisthorchis viverrini*
- Laing, Steven The investigation of ivermectin metabolism by nematodes using *Caenorhabditis elegans* as a model organism
- Layland, Laura A Comparative Phenotypic Analysis of Regulatory and Effector T cells induced during Chronic Helminth Infection
- Li, Ben-Wen Transcriptomes and pathways associated with infectivity, survival and immunogenicity in *Brugia malayi* L3
- Massey, Holman The SMART transposons of *Strongyloides stercoralis* and *S. ratti*

Poster Session Judges ~ Session 1
David Bird ~ Jan Bradley ~ Richard Martin

POSTER SESSION 2: MONDAY 15 SEPTEMBER, 18:10 - 20:00

Mc Carthy/Kopp	Altered expression of specific acetylcholine receptor subunit genes in pyrantel-resistant hookworm
Mikes, Libor	Serine peptidases in cercariae of the neuropathogenic schistosome <i>Trichobilharzia regenti</i> and its intermediate snail host <i>Radix peregra</i>
Mullen, Lisa	Functional analysis of the interaction of <i>B malayi</i> VAL-1 with peripheral blood leukocytes
Mulvenna, Jason	Characterising the host-parasite interface using novel fractionation techniques and tandem mass spectroscopy
Murray, Janice	Investigation into the potential immune function of the Bm-VAL-1 gene using a transgenic <i>Leishmania</i> system
Novelli, Jacopo:	Characterization of <i>C. elegans</i> glf-1 mutants reveals an important role for Galactofuranose metabolism in nematodes
Pearson, Mark	Neutralising monoclonal antibodies to the hookworm aspartic protease Na-APR-1: implications for a multi-valent vaccine against hookworm infection and schistosomiasis
Pettitt, Jonathan	The origin and evolution of nematode Wnt/beta-catenin signalling pathways
Pfarr, Kenneth	Mitochondrial encoded genes are up-regulated in response to depletion of essential <i>Wolbachia</i> endobacteria from <i>Brugia malayi</i>
Prazeres da Costa, C	Intestinal granuloma formation and egg expulsion requires host bacterial gut flora
Protasio, Anna	Short read sequencing technology applied to studying the <i>S mansoni</i> transcriptome: a new approach to gene modeling and quantitative gene expression analysis
Quack, Thomas	Involvement of a Rho/Dia/Src signalling complex in gonad differentiation of <i>S mansoni</i>
Rufener, Lucien	The <i>Haemonchus contortus</i> ACR-23 homologue of the acetylcholine receptor DEG-3 subfamily and its role in sensitivity to AADs
Samarasinghe, B	Function and Regulation of <i>Haemonchus contortus</i> genes
Schnieder, Thomas	Differential transcriptome analysis of free-living and parasitic L3 of <i>A. caninum</i>
Schramm, Gabreile	Omega-1, a glycoprotein secreted by <i>Schistosoma mansoni</i> eggs, drives Th2 responses
Siles Lucas, Maria	Proteomic analysis of non-activated and activated <i>Taenia solium</i> oncospheres
Strube, Christina	Transcriptome changes in the bovine lungworm <i>Dictyocaulus viviparus</i> during the transition from L1 to L3 stage
Tang, Shiau-Choot	The identification of novel Th2 inducing proteins in the secreted products of L3 stage of <i>Nippostrongylus brasiliensis</i>
Viney, Mark	What does it take to be a parasitic nematode – the Strongyloides view?
von Samson, Georg	Identification and localization of emodepside receptors of parasitic nematodes in <i>C. elegans</i>
Winter, Alan	Parallel studies of developmentally essential protein folding enzymes in the model nematode <i>Caenorhabditis elegans</i> and the filarial parasite <i>Brugia malayi</i>

Poster Session Judges ~ Session 2 ~ Al Scott ~ John Gilleard ~ Sara Lustigman

Traveling Fellowships

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**Through the generous support of the Burroughs Wellcome Fund, the following individuals were awarded traveling fellowships to attend the meeting.**

Bruce Bamber, University of Toledo, USA  
Chimeric nematodes as a new platform for anthelmintic drug screening

Julie-Anne Fritz, The Australian National University, Australia  
pWormgateMulti: Tissue-specific RNAi using Gateway® hpRNAi vectors

Jonathan Pettitt, Institute of Medical Sciences, UK  
The origin and evolution of nematode Wnt/beta-catenin signalling pathways

Alan Winter, University of Glasgow, UK  
Parallel studies of developmentally essential protein folding enzymes in the model nematode *Caenorhabditis elegans* and the filarial parasite *Brugia malayi*

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# INVITED SPEAKERS

Paul Sternberg, California Institute of Technology

Klaus Brehm, University of Würzburg

Jonathan Ewbank, Centre d'Immunologie de Marseille-Luminy

James Lok, University of Pennsylvania

Alan Scott, Johns Hopkins University

Charles Shoemaker, Tufts University

Nektarios Tavernarakis, Institute of Molecular Biology & Biotechnology- Heraklion



### **Paul Sternberg**

California Institute of Technology

<http://biology.caltech.edu/Members/Sternberg>

Paul Sternberg graduated from Hampshire College in 1978 and joined the Biology Department at M.I.T. for his Ph.D., which he received in 1984. His graduate work on the Genetic Control of Nematode Development was under the supervision of H. Robert Horvitz. He then pursued postdoctoral research on yeast mating type with Ira Herskowitz at UCSF, and returned to *C. elegans* when he joined the Caltech Biology Division faculty in 1987, where he is now Thomas Hunt Morgan Professor of Biology. He is an Investigator with the Howard Hughes Medical Institute, with whom he joined in 1989. He became lead-PI of WormBase in 1999 and started the Caltech branch of WormBase. He served on the board of directors of the Genetics Society of America from 2000-2003. At Caltech, he led the Biology graduate program for several years, helped found the BioEngineering graduate option, and is now director of the Center for Biological Circuit Design, part of the new Information Science and Technology initiative.

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Prof. Dr. rer. nat. Klaus Brehm

University of Würzburg

http://www.hygiene.uni-wuerzburg.de/forschung/ag_brehm/prof_dr_klaus_brehm/

Klaus "The Franconian" Brehm received a Ph.D. in Microbiology from the Faculty for Biology of the University of Würzburg in 1995 where he studied virulence factors of the intracellular bacterium *Listeria monocytogenes*. He then went to Madrid, Spain, to further pursue his studies on virulence gene regulation in *Listeria* at the Veterinary Faculty of the Universidad de Complutense. In 1997, he returned to Würzburg, stayed in infectious diseases, but completely changed his topic of interest away from bacteria to multicellular parasites. At the Medical Faculty, he started to work with the fox-tapeworm *Echinococcus multilocularis* and is doing so till today. His primary interests are molecular genetics and developmental biology of cestode larvae with emphasis on host-parasite interaction. In 2004, he received his 'Habilitation' in Medical Microbiology and in the very same year was appointed Associate Professor for Medical Parasitology at the Institute of Hygiene and Microbiology. He is active in teaching students of Medicine, Biology and Biomedicine and is member of the Würzburg Graduate School for Life Sciences (GSLs). He is also chairman of the informal WHO-working group 'Echinococcus basic biology', member of the international advisory board of the *Taenia solium* genome sequencing project and board member of the German Society for Parasitology. When not dealing with parasites, he's playing theater and involved in local government politics.



Jonathan Ewbank

Group Leader at the CIML

<http://www.ciml.univ-mrs.fr/Lab/Ewbank.htm>

http://www.ciml.univ-mrs.fr/EWBANK_jonathan/JE-CV.htm

Jonathan Ewbank is a research director at INSERM, the French medical research council. He received a MA in Biochemistry from the University of Oxford. Research for his PhD in Biophysics, from the University of Cambridge, was split between the MRC LMB and the EMBL in Heidelberg. After postdoctoral work at the Sloan-Kettering in New York, and McGill University, Montreal, he moved to the Centre d'Immunologie de Marseille Luminy (CIML) in 1997. In 1999, he started his own group at the CIML and has subsequently focused on host-pathogen interactions using the nematode *C. elegans* as a model.

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**James Lok**

University of Pennsylvania

<http://www.med.upenn.edu/camb/faculty/mv/lok.html>

James “Sparky” Lok received a Ph.D. in Medical Entomology from Cornell University in 1981 with primary emphasis on the interaction of filarial nematodes with their mosquito and black fly vectors. That same year he joined the Department of Pathobiology at the University of Pennsylvania as a postdoctoral fellow in parasitology. He was appointed to the standing faculty in 1984 and has continued at Penn ever since. He is currently Professor of Parasitology in the School of Veterinary Medicine. His research interests focus on the developmental biology of parasitic nematodes, particularly the events involved in formation of the infective and post-infective third-stage larva, and stress a comparative approach with *C. elegans* biology. He is active in the graduate teaching program at Penn and is a past Chair of the Graduate Group in Parasitology. He is also heavily engaged in the Veterinary curriculum and has received numerous awards for teaching including the Veterinary Dean’s Award for Basic Science Education and the Lindback Award for Distinguished Teaching, the University of Pennsylvania’s highest teaching honor.



**Alan Scott**

Johns Hopkins University

<http://faculty.jhsph.edu/?F=Alan&L=Scott>

Alan L. Scott: Received B.S and Ph.D. degrees from the University of Michigan and Auburn University, respectively. Professor in the Department of Molecular Microbiology and Immunology at the Johns Hopkins University Bloomberg School of Public Health. Director JHU Malaria Research Institute Gene Array Core Facility.

The major focus of the Scott lab is to understand the dynamics of the immunobiology of host-parasite interactions that result in the establishment of persistent helminth infections. It is anticipated that a detailed understanding of the cellular and molecular interactions that result in immune modulation and evasion of effector responses by nematodes will provide a basis to design effective vaccines and interventions to reduce pathology. In addition, this has potential to be translated into new approaches for disease management in the areas of autoimmunity and transplantation. The lab has a long-standing interest in the molecular mechanisms used by filarial parasites to persist in immunocompetent hosts. We have taken a molecular biology approach to define the molecules produced by the parasite that interact with the human immune response. In this regard, the lab is a charter member of the Filarial Genome Project, which has recently published the full genome of the human filarial parasite *Brugia malayi*. The lab also works in the *Nippostrongylus* system with a specific emphasis on defining the immunological consequences of larval migration through the lungs and how the modified pulmonary environment influences subsequent antigen challenges. In this research we use gene expression and cellular immunology approaches to define the mechanisms that control inflammation in the lungs and Th2 immunity in general. The approaches used to study pulmonary immunology in nematode infections have been extended to the study of malaria in the lungs.

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Charles Shoemaker

Tufts University

http://www.tufts.edu/vet/facpages/shoemaker_c.html

Charles B. Shoemaker, PhD, came to the Cummings School of Veterinary Medicine in 2003 to set up the Molecular Helminthology Laboratory with colleague Patrick Skelly, PhD. Shoemaker is intrigued by the remarkable lives of helminths, parasitic worms that infect millions of people worldwide. Some of these parasites can live for decades in a human host without being recognized by the immune system. Uncovering the mechanisms behind this cloaking ability has the potential not only to aid in the creation of vaccines against parasitic infections but also to advance our understanding of the human immune system.

Shoemaker also studies neurotoxins produced by the bacterium *Clostridium botulinum*. Botulinum toxins cause paralysis, asphyxia, and death. Because this bacterium survives only in anaerobic environments, the toxin is usually contracted through consumption of contaminated food (canned foods in particular). Although intoxication can be mitigated or prevented by antitoxin administration if the patient receives treatment shortly after exposure, no therapy exists for reversing botulism poisoning. A patient's only chance of survival is to be put on an artificial lung until the toxin dissipates, which can take weeks to months. Shoemaker leads several projects in the Botulism Therapeutics Program of the Microbiology and Botulism Research Unit, which is directed by Saul Tzipori. "Our primary focus is to try to develop therapies for treating people who have been exposed and have begun to show symptoms," says Shoemaker.

After receiving his PhD in biochemistry from the University of Iowa in 1979, Shoemaker played a principal role in elaborating the mechanism of retroviral DNA integration while working in David Baltimore's laboratory at MIT. Shoemaker was one of the original scientists at Genetics Institute, and he joined the Department of Tropical Public Health at Harvard University in 1987. From 1995 through 2003 Shoemaker split his time between Harvard and AgResearch Limited in New Zealand.

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### **Nektarios Tavernarakis**

Institute of Molecular Biology and Biotechnology  
Foundation for Research and Technology-Hellas, Heraklion  
<http://www.imbb.forth.gr/worms/>



Nektarios Tavernarakis heads the *Caenorhabditis elegans* molecular genetics group at the Institute of Molecular Biology and Biotechnology, in Heraklion, Crete, Greece. He earned his Ph.D. degree at the University of Crete, studying gene expression regulation in yeast, and received training in *C. elegans* genetics and molecular biology at Rutgers University, New Jersey, USA. In addition to necrosis/neurodegeneration and ageing, his research focuses on sensory transduction and signal integration by the nervous system. He is the recipient of an International Human Frontier in Science Program Organization (HFSP) long-term award, the Bodossaki Foundation Scientific Prize for Medicine and Biology, the Alexander von Humboldt Foundation Friedrich Wilhelm Bessel research award and is a European Molecular Biology Organisation (EMBO) Young Investigator.

**ABSTRACTS  
FOR ORAL PRESENTATIONS**

## **Nematode genomics and informatics: leveraging *C. elegans***

PAUL W. STERNBERG. CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CA

HHMI, Caltech and WormBase

*C. elegans* has emerged as a model organism, not only for classical biology but also for genomics and bioinformatics. With the emergence of additional genome informatic resources, the *C. elegans* and parasitic helminth communities can collaborate to greatly accelerate helminth research. WormBase ([www.wormbase.org](http://www.wormbase.org)) now includes sequences of other *Caenorhabditis* species as well as *Brugia malayi* and *Pristionchus pacificus*. Besides genome and EST sequences, WormBase curates a variety of experimental data such as microarrays, gene expression and regulation, phenotypes, and cell function. Many features of WormBase can accommodate biological information about other species. WormBook ([www.wormbook.org](http://www.wormbook.org)) is an open access online review that is indexed in PubMed. Textpresso ([www.textpresso.org](http://www.textpresso.org)) is an ontology-based, full-text search engine for biological literature that was developed by WormBase but has been extended to *Drosophila*, *Arabidopsis*, rat, and neuroscience, among others. Sentences are indexed according to categories of terms, for example, a "Reporter Gene" category includes "GFP," "luciferase," "Cyan Fluorescent Protein," and so forth, while a human disease gene category includes all names of human diseases. A pilot Textpresso-for-nematodes has been developed and help in developing the search categories is sought. WormBase handles Gene Ontology (GO; [www.geneontology.org](http://www.geneontology.org)) annotations for *C. elegans* and can help process annotation for other species; GO annotations are used in essentially every gene expression profiling experiment. A data integration pipeline to predict genetic interactions based on phenotypic, expression and interaction data in multiple species now includes *C. elegans*, *Drosophila* and mouse ([www.GeneOrienteer.org](http://www.GeneOrienteer.org)). In addition to these resources, three types of genomic experiments will be discussed: gene expression profiling during larval/juvenile growth and arrest, gene expression profiling of single cells using short-read sequencing technology, and comparative genomic analysis of cis-regulatory sequences.

## Parasitic helminth genome sequencing

M. BERRIMAN, WELLCOME TRUST SANGER INSTITUTE, HINXTON, CB1 3PT, UK

The availability of complete genome sequence data from both parasites and their mammalian hosts provides a unique opportunity to take a genomic approach to explore host/pathogen biology. The helminth genomes initiative at the Wellcome Trust Sanger Institute is aiming to produce reference genomes from a wide phylogenetic spectrum of helminths. Currently *Schistosoma mansoni*, *Haemonchus contortus*, *Echinococcus multilocularis* and *Strongyloides ratti*, are in active sequencing and additional projects for *Nippostrongylus brasiliensis*, *Onchocerca volvulus*, *Ascaris lumbricoides*, *Globodera pallida*, and *Trichuris muris* are just starting. Although, high levels of polymorphism and the lack of inbred lines remain serious problems, steps are being taken to decrease the complexity of the assembly task and produce higher quality drafts. These include using whole genome amplified material from single worms or using new, higher throughput (and lower cost), sequencing technologies to resolve haplotypes from complex mixtures. Data from the projects is made available through two main interfaces: GeneDB<sup>1</sup> – a gene centric database of reference genomes – and the TDR targets database<sup>2</sup> – designed to allow users to prepare their own prioritised and sorted list of molecular targets for drugs discovery, based on available validation criteria. <sup>1</sup> [www.genedb.org](http://www.genedb.org) <sup>2</sup> [www.tdrtargets.org](http://www.tdrtargets.org)

## The Genome Project of *Taenia solium*<sup>1</sup>

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A consortium of key laboratories at the National Autonomous University of Mexico is carrying out a full genomic project for *Taenia solium*. This project will provide powerful resources for the study of taeniasis/cysticercosis. The nuclear DNA content estimated through cytofluorometry on isolated cyton nuclei as well as two probabilistic calculations based on shotgun sequenced genomic clones, resulted in a size estimate for the haploid genome of about 130 Mb. A combined strategy with 454 and capillary sequencing is under process. So far, we have achieved

10X coverage by 454 pyrosequencing and 4.5X coverage by capillary sequencing. Results indicate that *T. solium* genome is not highly repetitive (< 7%). One small 53 bp tandem-repeat and different tetranucleotide repeats represented 0.5% and 4.5% of the genome, respectively. Current assemblage still shows tens of thousands contigs, however, several estimates suggest that 99% of the genes are already included.

Besides genomic sequencing, more than 34,000 ESTs have been obtained: 14,113 from adult cDNA libraries and 9,157 from larval libraries, which have been made public through GenBank. Additional 12,200 5' end sequenced ESTs from a larval full-length cDNA library are already available. Unique genes were identified by clustering all EST-fragments with an assembler (minimus). We have identified around 7,000 "genes", some of them are highly expressed in both adult and larvae stages. Thus, there are 349 "genes" with 10 or more sequences that account for 50% of all transcripts. Approximately one third (2,038) of the 7,000 genes have a significant match in SwissProt and about 27% of the genes have no match in SProt + TREMBL, and could constitute new genes. Gene identification in progress is allowing to elucidate some metabolic traits, signal transduction cascades and other physiological routes present in this tapeworm.

The consortium for the *T. solium* genome project wishes to use this International Congress on Helminth Parasites to make a call for collaborative research. <sup>1</sup> This project is supported by a special grant IMPULSA-UNAM.

### **Sequence of *Meloidogyne hapla*: A compact nematode genome for plant parasitism**

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Root-knot nematodes (*Meloidogyne* spp.) are widely distributed throughout temperate and tropical regions and are responsible for yield losses exceeding 10% on food and fiber crops. They also render plants more susceptible to drought stress and are a major contributing factor to a looming world food crisis. We have established *Meloidogyne*

*hapla* as a tractable model plant-parasitic nematode amenable to forward and reverse genetics. Based on sequencing from multiple libraries (3, 6, 8 kb shotgun libraries; 40 kb fosmids) we have obtained an assembled draft sequence with 10.4X coverage that spans  $\geq 98\%$  of the genome in 1,523 scaffolds. At 54 Mb, *M. hapla* represents not only the smallest nematode genome yet completed, but also the smallest metazoan, and defines a platform to elucidate mechanisms of parasitism by what is the largest uncontrollable group of plant pathogens worldwide. *Ab initio* gene discovery tools converge on an estimate of 14,494 protein-coding genes (freeze Mh1.1g). More than 20% of these gene models have been confirmed by analysis of transcripts, and over half encode

proteins with highly significant matches to proteins in GenBank ( $<1.0e^{-20}$ ). The *M. hapla* genome encodes significantly fewer genes and also shows overall compression of the genome compared to *C. elegans*. Disparity in gene number between parasite and free-living nematode is particularly striking in gene families. In some instances, most notably the G-protein coupled receptors, this family seems to have expanded in *C. elegans* to nearly 10-fold over *M. hapla*. Conversely, *M. hapla* encodes functions unique to its role as a plant-parasite, with some of these genes apparently acquired horizontally from bacteria and/or plants during its evolutionary history. Some of these genes (such as those encoding pectate lyase, an enzyme required for digestion of carbohydrates in the plant cell wall) appear to have undergone subsequent expansion and diversification; *M. hapla* encodes 22 pectate lyases. Although *M. hapla* and *C. elegans* diverged more than 500 million years ago, many developmental and biochemical pathways, including those for dauer formation and RNAi are strikingly conserved. Understanding how these generalized features of nematode biology have been adapted for a parasitic life-style is of particular interest to our group.

## The genome of *Pristionchus pacificus* and implications for the evolution of parasitism

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*Pristionchus pacificus* represents a nematode that shares with *Caenorhabditis elegans* many technical features and has been developed as a model system in evolutionary developmental biology. Many important differences on the genetic and molecular level of development, in particular vulva development, have been identified. Intriguingly, *Pristionchus* also occupies a distinct ecological niche. *Pristionchus* nematodes are associated with scarab beetles and have a necromenic life style: that is, worms invade the beetle as dauer larvae and wait for the insect's death to feed on the developing microbes on the carcass. It has been suggested that a close association with other organisms as seen in necromenic nematodes represent a pre-adaptation towards true parasitism.

With 169-Mb and 29,000 predicted protein-coding genes the *P. pacificus* genome is substantially larger than the genomes of *C. elegans* and the human parasite *Brugia malayi*. Comparative analysis with *C. elegans* revealed an elevated number of genes encoding cytochrome P450 enzymes, glucosyl-transferases and ABC transporters that were experimentally validated and confirmed. *P. pacificus* contains cellulase and diapausin genes and cellulase activity is found in *P. pacificus* secretions, the first time cellulases have been identified in nematodes beyond plant parasites. The increase in detoxification and degradation enzymes is consistent with the *Pristionchus* life-style and is a pre-requisite for parasitism. Thus, comparative genomics of three ecologically distinct nematodes offers a unique opportunity to investigate the correlation between genome structure and life-style.

Current studies involve developmental and genetic analyses of various traits associated with the specific ecological setting of *P. pacificus*, such as dauer formation, insect recognition by olfaction, mouth form dimorphism and bacterial association. Specificity of the *Pristionchus* beetle association is achieved by nematode interception of the insect sex pheromone/sex attractant system. For example, *P. maupasi* recognizes its cockchafer host by olfaction towards phenol, one of the cockchafers' sex attractants, whereas *P. pacificus* identifies the oriental beetle host by recognition of the sex pheromone (Z)-7-tetradecen-2-one. In contrast, analysis of the *Pristionchus* associated bacteria did not reveal any specificity so that the ultimate causation of the high specificity of the nematode beetle association remains open. Current analyses investigate a potential competition between *Pristionchus* and entomopathogenic nematodes.

*Pristionchus*, unlike many nematodes can feed on bacteria, fungi and other nematodes. This is because *Pristionchus* nematodes show a mouth dimorphism between a teethless bacterial feeding stenostomatous form and an eurystomatous form with teeth that can feed on fungi and nematodes as well. Genetic analysis suggests that the mouth dimorphism represents a case of phenotypic plasticity and that the ratio of the two forms is influenced by environmental factors, such as starvation. The genetic regulation of the mouth dimorphism is currently investigated and shares several genes with other plastic nematode traits.

### Genetics in the parasitic nematode genus *Strongyloides*

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The nematode genus *Strongyloides* consists of parasites that live as parthenogenetic females in the small intestines of their hosts. In addition to producing parasitic offspring, they can also form a facultative free-living generation with males and females (for review see Viney and Lok, 2007). While some species of *Strongyloides*, like *S. ratti*, and the human parasite *S. stercoralis*, employ an XX/XO sex determining system, *S. papillosus*, a parasite of farm ruminants, contains no true X chromosome. For this species it has been suggested that males are the product of a sex specific chromatin diminution event which leads to the elimination of a large portion of one of the homologues of a chromosomal pair during the single mitotic oocyte maturation division (for review see Streit, 2008). Contrary to earlier reports that were based on cytological observations, males of *S. ratti* (Viney et al., 1993) and *S. papillosus* (Eberhardt et al., 2007) do contribute genetic information to the next generation. We have generated molecular genetic markers for *S. ratti* and *S. papillosus* and we are analyzing their inheritance and linkage both genetically and molecularly (FISH). We have shown that in both species recombination within chromosomes occurs. All this indicates that these two species undergo standard sexual reproduction and are therefore amenable to classical genetic analysis and mapping. One of our goals is to complement the ongoing *S. ratti* whole genome sequencing with a genetic linkage map for this species. We are particularly interested in genetic differences between the two species, which relate to the different sex determining systems.

The ability to reliably distinguish between different species is an indispensable prerequisite for comparative studies. Generally all *Strongyloides* found in farm ruminants were considered to belong to the species *S. papillosus*, first described as a parasite of sheep. We have analyzed the sequences of the 18S rDNA and of three protein-encoding genes from *Strongyloides* individuals isolated from sheep and cattle from Germany, Mali and the USA. Our data clearly indicate that there exist at least two different, genetically isolated, sympatric populations that therefore should be considered different, relatively closely related, species. One species was the predominant *Strongyloides* in cattle and was found in this host only. The other species was the only species we found in sheep but did also occur in cattle at low numbers.

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### **Developing *Haemonchus contortus* as a model parasite for forward genetic studies of anthelmintic resistance**

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Genetic analysis has been central to the progress made in many areas of biological research ranging from model organism biology to human medicine. However, although there are many potential applications, genetic approaches have been under-exploited in parasitic helminth research with studies being largely limited to a relatively small number of population genetic studies. Although there has been significant progress in the use of forward genetic analysis to map genes associated with traits such as drug resistance and virulence in parasitic protozoa, similar approaches have yet to be applied to parasitic helminths. However, many parasitic helminth species should be amenable to forward genetic analysis and the vast amount of sequence data currently being generated from parasitic helminth genome projects provides major new opportunities in this area. The parasitic nematode of sheep, *H. contortus* is a good system in which to explore parasite genetics for a number of reasons. The ability to surgically transplant adult worms into the sheep abomasum, together the parasite's extremely high fecundity makes it possible to undertake genetic crosses. The availability of characterized, genetically differentiated drug resistant and susceptible isolates and the increasing amount of genome sequence available provides unprecedented opportunities for the analysis of genetic crosses. We have developed panels of autosomal and X-linked microsatellite markers and used them to study the basic genetics of this parasite to provide a framework for forward genetic analysis. We have shown female and male worms have XX and XO karyotypes respectively and that polyandrous mating occurs. A single female can contain the progeny of up to four male worms providing an additional mechanism of generating genetic diversity. We have developed an approach to produce highly inbred lines for genetic crossing and have validated the inbreeding process using genetic markers. We have also undertaken a series of backcrosses between the susceptible isolate MHco3(ISE) (the isolate being used for the genome sequencing) and two different ivermectin resistant isolates. Five generations of backcrossing these resistant isolates against MHco3(ISE) in the presence of ivermectin selection have been performed to introgress resistance genes from the two genetically divergent isolates into the MHco3(ISE) (susceptible) genetic background. The backcrossed isolates will be a valuable resource for genetic mapping and other molecular studies to identify ivermectin resistance loci.

### **Expression of potential control targets of parasitic nematodes using *Caenorhabditis elegans***

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Parasitic nematodes of livestock are a significant economic and welfare problem. Anthelmintic drugs are currently used to control infection, however the efficacy of these is decreasing due to the emergence of drug resistant nematodes and alternative controls are needed. For the sheep parasitic nematode *Haemonchus contortus*, a number of native proteins and protein complexes extracted from the parasite can induce significant protective immunity. However, protection trials using recombinant forms of these proteins expressed in bacteria, yeast or insect cells have demonstrated that the recombinant proteins are far less effective. This could be for a number of reasons, including the possibility that not all components of the native extract are present in the recombinant vaccines, as well as the difficulties associated with protein folding and correct glycosylation using standard expression systems. We are using *Caenorhabditis elegans* to express potential control targets of *Haemonchus*, with the aim of producing these in a form with similar conformation and glycosylation to the native proteins. We have expressed *H. contortus* cathepsin L cysteine protease in *C. elegans* and shown that the parasite gene can rescue *C. elegans* cathepsin L (*cpl-1*) mutants. The *C. elegans* expressed protease is active and glycosylated. We are now testing expression of previously identified protective proteins including the gut aminopeptidase H11 and cathepsin B protease gene family. Different *C. elegans* promoters and 3'UTRs are being used to try to optimise expression levels.

### Functional analysis of *Ostertagia ostertagi* vaccine candidates in *Caenorhabditis elegans*

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Previous vaccination trials against the abomasal nematode *Ostertagia ostertagi* in cattle have demonstrated the protective capacity of a protein fraction termed ES-thiol. Mass spectrometric analysis revealed that this fraction is highly enriched for two activation-associated secreted proteins (ASP-1 and -2) and contains, amongst others, a translationally controlled tumor protein (TCTP). The biological role of these molecules in *O. ostertagi* and other nematodes is still unclear. Therefore, the objective of this study was to further unravel the function of these molecules by using the model organism *Caenorhabditis elegans*. Database searches indicated that *C. elegans* contains around 36 ASP encoding genes of which VAP-1, T05A10.4 and T05A10.5 show the highest sequence similarity with the *O. ostertagi* ASPs. VAP-1 is a two domain ASP, transcribed in the amphid sheath cells and is upregulated during the aging of the worms. RNAi mediated knockdown of *vap-1* results in a significant extension of the lifespan of adult worms and has an effect on the stress response of the worms. Although T05A10.4 and T05A10.5 were predicted to be 2 separate genes, RT-PCR experiments showed that they are actually part of the same gene encoding for a second two domain ASP in *C. elegans*. The gene is expressed in the excretory duct cell and in some hypodermal cells of the tail. The localization and the observed phenotypic effects suggest a role of these ASPs in signal transduction. TCTP on the other hand seems to be a single-copy gene in nematodes. In *O. ostertagi*, the transcription pattern of TCTP indicated an upregulation in adult female worms and eggs. These results were confirmed by Western blotting and immunolocalisation, which showed that TCTP was highly present in the developing eggs of both *O. ostertagi* and *C. elegans*. RNAi mediated knockdown of *tctp* in *C. elegans* rendered a 90 % reduction in egg production, indicating a crucial role of this protein in nematode egg production and development.

### Functional expression of parasitic SLO-1 in *Caenorhabditis elegans slo-1* knockout mutants

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The calcium-gated potassium channel SLO-1 is of major importance for the effects of the new anthelmintic drug emodepside on the locomotory behaviour of *Caenorhabditis elegans*. In a recent study, *C. elegans slo-1* functional null mutants were shown to be highly resistant to emodepside, as they could be grown on agar plates containing 1 µM emodepside, a concentration, which immobilises wild-type worms. Expression of SLO-1 driven by either the neuronal *snb-1* promoter or the muscle-specific *myo-3* promoter reconstituted the susceptibility of the knockout mutants to emodepside. In the present study, we identified homologues of *slo-1* in the canine hookworm *Ancylostoma caninum*, the barber pole worm in sheep, *Haemonchus contortus*, and the bovine trichostrongyle *Cooperia oncophora*. The identities of the amino acid sequences were 96 to 98 % with each other and 88 % with *C. elegans* SLO-1. We expressed the *slo-1* coding sequences of *A. caninum* and of *C. oncophora* in the *C. elegans* null mutant *slo-1(js379)*. As promoters the neuronal *snb-1* promoter, which was also used in the previous study on *C. elegans*, and the putative *C. elegans slo-1* promoter (3 kb in size) were used. To determine susceptibility to emodepside, successfully transfected worms were incubated on agar plates containing emodepside and the locomotory behaviour was evaluated by counting the number of body bends per minute. Expression of SLO-1 from parasitic nematodes, driven by the *snb-1* promoter, in the *C. elegans* null mutant *slo-1(js379)* significantly increased susceptibility of the worms to emodepside. Furthermore, expression of SLO-1 under the *C. elegans slo-1* promoter in the *slo-1(js379)* mutant background restored the sensitivity of the mutant to emodepside to the same level as wild-type worms. Experiments were carried out with emodepside concentrations between 1 nM and 10 µM, and the observed effects were dose-dependent. These data confirm that emodepside signals through the calcium-activated potassium channel of both *C. elegans* and parasitic nematodes and the experiments provide *C. elegans* strains expressing the parasite target for further functional and pharmacological analysis.

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**Transgenesis in *Strongyloides stercoralis* and *S. ratti*: prospects for transposon-mediated gene transfer and applications in studies of gene function, immunology and sensory neurobiology**

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At Hydra 2005 we reported that regulated expression of a reporter transgene fusing the *era-1* promoter to the *gfp* coding sequence could be achieved in *Strongyloides stercoralis* by incorporating the 3' UTR for *era-1* into the construct. Since then, we have demonstrated that the *Ss-era-1* 3' UTR can act as a multi-purpose terminator for reporter transgene constructs with promoters driving either ubiquitous reporter expression (*Ss-rps-21*) or cell- or tissue-specific expression in such anatomical sites as the intestinal epithelium (*Ss-era-1*), body wall muscle (*Ss-act-2*) and amphidial neurons (*Ss-gpa-3*). We have demonstrated that F1 transgenic *S. stercoralis* are capable of infecting gerbils and maturing to parasitic females in the intestine while maintaining transgene expression. Transgene DNA can be detected in progeny of transgenic *S. stercoralis* to the F5 generation, but to date, none of our constructs is expressed after the F1 generation. Recently, we found that *Strongyloides ratti*, can be transformed, and exhibits cell- and tissue-specific transgene expression using constructs developed for *S. stercoralis*. We recently used our transgenesis system to investigate the function of the forkhead transcription factor encoded in *S. stercoralis* *fkf-1*. This factor is orthologous to DAF-16, which regulates dauer development and lifespan in *C. elegans*. Expression of recombinant FKTF-1 fused to GFP in *S. stercoralis* reveals that, like its *C. elegans* ortholog, this transcription factor is localized in cell nuclei of larvae destined for development to the dauer-like L3i. Moreover, expressing a putative dominant loss-of-function mutant FKTF-1 results in severe defects in pharyngeal morphology in transgenic *S. stercoralis*, suggesting a role for FKTF-1 in pharyngeal development. With an eye toward achieving heritable transgene expression, we are investigating transposons as means of integrating constructs into the genome of *Strongyloides* spp. To this end, we are evaluating an exogenous transposon, *PiggyBac*, which is capable of integrating DNA sequences into the genome of *Schistosoma mansoni*, and an endogenous transposon, designated SMART-1, which we have recently discovered in *S. stercoralis*. We are assessing the utility of transgenic *S. ratti* in studies of the immune response to infection in mice, where this parasite establishes a patent infection of low intensity and short duration. We have synthesized constructs designed to express avian ovalbumin (OVA) under the control of various tissue-specific promoters and are evaluating the OVA-specific response to worms carrying these transgenes in mice adoptively transferred with OVA-specific CD4<sup>+</sup> T cells. Finally, we are studying the feasibility of genetically targeted neuronal ablation in *Strongyloides* spp. In *C. elegans*, expression of caspases or mutant channel forming proteins under the control of neuron- or other cell-specific promoters has been applied in this manner. We intend to express such lytic or apoptotic factors under the control of neuron-specific promoters such as *Ss-gpa-3* as a means of assessing functions of chemosensory neurons in *Strongyloides* spp.

### **Regulation and function of *fktf-1* in transgenic *Strongyloides stercoralis***

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*Strongyloides stercoralis* has an unusual life cycle consisting of alternating free-living and parasitic generations. When appropriately triggered via external stimuli or internal genetic mechanisms, the post-parasitic larvae can develop either into a free-living generation, which can be cultured *in vitro*, or directly into the infective third stage. The developmental switch in post-parasitic L1s, the free-living generation and the morphological similarities to *Caenorhabditis elegans* make *S. stercoralis* an attractive model organism to study the development of parasitic infective larvae. Insulin-like signaling is one well-characterized mechanism directing *C. elegans'* larval development. Specifically, it negatively regulates the function of *daf-16*, a forkhead transcription factor that, when active, directs the development of the arrested third-stage larva. *fktf-1*, an *S. stercoralis* ortholog of *daf-16*, is hypothesized to regulate larval development in a similar manner to *daf-16*. Free-living *S. stercoralis* females were transformed with transgenes encoding *gfp* reporters under the direction of an *fktf-1 $\beta$*  promoter. Transgenic first-stage larvae were scored for GFP expression as well as tissue and sub-cellular localization. 60% of transgenic larvae showed GFP expression in either the hypodermis or the pharyngeal procorpus, 30% had expression in both sites. A GFP::FKTF-1b fusion protein under direction of the *fktf-1 $\beta$*  promoter was localized to the nuclei of hypodermal cells in transgenic larvae. In contrast, when GFP alone was expressed from the transgene it was cytoplasmic in hypodermal cells. *fktf-1b*, like *daf-16*, has three Akt/PKB phosphorylation consensus sites plus a fourth overlapping site in the forkhead domain. When phosphorylated, the transcription factor is inactivated and exported from the nucleus. We mutated two phosphorylation sites to phospho-mimetics and found that localization of the GFP::FKTF-1b(S238E/T240E) fusion protein was cytoplasmic in hypodermal cells, as opposed to nuclear with the wild-type sequence. In order to investigate the function of *fktf-1b* using transgenesis, we created a dominant-repressor construct to antagonize the function of the endogenous *fktf-1b*. First, alanine mutations were made in the Akt/PKB consensus sites to create a protein unable to be exported from the nucleus. Second, the transactivation domain was replaced with a repressor domain from *C. elegans'* *pie-1*. Finally, *gfp* was fused to the 5' end to easily visualize the protein. Transgenic larvae expressing the dominant-repressor version of GFP::FKTF-1b show defects in overall growth as well as defects in pharyngeal and intestinal morphology. The intestines are shrunken and appear to have a loss of granularity compared to age-matched larvae. The larvae survive as L1s, however they fail to grow or to develop into L3is. Using transgenesis, we have found that *fktf-1b* is active predominantly in tissues remodeled during infective larval development namely the hypodermis and pharyngeal procorpus, is localized to cell nuclei in larvae developing into L3is, and appears to be regulated post-transcriptionally via phosphorylation in a similar manner to *daf-16*. Furthermore, transgenic larvae expressing a dominant-repressor form of FKTF-1b have altered pharyngeal morphology and fail to develop into infective larvae. These findings support the hypothesis that *fktf-1b* regulates larval development in *Strongyloides stercoralis*.

### **Analysis of transcription and mRNA processing in *Brugia malayi* using a homologous transient transfection system**

THOMAS R. UNNASCH

The recent completion of the sequence of the *B. malayi* genome has provided a wealth of information regarding gene structure in this parasite. However, sequence data alone provide little information regarding the mechanisms that filaria use to regulate gene expression. Reverse genetic techniques, such as transfection, have proven to be quite useful in the study of the mechanisms of gene regulation in other organisms. We have developed a method to transiently transfect *B. malayi*, and have used this method together with a luciferase reporter assay system to begin to dissect the cis-acting factors that are necessary for transcription in *B. malayi*. An analysis of several *B. malayi* promoters using this system has revealed that the core promoter domains of this organism appear to lack many of the features that characterize a typical eukaryotic promoter. In addition, we have used the transient transfection system to study trans-splicing *in vivo* in *B. malayi*, and have demonstrated that trans splicing is dependent on cis-acting elements that are encoded downstream of the promoter domain. These studies provide evidence to demonstrate that transient transfection may be a useful tool to study both mRNA processing and gene regulation in the filarial parasites.

### **Integration of reporter transgenes into *Schistosoma mansoni* chromosomes mediated by pseudotyped murine leukemia virus**

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The recent release of draft genome sequences of two of the major human schistosomes has underscored the pressing need to develop functional genomics approaches for these significant pathogens. The sequence information also makes feasible genome-scale investigation of transgene integration into schistosome chromosomes. Retrovirus mediated transduction offers a means to establish transgenic lines of schistosomes, to elucidate schistosome gene function and expression, and to advance functional genomics approaches for these parasites. We investigated the utility of the Moloney murine leukemia retrovirus (MLV) pseudotyped with vesicular stomatitis virus glycoprotein (VSVG) for the transduction of *Schistosoma mansoni* and delivery of reporter transgenes into schistosome chromosomes. Schistosomules were exposed to virions of VSVG-pseudotyped MLV after which genomic DNA was extracted from the transduced schistosomes. Southern hybridization analysis indicated the presence of proviral MLV retrovirus in the transduced schistosomes. Fragments of the MLV transgene and flanking schistosome sequences recovered using an anchored PCR-based approach demonstrated definitively that somatic transgenesis of schistosome chromosomes had taken place and, moreover, revealed widespread retrovirus integration into schistosome chromosomes. More specifically, MLV transgenes had inserted in the vicinity of genes encoding immunophilin, zinc finger protein Sma-Zic and others, and also near the endogenous schistosome retrotransposons, the *fugitive* and *SR1*. Proviral integration of the MLV transgene appeared to exhibit primary sequence site specificity, targeting a gGATcc-like motif. Reporter luciferase transgene activity driven by the schistosome actin gene promoter was expressed in the tissues of transduced schistosomules and adult schistosomes. Luciferase activity appeared to be developmentally expressed in schistosomules with increased activity observed after one to two weeks in culture. These findings indicate the utility of VSVG-pseudotyped MLV for transgenesis of *S. mansoni*, herald a tractable pathway forward towards germline transgenesis and functional genomics of parasitic helminths, and provide the basis for comparative molecular pathogenesis studies of chromosomal lesions arising from retroviral integration into human compared with schistosome chromosomes.

## **Cestode stem cells: their role in host-induced larval development and their utilization to achieve parasite transgenesis**

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A population of totipotent stem cells, called germinal cells or neoblasts, is central to the developmental biology of flatworms. In free-living planarians, these cells are crucially involved in regeneration. In parasitic flatworms, a role of totipotent stem cells in asexual proliferation of larvae as well as 'never-ending' growth of tapeworms has been discussed. Investigating the role of germinal cells in larval development of the model cestode *Echinococcus multilocularis*, we were recently successful in isolating germinal cells and in establishing primary cultures that are devoid of contaminating host cells. When incubated in the presence of host hepatocytes, physically separated through a trans-well system, or hepatocyte supernatant, the germinal cells proliferated and formed cell aggregates which contained central cavities surrounded by the growing germinal layer. After six weeks of cultivation, complete and fully infective metacestode vesicles were present, indicating that the 'metamorphosis' of the oncosphere towards the metacestode within the host's liver (i.e. the initial phase of infection) can be mimicked in vitro by our system. Several hormones and cytokines which are present in high concentrations in the liver, such as insulin, significantly stimulated metacestode formation from germinal cells. Furthermore, isolated germinal cells expressed surface receptor kinases of the insulin-, EGF-, and FGF-receptor families which we have shown to be capable of interacting with the corresponding host cytokines. Signalling pathways that act downstream of receptor kinases, such as the MAP kinase cascade, were also stimulated within germinal cells in the presence of host insulin, EGF and FGF. Taken together, these data indicate that germinal cells, which are delivered to the host liver by the oncosphere, are able to sense the cytokine milieu of this host organ which finally results in germinal cell activation and metacestode formation at this specific site in the intermediate host.

The ability to generate infective parasite tissue from single cells now also allows the production of fully transgenic parasites once the methodology to introduce foreign DNA into germinal cells is available. Toward this end, we have successfully used transfection reagents to transiently express a heterologous reporter protein in cultivated germinal cells. Furthermore, we were able to stably introduce foreign DNA into the genome of germinal cells using heterotypic lentiviruses, which also resulted in heterologous reporter gene expression. Heterotypic lentiviruses and the in vitro germinal cell cultivation system are currently used in our laboratory to produce the first fully transgenic strain of *E. multilocularis*. In conjunction with the *E. multilocularis* whole genome sequencing project that is currently in an advanced stage, the established methods of in vitro larval and germinal cell cultivation as well as the ability to produce fully transgenic parasites will greatly facilitate molecular studies on *E. multilocularis* development and host-parasite interaction. Since the methods to in vitro-manipulate *E. multilocularis* cells should principally also be applicable to other tapeworms, the entire field of cestode molecular biology is expected to profit from our results.

### Signaling molecules involved in gonad differentiation of *Schistosoma mansoni*

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Schistosomes are the only members of the trematodes which live dioeciously. The sexual maturation of the female essentially depends on signals from the male inducing mitoses and differentiation processes in the vitellarium. Here, vitelline cells develop for the production of eggs, which are responsible for pathogenesis in the final host. Upon separation from the male, mitoses and vitelline cell differentiation in the female decline, and egg production stops. These effects are reversible upon re-pairing. Although known for a long time, the molecular basis of this phenomenon is poorly understood.

In an approach to identify molecules involved in the sexual maturation of schistosome females, we found among other signaling molecules Src and Syk tyrosine kinases (TK), and the transforming growth-factor  $\beta$  receptor 1 (TGF $\beta$ -R1) to play important roles during gonad differentiation. The Src-kinase inhibitor Herbimycin A, or the TGF $\beta$ -R1-kinase inhibitor blocked the activity of these signaling molecules in adult females kept *in vitro*. As revealed by the DAPI-staining/BrdU-incorporation technique, inhibitor treatment significantly reduced mitoses in paired females. In addition, egg production declined. A combined treatment with both inhibitors led to an additive effect indicating that at least two pathways cooperatively activate mitotic activity and egg production in paired females.

To unravel signalling cascades, in which the schistosome Src-kinase SmTK3 is involved, we searched for its binding partners by Yeast-Two-Hybrid (Y2H) analyses. For this we generated an Y2H-cDNA library on the basis of poly(A)<sup>+</sup>-RNA of male and female *S. mansoni*. Using SH3-domain constructs with or without the N-terminally located unique site of SmTK3 as baits for library screening identified the diaphanous homolog SmDia as the most prominent "downstream" binding partner. With the SH2 domain as bait to screen for potential "upstream" interaction partners, we identified the epidermal growth factor substrate protein 8 (SmEps8), a known multifunctional signaling molecule in eukaryotes. For characterization we used deletion constructs of SmEps8 and provide evidence for an atypical binding between SmTK3 and SmEps8 in Y2H interaction-studies. Since an Eps8-typical binding-domain for the epidermal growth-factor receptor (EGFR) exists also in SmEps8, we have started to investigate its binding activity to the known EGFR of *S. mansoni* (SER). Besides this, *in situ* hybridization experiments have demonstrated the transcription of the SmEps8 gene in the reproductive organs of both genders, which corresponds to the transcript localizations of SmTK3 and SmDia.

Since homologs of Eps8, diaphanous, and Src kinases have been shown to be involved in receptor signalling, cytoskeleton remodelling and cell proliferation in other eukaryotes, and since the schistosome homologs SmTK3, SmDia and SmEps8 interact in Y2H assay and colocalize in the reproductive organs of *S. mansoni*, we conclude that these molecules fulfil cooperative functions during gonad differentiation in this parasite.

## Developmental genes in the life cycle of a parasitic flatworm

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Tapeworms have exploited the regenerative and proliferative abilities of stem cells to extraordinary ends and provide many excellent models for understanding the genetic basis of morphological disparity and adaptation. From an evolutionary perspective, their typically segmented form is seen to have evolved in a step-wise fashion, first through the serial repetition of their hermaphroditic reproductive organs (ie. 'proglottization') followed subsequently by their somatic compartmentalization (ie. 'segmentation'). Whether these processes are novel or share a genetic programme homologous with other lophotrochozoans (eg. annelids and molluscs) or with the more basal, strobilate cnidarians (eg. jellyfish) is a question that can inform both flatworm development as well as the degree of genomic conservation in the animal kingdom. To address this, and to resolve more basic outstanding questions such as their correct anteroposterior (AP) orientation (ie. is the scolex in fact anterior?) and the possible change in polarity during metamorphosis from larval to adult form, information from Hox, ParaHox and other homeobox-containing genes is being examined. Using a fully segmented, adult model tapeworm (*Hymenolepis*), homeodomains of anterior (*Hox1/Lab*; *Hox4/Dfd*), central (*Lox4/AbdA*) and posterior-class (*Post-1/2*) Hox genes were recovered and their full transcripts characterized by PCR RACE. Full-length genes were then amplified, cloned and used as templates for reverse transcribing digoxigenin-labelled riboprobes, and the spatial expression patterns of the genes in adult worms examined by whole-mount *in situ* hybridization. Initial efforts achieved highly specific patterns for two of the genes that implicate potentially direct roles in the processes described above: the posterior gene *Post-2* is seen expressed in bands at the leading (ie. growing) margin of each segment, indicative of having a role in the process of segmentation, whereas expression of the central gene *Lox4* is associated strictly with the developing ovaries and thus either promotes ovarian development or alternatively, is being expressed by the ovaries themselves. It is thus implicated in playing a role in proglottization and, depending on the relationship between cause and effect, may be responsible for differences in the timing of development of male and female systems and for the loss or gain of hermaphroditism. Alternatively, *Lox4* may be acting to coordinate development in response to the maturing ovaries. Ongoing work aims to examine the expression of these and other homeoboxes throughout the complex ontogeny of cestodes and to arbitrate on cause and effect by inferring the functions of the genes using knockdown techniques. Once understood in the model system, this work can be extended to non-model groups exhibiting fundamentally different body plans and reproductive strategies and in this way be integrated with our existing knowledge of tapeworm phylogeny. The work also provides inroads for examining the roles of homeoboxes in parasitic flatworms more broadly and thus the ability to contrast these roles with those found in free-living groups whose regenerative abilities have made them long-serving models in developmental biology.

**The Syk-kinase SmTK4 from *Schistosoma mansoni*: upstream interaction partners and functional aspects in oogenesis and spermatogenesis**

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The parasitic helminth *Schistosoma mansoni* is the causing agent of schistosomiasis, one of the most prevalent parasitic infections in the world. Schistosomes, which represent the only family within the trematodes being dioecious, show unique biological features. Among these is the sexual maturation of the female, which depends on a constant pairing contact with the male. Upon pairing the reproductive organs of the female differentiate, a prerequisite for egg production leading to pathogenesis. Between the two genders, molecular communication processes occur, which probably include signal transduction cascades.

During our attempts to identify signaling molecules from *S. mansoni* we isolated the Syk-family kinase SmTK4, which is tissue-specifically transcribed in the ovary and in the testes of adult schistosomes. In other biological systems, Syk-kinases are members of signaling cascades controlling differentiation processes. To elucidate signaling cascades in which TK4 may be involved, we searched for interaction partners by yeast two-hybrid analyses.

First evidence is provided that SmTK4 is a member of a kinase complex interacting with the Src-kinase SmTK3 and with the newly detected Src/Abl-kinase SmTK6 from *S. mansoni*. Furthermore, an interaction of these three cellular tyrosine kinases with the cytoplasmatic part of the schistosome receptor tyrosine kinase SmVKR (SmRTK1) was demonstrated. *In situ* hybridization confirmed colocalization of the cellular kinases and the receptor kinase in reproductive organs. These results lead to the postulation of a receptor tyrosine kinase-mediated signaling pathway in the ovary and testes, probably involved in cell proliferation and/or differentiation.

In addition to the yeast two-hybrid experiments we performed inhibitor and RNAi experiments to investigate the function of SmTK4 in the reproductive organs. Treatment of adult schistosomes with a Syk kinase-specific inhibitor showed clear phenotypes in the ovary and the testes by confocal laser scanning microscopy. These results corresponded to data obtained by an RNAi approach aiming to knock-down SmTK4 transcription. From the phenotypic analyses we conclude that SmTK4 plays a central role in spermatogenesis and oogenesis of *S. mansoni*.

These data strengthen the concept that specific tyrosine-kinase inhibitors may be suitable candidates for novel chemotherapeutic strategies to fight schistosomes.

### **A diverse family of Kunitz inhibitors from *Echinococcus granulosus* involved in host-parasite cross-talk in echinococcosis**

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The successful establishment of a parasite relies, to a great extent, on the efficiency of secreted molecules acting through high affinity interactions with host counterparts at the initial phases of infection. We present a molecular and functional description of a family of Kunitz inhibitors (whose prototype is the trypsin inhibitor from bovine pancreas), predicted to be secreted by *E. granulosus* larval worms (protoscoleces), and provide evidence supporting their participation in parasite establishment in the duodenum of its definitive dog host.

Kunitz inhibitors were found to predominate among protoscolex secreted proteins: eight such molecules (*EgKU*-1 to *EgKU*-8) were identified while surveying the larval transcriptome. The predicted mature peptides contain a single 'Kunitz domain': about 50 amino acids cross-linked by three conserved disulphide bonds. *EgKU*-1 – *EgKU*-8 differ in MW (7-10 kDa), pI (5-10) and anti-proteinase site. Consequently, they could show diverse specificities if acting as serine protease inhibitors. Importantly, their expression is induced by exposure to pepsin/H<sup>+</sup>, a stimulus the worms naturally encounter upon ingestion by the dog. In addition, *EgKU*-1/*EgKU*-4, *EgKU*-3/*EgKU*-8 and *EgKU*-6/*EgKU*-7 represent three pairs of close paralogues that would have arisen from recent gene duplications. Interestingly, putative orthologues of *EgKU*-3, *EgKU*-4 and *EgKU*-8; and of *EgKU*-2 were respectively found among *E. multilocularis* and *Taenia solium* ESTs. Furthermore, genes encoding all eight members of the family were identified in the *E. multilocularis* draft genome. These sequences indicate that the genes encoding the pairs of paralogues are closely located in the genome.

Detailed kinetic studies carried out with *EgKU*-1 and *EgKU*-8 purified to homogeneity from protoscolex lysates showed that *EgKU*-8 is a powerful slow tight-binding trypsin inhibitor. Remarkably, *EgKU*-8 displayed higher affinity towards dog trypsin than towards the bovine enzyme. In contrast, no inhibition of a panel of serine proteases was observed with *EgKU*-1. Interestingly, molecular modelling revealed a clear structural similarity between *EgKU*-1 and the snake venom dendrotoxins, which are Kunitz proteins that selectively block specific subtypes of voltage-gated K<sup>+</sup> channels. We are currently studying whether *EgKU*-1 is indeed capable of acting in a similar way. We are also functionally characterising other members of the family using the corresponding recombinant proteins. Recombinant *EgKU*-3, in particular, was found to be a potent inhibitor of chymotrypsins.

Finally, we are analysing the presence of Kunitz proteins in the secretions of protoscoleces exposed to different stimuli, using 'intensity fading' MALDI-TOF MS, a methodology elegantly combining affinity purification and MS. So far, this approach has confirmed the presence of *EgKU*-3 and *EgKU*-8 in pepsin/H<sup>+</sup>-treated protoscolex secretions.

This family of secreted cestode molecules would provide a striking example of protein evolution, similar to the one described in animal toxin multigene families, where natural selection has diversified the duplicated genes, allowing the emergence not only of paralogues whose products would interact with paralogous proteins (*i. e.* specific inhibitors of particular enzymes); but also of a new function associated with the same molecular scaffold. We discuss the significance of the family in terms of putative targets of the Kunitz inhibitors in the protoscolex establishment scenario.

### **TGF-beta and hedgehog signalling pathways in *Trichinella spiralis***

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The recent release of genome sequence information is revolutionising the study of helminth parasites by providing important datasets for comparative genomics that will allow us to analyse the signalling pathways that regulate nematode parasite development. Much of our current knowledge of nematode signalling pathways is based on the study of the free-living model *Caenorhabditis elegans*. While most are conserved (e.g TGF-beta pathway), a number of pathways are missing in *C. elegans*, e.g. the JAK/STAT signalling pathway, or are incomplete. Most strikingly, the hedgehog signalling pathway in *C. elegans* is modified and lacks a bone-fide Hedgehog ligand. Studies indicate that other chromadorean nematodes (e.g. *Brugia malayi*) are likely to be similar to *C. elegans*. The recent availability of the draft genome sequence of the enoplean *Trichinella spiralis* has presented an opportunity to study signalling pathways in a basal nematode. We have undertaken an analysis of the hedgehog and TGF-beta signalling pathways in *T. spiralis* and have identified a gene encoding a bone-fide hedgehog ligand. Data on the Hedgehog expression and localisation will be presented. In addition we have identified 5 genes encoding TGF-beta-like ligands, three of which belong to the BMP subfamily and are closely related to the vertebrate BMP2/4, BMP5/8 and BMP3 proteins. The remaining two proteins clearly belong to the TGF-beta/activin subfamily. To date only one member (DAF-7) of this subfamily has been identified in other nematode species, including *C. elegans* and there is some support for grouping one of the *Trichinella* proteins with the nematode Daf-7-like proteins. In contrast, the second protein shares significant amino acid identity with myostatin, a TGF-beta ligand involved in regulating muscle mass in adult vertebrates. The identification of a myostatin in *Trichinella* is unique among the nematodes to date and raises the possibility that it may play a role in altering gene expression in skeletal muscle cells post-infection. TGFbeta signaling in schistosomes

### **TGFbeta signaling in schistosomes**

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Schistosomes are complex metazoan pathogens that belong to an early diverging branch of the Bilateria, the Lophotrochozoans. Little is known of the molecular basis of cell to cell communication in animals in this group, but targeted studies and genome sequencing efforts have revealed that, not surprisingly, schistosomes contain some of the intercellular signaling systems that are found in higher order metazoa. Amongst these, we are particularly interested in the transforming growth factor beta (TGFbeta) pathways. Members of the TGFbeta superfamily can be split into two main subfamilies based on sequence homology and the different downstream pathways they activate, namely, the TGFbeta/activin/nodal subfamily and the BMP/GDF/MIS (bone morphogenetic protein/growth and differentiation factor/Muellerian inhibiting substance) subfamily. The basic TGFbeta signaling mechanism involves the binding of the extracellular TGFbeta homologue to a heterodimeric receptor complex resulting in the activation of specific cytoplasmic proteins (Smads) that translocate to the nucleus to influence transcriptional responses. Several components of the TGFbeta signaling pathway have been characterized from *S. mansoni* including TGFbeta receptors SmRK1 (also known as SmTbRI) and SmRKII (also known as SmTbRII); several Smad proteins, and one homologue of the TGFbeta subfamily, SmlnAct. Analysis of the Wellcome Trust's Sanger Institute's *S. mansoni* genome sequence database has revealed that schistosomes possess at least two additional Type 1 receptors, one additional Type 2 receptor and one homologue of the BMP subfamily (SmBMP). With the isolation of the BMP homologue from *S. mansoni*, we now have representatives from each major subfamily of the TGFbeta superfamily in this parasitic flatworm. The current state of the *S. mansoni* genome sequence suggests SmBMP and SmlnAct are the only two TGFbeta-like ligands present. The functions of SmlnAct and SmBMP, as deduced from studies using RNAi and specific inhibitors of TGFbeta signaling, will be discussed.

### **The nicotinic acetylcholine receptors of *Ascaris suum***

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Nematodes of the genus *Ascaris* are gastrointestinal parasites of medical and veterinary importance; human parasite *Ascaris lumbricoides* infects ~1 billion people globally, and the pig parasite *Ascaris suum* causes significant animal welfare problems and has economic implications for farmers. Nicotinic acetylcholine receptors are important targets of the anthelmintic drugs levamisole and pyrantel, and more recently, the AADs. The large size of *Ascaris* has allowed studies of nematode nAChR pharmacology to be carried out using *Ascaris* muscle. In contrast to this, most molecular studies of nematode nAChRs have been carried out using *C. elegans*, which has the largest known nAChR gene family.

Using a bioinformatics approach applied to the genomes of *Brugia malayi* and *Trichinella spiralis*, we have demonstrated that these parasites have very few nAChR genes (9 and 8 respectively). Certain genes encoding neuromuscular nAChR subunits are absent from these parasites (notably *lev-1*, *lev-8* and *acr-16*) although other proposed components of the levamisole-sensitive neuromuscular nAChR (*unc-38*, *unc-29* and *unc-63*) are remarkably well conserved throughout the Nematoda. Additionally, there is one subunit gene, *acr-26*, which is present in *B. malayi*, but is absent from *C. elegans* and other free-living nematode species examined.

We have cloned cDNAs encoding UNC-29, UNC-38, and UNC-63 (partial) from *A. suum* and raised antibodies against these subunits. *Ascaris* UNC-29, UNC-38 and UNC-63 are expressed on body-wall muscle cells, and UNC-29 and UNC-38 clearly co-localise. When co-expressed in *Xenopus* oocytes, *Ascaris* UNC-29 and UNC-38 form heteromeric channels gated by levamisole, acetylcholine and nicotine. Order of agonist potency is levamisole > acetylcholine > nicotine, which matches the described pharmacology of the native receptors present on *Ascaris* muscle cells.

### **Secreted proteins of *Trichinella spiralis* modulate purinergic signalling in immune cells**

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Nucleotides accumulate at sites of inflammation and act as endogenous danger signals to the immune system. Extracellular nucleotides ligate to purinergic receptors and are involved in activation/deactivation of many different immune responses. We have shown that *Trichinella spiralis* secrete enzymes which can metabolise extracellular nucleotides and thus have the potential to alter host purinergic signalling and the ensuing immunological response. Murine bone marrow-derived dendritic cells (mBMDC), mast cells of a mucosal phenotype (mBMMC) and platelets were used to investigate the effect of *T. spiralis* secreted proteins on nucleotide-induced effector functions.

We showed that ADP is responsible for inducing chemotaxis of both immature and mature mBMDC via activation of the P2Y<sub>12</sub> purinergic receptor. ADP-induced chemotaxis was inhibited by *T. spiralis* secreted proteins, and we identified the secreted 5'-nucleotidase as the enzyme responsible. Adenosine, generated by the action of this enzyme, activates P1 receptors and inhibits chemotaxis not only towards nucleotides but also towards MIP-1 $\alpha$  and MIP-3 $\beta$ .

Adenine nucleotides induced mBMMC exocytosis, resulting in the release of mouse mast cell protease-1 (mMCP-1) and  $\beta$ -hexosaminidase, and this was inhibited by *T. spiralis* secreted proteins. Extracellular nucleotides were unable to directly stimulate cytokine production in mBMMC, but stimulated inhibitory pathways that led to the down-regulation of LPS-induced TNF- $\alpha$ , a process that was again inhibited by *T. spiralis* enzymes.

It is well established that ADP causes platelet aggregation via activation of the P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors. *T. spiralis* inhibits ADP-induced platelet aggregation and P2Y receptor activation via the action of the secreted 5'-nucleotidase.

Overall, our data suggest that nucleotide-metabolising enzymes secreted by *T. spiralis* can counterbalance inflammatory stimuli and potentially downregulate inflammatory responses.

## Worm-Induced Modulation of Lung Immunity

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A number of important helminth parasites have incorporated a short-term residence in the lungs as an obligate phase of their life cycle. While the significance of the lung phase to parasite development is not clear, this short-term exposure is thought to have a lasting impact on the immunobiology of the lung. We have exploited the transient pulmonary phase of *Nippostrongylus brasiliensis* (Nb) to study the innate immune responses as well as the long-term consequences of this infection on the immunological status of the lungs. Nb induces a rapid and robust Th2-dominated innate response in the lungs resulting in eosinophil infiltration, goblet cell hyperplasia and alternate activation of the lung macrophage population. While eosinophil levels and goblet cell hyperplasia are transient, there are marked Nb-induced, T cell-dependent changes to the lung that persist long after the adult worms are expelled. Weeks after infection, Th2 and Th1 cytokines are constitutively elevated, lung macrophages are maintained as alternatively activated cells with distinct morphological, surface and functional phenotypes and there is a significant reduction in airway responsiveness and inflammation to subsequent allergen challenge. The importance of IL-4/IL-13-mediated signaling to the induction and maintenance of the altered lung environment was studied in STAT6<sup>-/-</sup> animals. In the absence of STAT6, Nb induces a robust Th17 response indicating that STAT6 signaling in the lungs is a key component of the mechanism that regulates Th17 immunity and pathology in the lungs. In addition, Nb infection of STAT6-deficient animals also reveals a complex pattern of regulation of Arg1, Fizz1 and Ym1 in the lungs. The results demonstrate that even a transient exposure to a helminth parasite can affect significant and protracted changes in the immunological environment of the lung and that these complex molecular and cellular changes are likely to play a role in modulating subsequent responses to antigen challenge.

## miRNAs in *Brugia malayi*

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MicroRNAs and other small RNAs play a major regulatory roll in eukaryotic cells. We are characterizing the endogenous small RNAs and the enzymes that generate them in *Brugia malayi*. We have identified Dicer, Drosha and RNA dependent RNA polymerase, which are involved in the cleavage and propagation of small RNAs. Comparison of the RNase III domains of Dicer and Drosha suggests that these enzymes have an ancient origin that predates the divergence of plants from animals. Though the processing enzymes, Drosha and Dicer, are conserved there is no evidence in *B. malayi* of a homologue for SID-1, which is the protein involved in spreading dsRNA between cells in *C. elegans*. This could have implications for the use of exogenous dsRNA for RNAi in filarial parasites.

MicroRNAs and other small RNAs in *B. malayi* were identified using both bioinformatics and small RNA cloning. Of the 30 miRNAs so far identified the majority have close homologues in *C. elegans*, however, three are not in miRBase and appear to be *B. malayi* specific. Two members of the miR 100 family identified in *B. malayi* are more closely related to the vertebrate and insect miR-100 families than they are to the related miRNA in *C. elegans*. One microRNA identified in *Brugia*, miR-153, has no homologues in protostomes, that includes nematodes and insects but is found in deuterostomes, that includes chordates. It is not known how this miRNA was acquired by *Brugia*. In addition to miRNAs we have also cloned small RNAs that are coded by the Pao retrotransposon family. These RNAs may be required to activate the RNAi pathway and prevent transposon hopping which would cause genomic instability. Deep sequencing of small RNAs from *Brugia* will allow a detailed comparison of the miRNAs in a free living nematode, *C. elegans*, to those of a filarial parasite. Characterization of the diversity of miRNAs is an essential step in identification of the target mRNAs that these small RNAs regulate.

### **Development of RNA interference in *Nippostrongylus brasiliensis***

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Exploitation of RNA interference (RNAi) has revolutionised work on *Caenorhabditis elegans*, and considerable success has been reported in the last few years with plant-parasitic nematodes. Somewhat surprisingly, it has proven difficult to transfer this technology to animal parasitic species, with a few reports of successful knockdown which appear to be gene specific. We are attempting to develop conditions for RNAi in the strongylid nematode *Nippostrongylus brasiliensis*. Although we initially reported suppressed expression of secreted acetylcholinesterase by soaking adult worms in double stranded RNA (dsRNA), RNAi should ideally be applied to larval stages in order to assess effects on infectivity and parasite development in vivo. Culture of *N. brasiliensis* on *E. coli* supports development from L1 to infective L3, however growth of larvae on *E. coli* HT115 expressing different target mRNAs gave no consistent reductions in transcript levels. Infective L3 do not feed or ingest dsRNA, but can be induced to do so by activation at 37°C in a serum-independent manner. The susceptibility of activated L3 to RNAi via soaking and electroporation with dsRNA and siRNA was nevertheless still observed to be irreproducible. Use of mRNA encoding firefly luciferase identified uptake into larvae or adult worms as a major impediment. Defects in RNA uptake and/or spreading in parasitic nematodes have been suggested by others, and are consistent with the absence of sequences homologous to *sid-1* and *sid-2* in *Haemonchus contortus* and *Brugia malayi*, two species for which close to complete genome sequence is available. Development of a delivery system will therefore most likely be required to yield robust methods for RNAi in several animal parasitic species.

### **RNA Metabolism and Molecular Adaptation in *Ascaris* Embryos**

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Embryos of the nematode *Ascaris* provide a powerful system for developmental and molecular studies. Unlike *C. elegans*, large amounts of synchronized populations of developing embryos can be obtained, enabling a variety of staged molecular and proteomic analyses. Molecular and biochemical tools developed in *Ascaris* embryos include in vitro RNA transcription, splicing, translation, and decay systems, as well as biolistic transfection of both DNA and RNA.

Using these *Ascaris* embryo tools, we have examined the functional contribution of spliced leader trans-splicing to mRNA translation and decay. Both in vitro and in vivo studies demonstrate that spliced leader trans-splicing (the addition of a new cap and 22 nt sequence to the 5' end of mRNAs) does not confer any measurable advantage to mRNA translation or stability when compared to non-trans-spliced mRNAs. The new trimethylguanosine (TMG) mRNA cap derived from trans-splicing alone does not efficiently support translation. Efficient translation of TMG capped mRNAs requires the accompanying spliced leader sequence added with the new cap to the 5' end of the mRNA. We have identified sequences within the spliced leader that are necessary and sufficient for efficient translation of mRNAs with the TMG cap. Interestingly, sequences required for the efficient translation of TMG capped mRNAs, also inhibit mRNA decapping, an initiating event in mRNA decay. Efforts are underway to mechanistically determine how and through what proteins the spliced leader sequence influences these post-transcriptional processes.

Many cap-interacting proteins in *Ascaris* have adapted to recognize and act on the atypical trans-spliced TMG cap present on the majority of nematode mRNAs. These cap-interacting proteins, such as the translation initiation factor eIF4E, are essential in nematodes, and as they have a unique ability to recognize the TMG cap compared to their mammalian hosts, are potential targets for rational drug design. Crystallographic and NMR approaches are underway to characterize the biochemical and biophysical basis for helminth eIF4E recognition of the TMG cap.

Finally, we have initiated small RNA discovery and profiling during early *Ascaris* development using "deep sequencing". These studies have identified novel *Ascaris* embryo miRNAs and other small RNAs that are not present in *C. elegans*. The expression profiles of these small RNAs have been characterized in fertilized embryos through L2 larvae. Several of these small RNAs are maternally contributed, whereas the transcription of others begins as early as the 1-2 cell stage, earlier than most nematode embryo transcription was thought to occur. Efforts are underway to characterize the transcription of these small RNAs, to identify the mRNA targets for these small RNAs, and to develop competent in vitro systems to examine mechanisms of mRNA repression that contribute to early nematode development.

### **Cellular energy metabolism and ageing in *Caenorhabditis elegans***

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The biogenesis and function of mitochondria, the energy-generating organelles in eukaryotic cells, are primary longevity determinants. However, the molecular mechanisms regulating mitochondrial energy metabolism during ageing are poorly understood. Prohibitins are ubiquitous, evolutionarily conserved proteins, which form a ring-like, high molecular weight complex at the inner membrane of mitochondria. Prohibitin function has been implicated in carcinogenesis and replicative senescence. We have found that the mitochondrial prohibitin complex promotes longevity and tumor growth by moderating fat metabolism and energy production in the nematode *Caenorhabditis elegans*. Prohibitin deficiency shortens the lifespan of otherwise wild type animals. In sharp contrast, knockdown of prohibitin promotes longevity under dietary restriction and in diapause mutants. In addition, prohibitin deficiency extends the lifespan of animals with compromised mitochondrial function or fat metabolism and restores normal lifespan in mutants with lethal germline tumors. Depletion of prohibitin influences ATP levels, adipose tissue fat content and mitochondrial proliferation in a genetic background and age-specific manner. Together, these findings reveal a novel mechanism of regulating mitochondrial energy metabolism and fat utilization during diapause and ageing.

### **Levamisole channel currents and resistance in adult *Caenorhabditis elegans* muscle**

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Nematode nicotinic acetylcholine receptors (nAChRs) are the site of action of the anthelmintic levamisole. We have used single-channel recording techniques to examine these receptors in the model nematode *C. elegans*. Although single-channel current recordings from these receptors have been possible using cultured embryonic muscle cells, *in situ* recordings from adult *C. elegans* have not yet been possible because the receptors are aggregated at the synapse and are inaccessible. In order to record channel currents *in situ* and to investigate the resistance at the channel level we exploited a *lev-10* mutant. LEV-10 is a receptor associated protein in *C. elegans* which is required for postsynaptic clustering of the levamisole-selective receptor but not the nicotine-selective receptor. Previous observations had suggested in *lev-10* mutants that the levamisole receptors distribute extra-synaptically. We tested this hypothesis by observing single-channel currents in extra-synaptic muscle membrane patches from wild type and *lev-10* mutants and found a population of 30 pS channels activated by acetylcholine and levamisole that increased in number in *lev-10* knockouts. We were then able to examine at the single-channel level, the *in vivo* effects of removing either the LEV-8 subunit or the LEV-1 subunit using the double mutants *lev-10;lev-8* and *lev-10;lev-1*. In *lev-10;lev-8* mutants the mean closed time of the channels increased (control 133ms, *lev10;lev-8* 410ms) without significant change in channel conductance or mean open-time. In *lev-10;lev-1* mutants the channel properties were unaltered but the number of functionally expressed channels was reduced. Our observations using the LEV-10 knockouts provide an explanation for levamisole resistance produced by LEV-8 and LEV-1 mutants at the single-channel level. They also allowed a direct comparison with levamisole activated channels from the parasitic nematode *Ascaris suum* which we have already described. The channels from *C. elegans* had shorter mean open-times, were not activated by nicotine, and did not have as big a conductance range. These differences may arise from differences in the subunit composition of the levamisole receptors between the two species.

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**MITR-1: a conserved novel nematode-specific protein required for mitochondrial respiration in *C. elegans***  
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Mutations affecting components of *C. elegans* mitochondria have a variety of effects on the worm, from increased susceptibility to oxidative stress and developmental defects, to the extension of life span. We have identified a novel, nematode-specific nuclear gene required for mitochondrial function in *C. elegans*. *mitr-1* (mitochondrial respiration defective) plays an essential role in mitochondrial respiration and energy production and has many of the hallmarks of the *clk* genes, including an increased life span following gene knockdown. *mitr-1*(RNAi) worms are small, slow growing, have small brood sizes and display a reduced rate of some rhythmic processes such as defecation and egg-laying, which manifests as a highly penetrant lethal egg-laying defect (Egl). Those worms that do not die from Egl have significantly extended life spans in a manner independent of the insulin-like/DAF-2 signalling pathway. MITR-1::GFP is localised to mitochondria throughout the worm and is expressed at all development stages. Biochemical analysis reveals that MITR-1 is required for mitochondrial respiration: *mitr-1*(RNAi) worms have low oxygen consumption rates and low free-ATP levels. Consistent with this, these worms display a heightened sensitivity to hypoxic stress. Silencing of *mitr-1* also results in expression of *hsp-6*::GFP, which is a reporter of mitochondrial stress and protein instability. In an attempt to better understand how MITR-1 contributes to mitochondrial respiration, we performed yeast two-hybrid screening to identify protein interactors of MITR-1. A putative physical interaction between MITR-1 and components of the E1 subunit of the pyruvate dehydrogenase complex (PDC) was identified and confirmed by GST pull-down experiments. This implicates MITR-1 at the site of a critical metabolic reaction: the production of acetyl CoA from pyruvate. Homologues of *mitr-1* have been identified in *C. briggsae*, *Haemonchus contortus* and *Brugia malayi*, which suggests that *mitr-1* might play a similar role in other nematodes, including parasites.

**A hypodermally expressed Haem peroxidase is critical for viability and cuticle formation in *C. elegans***  
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The nematode cuticle is a collagenous extracellular matrix that is repeatedly shed and re-synthesised throughout post-embryonic development via the moulting cycle. We study the enzymes involved in the synthesis, modification, processing and crosslinking of the cuticle components in the tractable model *C. elegans*. Using a combination of genetics and biochemistry we have identified an essential peroxidases called MLT-7 that in combination with the dual oxidase BLI-3 is responsible for cuticle cross-linking, maintenance of structural integrity and viability of *C. elegans*. Orthologues of these essential enzymes exist in the genomes of the human parasitic nematodes *Brugia malayi* and the sheep parasite, *Haemonchus contortus* We will discuss the individual and combined functions of these enzymes with respect to cuticle formation in nematodes.

### **Molecular mechanisms of drug resistance in *Haemonchus contortus***

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The nematode *Haemonchus contortus* is a gastro-intestinal parasite of ruminants. Infections are controlled primarily with three classes of anthelmintics: the benzimidazoles, imidazothiazoles, and macrocyclic lactones. However, the genetic versatility of *H. contortus* combined with the large numbers of produced offspring promote the emergence of drug resistance and recently, *H. contortus* field isolates were described that are cross-resistant to all current anthelmintics. Such mutants present an existential threat to sheep farms. We are investigating the molecular mechanisms of drug resistance in *H. contortus* in order to (i) devise tests for early recognition of resistance alleles in the gene pool of the parasites and (ii) learn more about the mode of action of novel anthelmintics. Of particular interest are the amino-acetonitrile derivatives (AAD), a promising class of compounds with strong activity against nematodes and good tolerability in mammals. Based on the molecular characterization of worms selected for AAD-resistance, the targets appear to be nicotinic acetylcholine receptors of the DEG-3 subfamily which only occurs in nematodes. This provides a possible explanation for the selective action of the AADs.

### **Anti-wolbachial chemotherapy of onchocerciasis – a macrofilaricide at last?**

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The treatment of human onchocerciasis has long been hampered by the fact that ivermectin, the only drug that is in use, is mainly microfilaricidal and only shows moderate sterilising activity on the level of female worms and practically no macrofilaricidal effect. Our group has earlier introduced the concept of chemotherapy by antibiotics that target the *Wolbachia* endosymbionts, with doxycycline as the best usable drug, but also rifampicin showing effects in animal models. While in lymphatic filariasis, we had previously demonstrated that doxycycline given for 4 or 6 weeks had a strong macrofilaricidal effect (> 90%) and also ameliorates lymphatic pathology, whereas in onchocerciasis the effect seemed to be a long-term sterilising only. However, in a placebo-controlled study, doxycycline for 4 or 6 weeks at a daily dose of 200 mg clearly demonstrated a macrofilaricidal effect of up to 70%. A further open study also showed a macrofilaricidal effect when doxycycline was given at the lower dose of 100 mg/d for 5 weeks. The reason for our inability to detect a macrofilaricidal effect of doxycycline in earlier studies seems to be that this effect occurs only after a comparatively long observation period (18-20 months after doxycycline).

The chemotherapy with doxycycline is currently limited to individual treatment, e.g. in patients presenting to outpatient clinics. However, first results from Cameroon suggest that provided there is sufficient training it is also possible to administer doxycycline within the activities of the so-called community-directed intervention (ComDT). In addition, new data from animal studies and humans suggest that combination with rifampicin may augment treatment efficacy or shorten total treatment time. We are collaborating in a large consortium to exploit the *Wolbachia* genome for the detection of new anti-*Wolbachia* drugs against filariasis using different new approaches such as bio-informatics and aptamer technology (SELEX).

**Filarial *Wolbachia* lipoprotein stimulates innate and adaptive inflammatory responses through TLR2 and TLR6 and induces disease manifestations of lymphatic filariasis and river blindness**

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The filarial nematodes that cause lymphatic filariasis and river blindness are host to a symbiotic bacterium, *Wolbachia*, which contributes to the inflammatory pathogenesis of filarial disease. Using siRNA knockdown of constitutive TLR1 or TLR6 expression in HEK-TLR2 cells and TLR1<sup>-/-</sup> and TLR6<sup>-/-</sup> mice we determined that extracts of *Brugia malayi* containing *Wolbachia* (BMFE), but not those derived from antibiotic treated nematodes (BMFEtet), induce inflammation through recognition by TLR2/TLR6 and to a marginal extent TLR2/TLR1 heterodimers. Removal of lipids or proteins from BMFE eliminated inflammatory activity. We used the TLR recognition profile to focus bioinformatic searches of wBm for putative lipoproteins. We identified the lipoprotein biosynthesis-encoding genes: *Ltg*, (prolipoprotein diacylglyceryl transferase) and *Lspa*, (lipoprotein signal peptidase II), but an absence of *Lnt*, (apolipoprotein aminoacyl transferase), the enzyme responsible for tri-acylation of the N-terminus amide group in most bacteria. Thus diacylation of *Wolbachia* lipoproteins occurs but further acylation is prevented through lack of *Lnt*, accounting for the predominant recognition by TLR2/6. Searches of three predictive lipoproteins databases revealed from 3-11 potential lipoproteins from wBm. Two proteins, peptidoglycan-associated lipoprotein (PAL) and VirB6, type IV secretion system protein were predicted by all three programs and *Wolbachia* PAL (WoLP1) was selected for further characterization. Antibodies raised to WoLP1 stained the surface of bacteria and detected a single 18kDa band in BMFE. Synthetic 20-mer peptides of the N-terminus of WoLP1, which were either diacylated or triacylated at the N-terminal cysteine residue, showed that the diacylated peptide had a near identical TLR2/TLR6 and TLR2/TLR1 receptor usage compared with native *Wolbachia*-containing BMFE. In contrast triacylated WoLP1 displayed no dependency on TLR6 and partial dose-dependency on TLR1. Injection of diacylated WoLP1 into mice resulted in systemic TNF $\alpha$  production and neutrophil mediated keratitis in a murine model of river blindness, which was significantly reduced in TLR6<sup>-/-</sup> but not TLR1<sup>-/-</sup> mice. BMFE and WoLP1 synergise with sub-inflammatory levels LPS and activate monocytes to induce up-regulation of podoplanin on lymphatic endothelial cells. WoLP molecules are also responsible for driving enhanced dendritic cell (DC) maturation and induction of DC cytokines, IL-12/IL-23 p40 monomer/monodimers, IL-12p70 and IL-23p40/p19 heterodimers and TNF $\alpha$ . DC primed with BMFE generated a mixed Th1/Th2 CD4+ T cell profile, whereas DC primed with BMFEtet polarised to only to a Th2 profile. Mice inoculated with BMFE generated IgG1 and IgG2c antibody responses. TLR2<sup>-/-</sup> or MyD88<sup>-/-</sup> mice generated similar levels of IgG1 but failed to produce IgG2c responses suggesting *Wolbachia* lipoproteins drive IFN $\gamma$  dependent antibody switching to filarial antigens. Together our results support a role for *Wolbachia* lipoproteins as the TLR2/TLR6 ligands associated with the activation of innate and adaptive inflammatory immune responses associated with the pathogenesis of filarial disease.

### **Atypical modulation of calcium currents by a schistosome calcium channel beta subunit**

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Voltage-gated  $\text{Ca}^{2+}$  ( $\text{Ca}_v$ ) channels are members of the voltage-gated ion channel superfamily that underlies electrical excitability in cells.  $\text{Ca}_v$  channels couple membrane depolarization to entry of extracellular  $\text{Ca}^{2+}$  and represent likely targets for new anthelmintics, as well as candidate targets for praziquantel, the current drug of choice against schistosomiasis.  $\text{Ca}_v$  channels consist of a main pore-forming and voltage-sensing  $\alpha_1$  subunit and auxiliary subunits such as  $\beta$  and  $\alpha_2/\delta$ . The function of  $\text{Ca}_v$  channels greatly depends on functional coupling to the cytoplasmic accessory  $\beta$  subunit, which promotes surface expression of the  $\alpha_1$  subunit, and also modulates several gating and kinetic parameters of  $\text{Ca}^{2+}$  currents. Thus, when co-expressed with  $\alpha_1$  subunits,  $\text{Ca}_v$  channel  $\beta$  subunits typically increase currents and modulate other biophysical aspects of the channel. We have previously shown that schistosomes and other platyhelminths express two  $\beta$  subunit subtypes: a conventional  $\beta$  subunit ( $\text{SmCa}_v\beta$ ) and a variant  $\beta$  subunit ( $\text{SmCa}_v\beta_{\text{var}}$ ) that has unusual functional properties and appears to be involved in the action of the antischistosomal drug praziquantel. We have recently been using whole-cell patch clamp to characterize the functional modulation of the conventional  $\text{SmCa}_v\beta$  subunit on the human  $\text{Ca}_v2.3$   $\alpha_1$  subunit stably expressed in HEK-293 cells.  $\text{SmCa}_v\beta$  dramatically increased  $\text{Ca}_v2.3$  currents, slowed macroscopic inactivation and shifted the steady state inactivation in the hyperpolarizing direction, effects similar to those found for mammalian  $\text{Ca}_v\beta$  subunits. Interestingly, currents produced by the  $\text{Ca}_v2.3/\text{SmCa}_v\beta$  complex run down to about 75% of their initial amplitudes within two minutes of establishing the whole-cell configuration, while currents produced by  $\text{Ca}_v2.3$  subunits alone or with mammalian  $\text{Ca}_v\beta_{2a}$  or schistosome  $\text{SmCa}_v\beta_{\text{var}}$  were stable over the same time frame. The kinetics of inactivation and steady-state properties were the same before and after rundown, indicating that rundown is not the result of the loss of interaction between  $\text{Ca}_v2.3$  and  $\text{SmCa}_v\beta$ . Recordings using  $\text{Ba}^{2+}$  as the charge carrier run down at the same rate, indicating that regulation of rundown does not distinguish between  $\text{Ca}^{2+}$  and other divalent cations. Interestingly, rundown does not occur in the absence of  $\text{Mg-ATP}$  or  $\text{Na}^+$ . Deletion analysis indicates that the molecular substrate for this  $\beta$  subunit-dependent rundown is within the highly acidic N-terminal domain of  $\text{SmCa}_v\beta$ , a domain not found in other  $\beta$  subunits. We are currently exploring in greater detail the cellular factors that regulate this unusual modulatory effect and the role of this atypical domain in signal transduction.

### **Identification of new drug leads targeting redox biochemistry for the control of schistosomiasis**

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Treatment for schistosomiasis depends almost exclusively on praziquantel. Tens of millions of people are treated annually with praziquantel and drug resistant parasites are likely to evolve. Phosphinic amides and oxadiazole 2-oxides identified from a quantitative high-throughput screen were shown to be inhibitors of a parasite enzyme, thioredoxin glutathione reductase (TGR), with activities in the low micromolar to low nanomolar range. Incubation of parasites with these compounds led to rapid inhibition of TGR activity and parasite death. The activity of the oxadiazole 2-oxides was associated with a donation of nitric oxide. Treatment of experimental schistosome infections with 4-phenyl-1,2,5-oxadiazole-3-carbonitrile-2-oxide led to dramatic reductions in worm burdens from treatments against multiple parasite stages. Highly significant reductions in egg-associated pathologies also resulted. In addition, this compound was active against the three major schistosome species infecting humans. These protective effects exhibited by this compound exceed benchmark activity criteria set by the WHO for lead compound development for schistosomiasis. We also discuss the development of these lead structures for potency and definition of mechanism.

### Probing the helminth host/parasite interface with phage-displayed antibodies

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Helminth parasites largely interact with hosts through their exposed surfaces, and these surfaces are the primary sites where the parasite must defend itself against immune recognition and effector action. It is also this surface that is the target of the few successful examples of metazoan parasite vaccines (e.g. *Boophilus microplus*, *Haemonchus contortus*, *Taenia ovis* and *Echinococcus granulosus*). Hosts of helminth parasites mount widely variable antibody responses against antigens exposed at the host/parasite interface, and these differences often strongly correlate with differences in the ability of the hosts to reject infection. We are using phage-displayed antibody methods to identify the surface antigens that are selectively recognized by antibodies from helminth resistant hosts. We hypothesize that exposed epitopes on antigens selectively recognized by resistant hosts are excellent targets of protective immunity. Using display methods, we found that mucosal antibodies from sheep that have been protectively immunized to gastrointestinal strongylid parasites by multiple truncated infections almost exclusively recognize only two antigens present on the L3 stage cuticle. One antigen, the glycan CarLA, displays remarkable intraspecific variation in which different anti-CarLA clonal Abs recognize different worm subsets, suggesting a novel immune evasion strategy possibly involving carbohydrate remodeling. The second antigen, the glycoprotein P6, turns over or sheds rapidly at the L3 surface in some strongyle species. Interestingly, P6 is the predominant hookworm L3 surface antigen recognized by dogs and hamsters successfully immunized by truncated infections or irradiated larvae. In schistosomiasis models, Fisher rats reject *S. mansoni* infections after about four weeks as compared to mice that are fully susceptible. The resistant rats mount a significantly more robust antibody response against the schistosome tegument than mice. We identified a panel of phage displayed antibodies from immune rats that recognize epitopes at the host-interactive surface of living schistosomes. The recombinant antibodies recognize both protein and non-protein antigens. In two cases, we identified the protein targets as Sm-TSP-2 and Sm29, which are among the small subset of schistosome proteins found to be exposed on the tegument by proteomic studies. We find that both antigens are selectively recognized by schistosome immune Fisher rats as compared to susceptible mice. Both proteins are also selectively recognized by a small subset of individuals in Brazil that appear potentially resistant to schistosome infection as compared to the general population. Identification and characterization of helminth host-interactive surface antigens selectively recognized by immune animals should lead to new insight into host/parasite interaction and could identify novel and promising vaccine antigens.

### Functional characterization of the schistosome surface

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The tegument of intravascular schistosomes is a syncytium that is bounded on the exterior by a double lipid bilayer. This surface constitutes a major site of interaction with the host. In recent years proteomic analysis has been used to catalog the protein composition of the tegumental surface bilayers. The precise functions played by many of the molecules found in the tegumental surface membranes, and how they avoid being targeted by effective host immunity, are largely unknown. We are using RNA interference (RNAi) to suppress the expression of genes whose products are found in the host-exposed tegumental membranes. In this manner, we have begun a functional characterization of the schistosome surface. For example, we have identified a cDNA potentially encoding a 304 amino acid protein (SmAQP1) that is expressed in the tegumental membranes and has sequence similarity to members of the aquaporin protein family. Treatment of schistosomes with short interfering RNAs (siRNAs) targeting SmAQP1 results in potent (>90%) gene suppression 48 hours later but leads to no overt phenotype in parasites cultured for up to 14 days in rich medium. However, when these parasites are placed in hypotonic solution they resist swelling, unlike their control counterparts which rapidly double in volume. These experiments reveal a heretofore unrecognized role of the tegument in controlling water movement into the parasites and thus in osmoregulation. In a second example, treatment of parasites with siRNAs targeting either the alpha or the beta subunit of schistosome tegumental NaKATPase (SNaK1) leads to decreased parasite viability *in vitro*. In addition, significantly fewer parasites (~10%) are recovered from mice infected with schistosomes whose SNaK1 alpha and beta genes have been suppressed by siRNA treatment compared with controls, and recovered parasites exhibit a stunted appearance. In agreement with the work of other groups on the proposed role of tegumental molecules in signal transduction, our preliminary evidence suggests that SNaK1 in the schistosome tegument can act to transduce signals in response to the endogenous steroid hormone, ouabain. Finally, we are examining the role of the tegument in immune modulation by silencing the expression of genes whose tegumental products we hypothesize can act to diminish host immune signaling through DAMPs (Danger Associated Molecular Patterns). These experiments are designed to generate a comprehensive understanding of the role of the schistosome tegumental surface in promoting parasite survival through immune modulation, signaling and by controlling the movement of metabolites into and out of the tegument.

### Hsp90 and the biology of parasitism

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The molecular architecture of Hsp90 is highly conserved throughout evolution. However, *C. elegans* Hsp90 is unique amongst eukaryotes because of its inability to bind to the Hsp90 inhibitor Geldanamycin (GA). In contrast Hsp90 from the parasitic nematode *Brugia pahangi* specifically binds to GA. We have investigated the GA-binding characteristics of Hsp90 from an additional 23 species of nematode. The results demonstrate that Hsp90 from both free-living and parasitic species belonging to Clade V are non-binding while Hsp90 from *T. spiralis* (Clade I) and from Clade III nematodes, including other filarial worms and ascarids, can bind to GA. Thus the life history of the species may determine whether or not Hsp90 binds to GA; species that have a free-living larval stage in the soil do not bind GA, while those species which are obligate parasites (*Trichinella* and the filarial worms), or which are enclosed within a protective egg shell while in the environment (Ascarids), possess an Hsp90 that has retained GA binding. Our results support the concept of the adaptive evolution of Hsp90. GA is synthesized by a soil-dwelling *Streptomyces* species and, as Hsp90 is essential in all eukaryotes, it would clearly be disadvantageous for worms sharing the same ecological niche to possess a GA-sensitive Hsp90. Current efforts are focused on understanding the molecular basis of GA sensitivity.

### **Transcriptomic analyses of schistosome biology: what we have learned and where we are headed.**

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Unquestionably, DNA microarrays have positively contributed to a variety of research programmes studying helminth and nematode organismal biology. Developing and utilizing these tools in our laboratory has allowed us to ask fundamental questions regarding schistosome conjugal biology and sexual maturation. Over the past several years we have elucidated a transcriptional basis underlying gender in adult *S. mansoni* and *S. japonicum* worms, have illustrated that adult worm gene expression in both sexes is dependent upon dioecious interactions and have demonstrated that strain-specific transcription can be detected in adult *S. japonicum* worms originating from different Chinese provinces. Collectively, these high-throughput transcriptional studies logically led to more hypothesis-directed investigations, which culminated in the characterization of new drug targets (schistosome tyrosinases) involved with the egg laying machinery and novel vaccine candidates (schistosome venom allergen like molecules) associated with cercarial penetration and schistosome development.

In addition to continuing these focused investigations on gene products identified from the study of adult schistosome conjugal biology, we have recently applied DNA microarrays to the specific analysis of cercariae dioecy. Interestingly, these studies have demonstrated that morphologically identical, but chromosomally distinct, cercariae do express divergent gene repertoires. Subsets of these differentially expressed genes are: **1)** characteristic of ones previously described in functionally-distinct dimorphic adults, **2)** likely instrumental in explaining male-biased sex ratios commonly observed during definitive host infection and **3)** possibly associated with processes involved in maintaining long-term female genomic stability. These results suggest that the short-lived cercarial life stage is not only transcriptionally active, but more importantly, is starting to establish gender-associated patterns of gene expression critical for labour division in the adult.

Finally, we will present our current transcriptional understanding of schistosome development as inferred from an intensive DNA microarray analysis of the parasite's lifecycle. We will demonstrate how this data is currently being linked to SchistoGeneDB and will discuss how future developments can dramatically improve community access. We will additionally discuss how interrogation of this lifecycle dataset is contributing to our continued efforts in identifying urgently needed novel drug and vaccine candidates.

### **Use of the *Caenorhabditis elegans* model system to identify the receptors in parasitic helminths modulating serotonin-stimulated paralysis**

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Almost all anthelmintics act as agonists to stimulate inhibitory inputs into locomotion and/or pharyngeal pumping. Similarly, exogenous serotonin (5-HT) and dopamine paralyze nematodes from all clades, suggesting that serotonergic/dopaminergic signaling includes potential targets for anthelmintic development. Using the well characterized *C. elegans* model system, we and others have identified 16 putative biogenic amine receptors in *C. elegans* and characterized their pharmacology and coupling after heterologous expression in a variety of systems. Using this approach, four *C. elegans* 5-HT G-protein coupled receptors and at least one 5-HT-gated channel have been identified. Surprisingly, null mutations in the genes encoding each of these receptors reduce 5-HT dependent paralysis, and *ser-4;mod-1* null animals are completely resistant. Based on *promoter:gfp* translational fusions, both *ser-4* and *mod-1* appear to be expressed in interneurons, and not in motoneurons or muscle, suggesting that 5-HT acts at the level of the animals decision to move and rather than its ability to move. Genes encoding 14 of these 16 putative biogenic amine receptors have been tentatively identified in the *Ascaris suum* genome. Based on work from *C. elegans*, we are currently heterologously expressing the *A. suum* and *Brugia malayi ser-4/mod-1* homologues, and attempting to rescue *C. elegans* null mutants with receptors from the parasites to confirm the predicted roles of these receptors. In addition, we are focusing on the identification of the signaling pathways and ion channels downstream from these receptors. Certainly, many differences between *C. elegans* and the parasites are to be anticipated, but *C. elegans* has proven to be a useful tool for identifying core signaling pathways. Indeed, this approach has been used to identify molecular targets for most anthelmintics and should have even increased utility in the future. This work is supported by NIH AI 0145147 and the Julius Jacobson Professorship in Biomedical Research Fund.

### **Innate immunity in *C. elegans***

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A substantial number of pathogens that can infect *C. elegans* have been described, including some that co-exist with the nematode in its natural environment. In this presentation, I shall describe the genetic and functional genomic methods being used to study the different innate immune responses of *C. elegans*, and present a summary of our current understanding of its defence mechanisms. I will focus on the main signalling pathways that have been identified and highlight the way in which certain molecular cassettes have both immune and developmental functions.

### **The mechanism by which mannose-binding lectin deficiency prevents parasite clearance and development of anti-parasite IgM**

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Mannose binding lectin (MBL) is a serum pattern recognition receptor of the collectin family, which forms part of our innate immunological defence. There are two types of mannose binding lectin in mice, MBL-A and MBL-C. Recently we showed the first clear phenotype for mice that lack MBL-A alone. MBL-A is necessary for clearance of *Brugia malayi* microfilariae (Mf) in mice (J.Immunol. 2007, 78, 5116-23). Intriguingly MBL-A<sup>-/-</sup> mice showed a distinct defect in Mf-specific IgM production. In addition our preliminary studies show that MBL-C deficiency also reduces clearance of microfilariae and decreases anti-Mf IgM responses. Antibody responses to T-dependent antigens and T-independent type I antigens are unaffected in the absence of either MBL-A or MBL-C. However both MBL-A and MBL-C deficient mice have reduced ability to produce anti-phosphorylcholine IgM and IgG3 antibodies. B cell populations that make natural IgM, for example B1 and MZB cells appear to be normal in these mice, although MBL<sup>-/-</sup> mice have significantly increased numbers of follicular B cells in the spleen and a decreased proportion of pre-B cells in the bone marrow. Because production of Ab by B cells even against T-independent Ag requires two signals, one via the Ig receptor and the second via cytokines or anti-apoptotic factors produced by Ag-activated accessory cells, we are also investigating the effect of MBL on activation of accessory cells. Recently human MBL has been shown to be necessary for trafficking of bacteria into the early phagosome of macrophages where it associates with TLR2 and enhances cytokine production. We will present data on the ability of dendritic cells from MBL deficient mice to mature and upregulate co-stimulatory molecules, and on antigen uptake and intracellular processing in the presence or absence of MBL-A and/or MBL-C.

### **Polyclonal and specific antibodies mediate protective immunity against enteric helminth infection.**

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Helminths represent an evolutionary ancient species that have co-evolved with, and possibly asserted evolutionary pressure upon, the mammalian immune system. The enteric helminth *Heligmosomoides polygyrus* (Hp) is a natural murine parasite that establishes a chronic infection in wildtype mice. Anti-helminth immunity has long been recognized to involve CD4 T cells, yet the precise effector mechanisms responsible for parasite killing or expulsion remain elusive. We now show an essential role for antibodies in mediating immunity against *H. polygyrus* infection. Polyclonal IgG antibodies, present in naïve mice and produced following *H. polygyrus* infection, functioned to limit egg production by adult parasites. Comparatively, affinity matured parasite-specific IgG and IgA antibodies were required to prevent adult worm development, although these only developed after multiple infections. These data reveal previously unrecognized complementary roles for polyclonal and affinity matured parasite-specific antibodies in preventing enteric helminth infection by limiting parasite fecundity and providing immune protection against re-infection, respectively.

### **The lung is a key site for protective immunity against gastrointestinal helminth parasites**

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The rodent helminth *Nippostrongylus brasiliensis* (Nb) infects its host by penetrating the skin migrating to the lungs with eventual colonisation of the gut via tracheal/esophageal migration. Once in the gut the L5 stage worms matures and produces eggs, prior to expulsion at day 10 after infection. Mice appear to be protected from a re-infection exhibiting a 95% reduction in gut worm burden and 75% reduction in worm burden in lung tissue. Using *in vitro* and *in vivo* approaches, we demonstrate that this immune protection does not occur at the skin site. Furthermore, priming of the immune response in the gut is not sufficient to confer protection in the lung despite generating a locally protective response. Most importantly we show that the lung is the key site for protective immunity and using mice that are deficient in key immune components, we show that the requirements for protection in the lung and gut site are different. Thus, requirements for protection from reinfection with Nb are dependent on lung priming, as well as polarity of the cellular response. The importance of these findings for vaccination strategies targeting helminth parasites will be discussed.

### **S. mansoni egg excretory/secretory proteins – major modulators of the host's immune response**

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Schistosomiasis pathology mainly results from the immune response to the egg stage of the parasite. Schistosome eggs have a dual effect on the host's immune system: they are triggering a focal inflammation necessary for their excretion that goes along with a Th2 response and later-on they are causing a down-modulation of inflammation. The fact that Th2 induction occurs rapidly after egg deposition suggests that excretory/secretory rather than structural egg components are responsible for this effect. We have systematically investigated the three major immunogens secreted from *Schistosoma mansoni* eggs, namely omega-1, IPSE/alpha-1 and k-5. Omega-1, a hepatotoxic T2 RNase, was found to be an IL-4-independent Th2 initiation factor both *in vitro* and *in vivo*, while IPSE/alpha-1, an immunoglobulin-binding factor, seems to amplify the Th2 response by triggering IL-4 and IL-13 production from human and murine basophils. It is known from the work of several groups that animals deficient in IL-4 or IL-4 receptor alpha chain are dying early of fatal inflammation suggesting a role of these cytokines in the regulation of inflammation. Although the functional role of k-5 has still to be elucidated, this molecule turned out to be a major schistosome "allergen", which, in contrast to omega-1 and IPSE/alpha-1, elicits a nearly exclusive IgE antibody isotype response in schistosome-infected human individuals. The further characterisation of the functional properties and the mutual interaction of the three major secreted schistosome egg immunogens should improve our knowledge about the immunomodulatory strategies of *S. mansoni*.

### **Gastrointestinal nematode infection inhibits experimental allergic airway inflammation but not atopic dermatitis**

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Over the last years helminth infections have been associated with the prevalence of allergic disorders depending on the worm species, intensity and phase of infection, and the type of disease. The aim of this study was to analyse the influence of a gastrointestinal nematode infection, *Heligmosomoides polygyrus*, in two distinct murine models of allergic airway disease and atopic dermatitis, respectively. Mice were infected with *H. polygyrus* and then systemically sensitized with the model allergen ovalbumin. Subsequently, the animals were challenged with allergen either via the airways for induction of airway disease or via skin patches for induction of dermatitis. Mice concomitantly infected with *H. polygyrus* showed reduced airway hyperreactivity, diminished eosinophil recruitment into the lungs, and decreased allergen-specific IgE levels when compared to sensitized and airway challenged controls. In contrast, no significant differences in phenotypical signs of dermatitis, such as severity of eczematous skin lesions, were observed between infected and control animals in the atopic dermatitis model. Although *H. polygyrus* reduced CD8<sup>+</sup> and CD4<sup>+</sup> T cell infiltration into the skin and production of allergen-specific IgE, mast cell recruitment was significantly increased in worm-infected mice in the dermatitis model. The worm infection was associated with significantly elevated numbers of Foxp3<sup>+</sup> regulatory T cells in peribronchial lymph node cells in *H. polygyrus*-infected sensitized and airway challenged mice, whereas Treg cells were basically absent in eczematous skin and not upregulated in skin-draining lymph node cells in mice with experimental dermatitis. These data indicate that infection with gastrointestinal nematodes leads to significant inhibition in mucosa-associated but not cutaneous allergic reactions and this might be an important aspect for future considerations of helminths for treatment of allergic diseases.

### Induction and maintenance of effector and regulatory T cell responses during filarial infection

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Human helminth infections are synonymous with suppression of the host immunity resulting in parasite survival and the maintenance of chronic infections. Using a murine model of filariasis, *Litomosoides sigmodontis* infection of susceptible (BALB/c) and resistant (C57BL/6) mice, we have shown that T cell regulation occurs at two levels; through a CD4<sup>+</sup>Foxp3<sup>+</sup> regulatory T (Treg) cell response and the development of CD4<sup>+</sup> effector T (Teff) cell hypo-responsiveness. The CD4<sup>+</sup>Foxp3<sup>+</sup> Treg cell response is initiated by the infective L3 stage rapidly upon contact with the host, with increased CD4<sup>+</sup>Foxp3<sup>+</sup>CD25<sup>+</sup> Treg cell proliferation *in vivo* resulting in a dominant expansion of CD4<sup>+</sup>Foxp3<sup>+</sup>CD25<sup>+</sup> T cells. Depletion of CD25<sup>+</sup> Treg cells prior to infection enhances parasite clearance indicating that the Treg cell response inhibits protective immunity and is mainly recruited from the pre-existing pool of natural CD4<sup>+</sup>Foxp3<sup>+</sup> Treg cells. The second level of T cell regulation develops as infection establishes and the CD4<sup>+</sup> Teff become intrinsically hypo-responsive to antigenic stimulation. This is associated with enhanced expression of CTLA-4, GITR, and PD-1. Once established, infection-induced suppression can be overcome by depleting CD25<sup>+</sup> Tregs, but only if combined with restoring Teff cell responses by providing co-stimulation through GITR, or blocking co-inhibition through CTLA-4. As yet, it is not known what factors drive the initial bias towards a Treg response or the later Teff cell hypo-responsiveness. Our hypothesis is that the balance of co-stimulatory/ inhibitory signals during T cell priming and maintenance determines whether regulatory or effector responses prevail, with a lack of co-stimulation or a bias towards co-inhibition resulting in immune suppression. Initial work shows an important role for GITR in Th2 cell priming as blocking GITRL in resistant C57BL/6 mice ablates the Ag-specific Th2 response (IL-4, IL-13) and results in a Th1 phenotype (increased IFN- $\gamma$ ). Additionally, co-stimulating susceptible BALB/c mice with an agonistic anti-GITR mAb enhances their Ag-specific Th2 response. We are currently investigating whether a bias towards co-inhibition favors a regulatory environment using blocking antibodies against PD-1 and its ligands. To further delineate Th2 responses following infection and treatments, as well as interactions between Treg and Teff cells, we are using BALB/c 4get IL-4gfp mice to track and quantify Th2 cells. Overall we believe that the initial T cell priming to filarial helminths is critical in determining whether the host will succumb to or resist parasite immunomodulation. Co-stimulatory/inhibitory signals play a role in the development of T cell responses against *L. sigmodontis* and therapeutic manipulation of these pathways could be used to enhance immune priming and restore protective immunity.

### **Murine filarial infection induces protection against malaria and inhibits allergic asthma**

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Chronic helminth infections such as filariasis in human hosts can be life long, since the parasites are equipped with a repertoire of immune evasion strategies. It is likely that the infection may modify immune reactions to other pathogens or allergens. However, mechanisms of such interactions are poorly understood. Here we show that infection of mice with the rodent filaria *Litomosoides sigmodontis* leads to protection against a subsequent *P. berghei* infection as well as inhibition of asthma induction upon allergenic challenge with ovalbumin.

Filaria infected C57BL/6 mice did not develop symptoms of cerebral malaria (CM) upon subsequent infection with *P. berghei*, nor did they display weight loss and splenomegaly. These mice survived this otherwise deadly inflammation induced by the malaria parasite. CD8<sup>+</sup>-T cells, the major effector cell type responsible for CM induction, were reduced in the brain and spleen of co-infected mice. This was essentially dependent on IL-10, as *L. sigmodontis* led to an increase of plasma IL-10 levels and protection from CM was abrogated in IL-10 deficient mice.

Infection with *L. sigmodontis* prior to allergic sensitisation with ova reduced all aspects of the asthmatic phenotype: antigen specific Ig production, pulmonary eosinophilia as well as airway reactivity to inhaled metacholine. In addition, recall proliferation and Th2 cytokine production was significantly reduced in *L. sigmodontis*-infected and allergen-sensitized mice. This was associated with an increase in splenic TGF- $\beta$ -producing CD4<sup>+</sup>-T cells and mononuclear cells. Administration of antibodies blocking TGF- $\beta$  or depleting regulatory T cells in infected animals before allergen sensitization and challenge reversed the suppressive effect with regard to airway hyperreactivity.

These data point toward an induction of regulatory mechanisms as an important mechanism of *L. sigmodontis* mediated immunosuppression. Filarial parasites are therefore able to modify the immunological balance to other diseases.

### **Secreted products from *H. polygyrus* which induce regulatory T cells**

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Several helminth infections have now been associated with expansion of Regulatory T cell populations, either numerically or in terms of heightened immunosuppressive function. One of these, *Heligmosomoides polygyrus*, establishes a long-term (>2 months) chronic infection in the intestine of mice. We had previously shown that Tregs from infected mice are able to suppress airway allergy to an unrelated antigen, and indeed could confer protection from allergy on transfer to an uninfected recipient. Because Treg expansion is associated with live parasites, we investigated whether molecules secreted by adult worms when maintained in serum-free medium in vitro would be able to stimulate Treg activity. Purified secreted proteins, termed HES (*H. polygyrus* Excretory-Secretory products) were found to increase the proportion of CD4<sup>+</sup> T cells expressing Foxp3 (the regulatory T cell master transcription factor) substantially increased in vitro in the presence of HES. This expansion was dependent on co-incident TCR ligation (with Con A or anti-CD3), did not require APC, and was lost on heat treatment of HES. Importantly, by adding HES to GFP-ve CD4<sup>+</sup> T cells from a GFP-Foxp3 reporter mouse, it was shown that de novo induction of Foxp3 by naive non-regulatory precursors occurred. To dissect the molecular pathway of Treg induction, we compared HES with mammalian TGF- $\beta$ , a known stimulant of Foxp3 expression. HES was found to act in a highly similar manner to TGF- $\beta$ , failing to induce Foxp3 gene expression in T cells carrying a dominant negative TGF- $\beta$  receptor, or in cultures containing a specific inhibitor of TGF- $\beta$  signalling. Importantly, monoclonal antibody to mammalian TGF- $\beta$  did not interfere with the ability of HES to drive Foxp3, while abolishing all activity of TGF- $\beta$  itself. Thus HES contains a functional homologue of TGF- $\beta$  able to ligate and signal through the TGF- $\beta$  receptor. To appraise the importance of TGF- $\beta$  during infection in vivo, we found that while monoclonal antibodies to host TGF- $\beta$  did not affect parasite worm loads, the signalling inhibitor (which blocks downstream effects of both host and parasite ligands) was able to significantly reduce parasite numbers. Hence, this parasite has evolved to manipulate the host pathway of Treg induction by TGF- $\beta$ , in order to accentuate Treg activity which is now implicated in the persistence of many chronic helminth parasite infections.

### **Proteomics and vaccine candidate discovery – the old and the new**

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We are attempting to identify effective antigens for vaccine development against major gastrointestinal nematode infections of livestock. Pioneering work in the 1980s (Ed Munn, U.K) led to the discovery of contortin, a helical polymeric protein found between the microvilli of intestinal cells of the sheep blood-feeding nematode *Haemonchus contortus*. The protein could be partially purified by differential centrifugation and was a highly effective immunogen against *Haemonchus* in lambs. The partially purified protein contained the highly protective H11 antigen and work has focused on the latter. However, the nature of major protein in contortin was undefined and its protective properties never tested in a pure form. Here, we have used proteomic and follow-up enzymological techniques to show that this is a prolyl-carboxypeptidase with potent anticoagulant potential, being able to degrade the C-terminal end of the alpha-fibrinogen chain extremely rapidly and thus disrupt clot formation.

In addition, we have used proteomics to identify larval excreted/secreted antigens recognized by efferent lymph from lambs infected with, and immune to, another sheep abomasal nematode, *Teladorsagia circumcincta*, in this case not an obligate blood feeder. Data from these studies will be presented.

### **The immunopotentiating properties of the *Onchocerca volvulus* recombinant Ov-ASP-1 protein**

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Protozoan and helminth parasites have proven to be a source of molecules which have potent regulatory and, sometimes, stimulatory effects on the immune systems of their hosts. We have identified a naturally occurring protein with intrinsic immunostimulatory properties from *Onchocerca volvulus*. In preliminary studies, we found that the LPS-free recombinant Ov-ASP-1 protein (rOv-ASP-1) is highly immunogenic in mice without an adjuvant and highly immunostimulatory (Th1 biased) for naïve human PBMCs *in vitro*. We consequently explored its ability to function as an adjuvant for bystander antigens and whether it could augment cellular responses against human pathogen antigens *in vitro*. Using several antigen vaccination models we found that rOv-ASP-1 can stimulate both antibody and Th1 responses that exceed those generated against the antigens in the presence of Alum or MPL+TDM adjuvants. Moreover, rOv-ASP-1 improved the immune efficacy, including IgG1 and IgG2a isotype responses, of commercial inactivated vaccines when used as a single vaccine or in a combination of three vaccines with reduced doses. *In vitro*, rOv-ASP-1 was able not only to induce the secretion of IFN- $\gamma$  from naïve PBMC but also to enhance IFN- $\gamma$  recall responses to tetanus toxoid (77.3%) and HCV core (88.9%) antigens of normal healthy donor and of chronic hepatitis C virus infected patient PBMCs, respectively. The recall response appeared to be dependent on contact between CD56+ and CD56- fractions of the PBMCs. Our most recent data establish that rOv-ASP-1 immunopotentiating activities are mediated by its ability to activate APCs such as DCs and B cells. The rOv-ASP-1 can induce phenotypic maturation of human monocyte-derived DCs as well as of purified murine B cells (>99% B220+). In addition, rOv-ASP-1 induced the secretion of Th1/immunostimulatory cytokines and that of IL-10. Moreover, rOv-ASP-1-primed normal human DCs were able to induce IFN- $\gamma$  secretion from naïve autologous PBMCs, while rOv-ASP-1-primed murine B cells normal were able to induce IFN- $\gamma$  secretion from naïve autologous murine T cells. Significantly, the rOv-ASP-1-induced IFN- $\gamma$  secretion from human PBMCs could be inhibited by antibodies against human TLR2 and TLR4. Using TLR-transfected HEK293 cells, we further demonstrated that rOv-ASP-1 was able to trigger cellular activation via TLR2 and TLR4 signaling.

The rOv-ASP-1 is therefore a new addition to a number of microbial products thought to function as effective innate adjuvants due to their effects on APCs, which in turn can boost the activation of an adaptive immune response. We believe that rOv-ASP-1 offers new advantages for vaccine development and possible therapeutic applications against well-defined pathogen antigens because of its biased Th1 immunostimulatory properties.

**The immunomodulatory properties of hOv-FAR 1; the fatty acid and retinol binding protein of *Onchocerca volvulus***

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Ov-FAR-1 (formerly known as Ov20) is a nematode-specific, structurally novel small helix-rich secreted protein with the capacity to bind fatty acid and retinol (Vitamin A), produced by the filarial parasite *Onchocerca volvulus*. Homologues have been described in other parasitic and free living nematodes and many have been shown to also bind retinol. Retinoids affect dendritic cell viability, maturation and cytokine secretion and retinoic acid induce growth and differentiation of T regulatory cell and elicitation of gut-homing T and B cells. We therefore have investigated if FAR proteins may have immunomodulatory properties and if these could due to their capacity to interact with retinol. Ov-FAR1 induced upregulation of maturation markers and cytokine production in mouse bone marrow-derived dendritic cells. The increased production of IL-10 and reduced production of IL-12 by these cells, compared to LPS-treated DC, suggest that Ov-FAR-1 might trigger an anti-inflammatory maturation program on DC. We are currently investigating the phenotype of co-cultured transgenic T-cells and evaluating the effects of RXR/RAR antagonists in this system.

**Human sensitization and desensitization to allergen-like proteins from *Schistosoma mansoni* depends on their expression patterns through the parasite life-cycle**

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IgE is central to allergic disease processes, but must have evolved as a defence against parasitic worms. Environmental and food allergens are found in a relatively few protein families and this is also true of IgE target antigens in *Schistosoma mansoni*. The principle human IgE target antigen in *S. mansoni* is the tegumental protein Sm22.6 that, because it contains a [EF-hand]<sub>2</sub> domain, has structural similarities with major pollen and food allergens. Human IgE directed against tegument protein Sm22.6 is associated with age-dependent immunity to *S. mansoni* that is observed in adults, but not younger children, living schistosomiasis endemic areas. We now know that Sm22.6 (SmTAL1) is a member of a gene family, the tegument-allergen-like (SmTAL) proteins, a group of highly similar molecules that have different life-cycle expression patterns. SmTAL5 is only present in infectious larvae, while SmTAL1 is an internal antigen of adult worms and is only released occasionally when long-lived worms die in the blood stream. SmTAL2, which is expressed in both the adult worm and the parasite's egg, is continuously released from eggs trapped in the host's tissues. We have expressed recombinant SmTAL1, 2, 4, 5 and 8 and have examined antibody isotype responses to these allergen-like proteins in both infected mice and in human populations living in schistosomiasis endemic areas. This clearly shows that these contrasting life-cycle expression patterns result in differential up- and down-regulate of IgE to different TAL antigens during chronic infection. These regulatory events provide a possible explanation for the patterns of age-dependent susceptibility and immunity to the schistosomiasis observed in human populations living in endemic areas, while future studies could also inform rational improvements to empirically derived allergen-desensitisation therapies.

**Apical membrane proteins as recombinant vaccines against *Schistosoma mansoni* – from men to mice then back to men**

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Vaccines against schistosomes, like other unicellular and multicellular parasites of humans, are proving elusive. However, much of the genome sequence is now publicly available, and the major proteins exposed on the surface of live adult worms have been revealed using proteomics. We believe that researchers are now armed with the most efficacious antigens on which to base a schistosome vaccine, and the major hurdles now facing the field are how best to synthesize, formulate and deliver such a vaccine. Our approach has been to focus exclusively on apical membrane proteins that are accessible to biotinylation on live worms, and therefore presumably accessible to antibodies *in vivo*. Using ultra-sensitive mass spectrometry methods, we have shown that the number of biotinylated apical membrane proteins on the adult worm surface is larger than initially described by others. We have, however, chosen to restrict our initial vaccine development efforts to the most abundant tegument membrane proteins, including the tetraspansins (TSP-2, -3 and -4) and Sm29, and chimeric fusions of these antigens. Data will be presented on the pre-clinical development of these vaccines, including human immuno-epidemiological studies, murine challenge trials, and the progression towards phase I clinical trials in Brazil for the *Sm*-TSP-2 Schistosomiasis Vaccine.

# ABSTRACTS FOR POSTER PRESENTATIONS

## SESSION 1

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PLOS Neglected Diseases has sponsored prizes for the two Poster Sessions



## Chimeric nematodes as a new platform for anthelmintic drug screening

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Parasitic nematodes pose a significant human health threat worldwide. Anthelmintic drugs can effectively treat nematode infections, but existing therapies have appreciable toxicity and drug –resistant strains are emerging rapidly. For these reasons, development of new anthelmintics is a critical priority in infectious disease research. A major problem is that parasitic nematodes cannot be cultured in the laboratory, so it is difficult to screen candidate compounds for anthelmintic properties directly.

Genome sequencing of the parasitic nematodes *Ascaris suum* and *Brugia malayi* provides important information to advance anthelmintic drug development. Potential target genes from these parasites can now be cloned and expressed in recombinant systems for high-throughput screening of chemical libraries. Unfortunately, existing recombinant systems usually fail to preserve the native organismal context of the target protein being expressed. For example, anthelmintics often activate neurotransmitter receptors at synapses, but synapses do not form in recombinant expression systems. Furthermore, post-translational modifications, protein-protein interactions, and intracellular regulation will not be the same, so the target protein may function quite differently in the recombinant system compared to its native environment. These factors compromise the ability of high throughput screens identify potent anthelmintics.

My laboratory is presently developing an alternative approach, using the free-living model nematode *Caenorhabditis elegans* as an expression system for parasitic nematode drug targets. *C. elegans* should provide an expression milieu that better reproduces the native functional context of a parasite nematode target protein. Because it is easy to culture and study in the laboratory, *C. elegans* can be used to screen for drug sensitivity with moderate to high efficiency. The endogenous *C. elegans* homolog of the parasite target, which may have substantially different pharmacology, can be eliminated by gene-knockout techniques so it does not interfere. It should also be possible to make chimeras containing multiple parasite genes, and cassettes of functionally-related genes may also be transferred, to reconstruct entire pathways from parasites in *C. elegans*. As proof of principle, we are working toward expressing the *Ascaris suum* body wall muscle GABA receptor in *C. elegans*. This receptor is the target of the anthelmintic piperazine. We have determined that piperazine also targets the GABA receptor encoded by the *unc-49* gene in *C. elegans*, and we have identified *unc-49* homologs in the genomes of *Ascaris* and *Brugia malayi*. We are presently cloning the *Ascaris unc-49* homolog and will express it in *C. elegans* mutants lacking endogenous *unc-49* to generate a chimeric *C. elegans* strain for use in anthelmintic screens. By making parasite-*C. elegans* chimeras in this way, a high-fidelity representation of parasitic nematode biology can be reproduced in an easy-to-screen platform.

**Type 1 hypersensitivity reactions in a phase 1 trial of the *Na*-ASP-2 hookworm vaccine in previously-infected Brazilian adults**

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*Necator americanus* secretory protein-2 (*Na*-ASP-2) is secreted by hookworm larvae upon entry into the host and is thought to play a role in parasite development. Vaccination of laboratory animals with *Na*-ASP-2 reduces hookworm infection and antibodies to ASP-2 in naturally-infected humans are associated with reduced worm load. Recombinant *Na*-ASP-2 was expressed in *Pichia pastoris* and formulated with Alhydrogel. After no significant vaccine-related adverse events were seen in a Phase 1 study of the vaccine in hookworm unexposed adults living in the US, a double-blind, dose-escalation Phase 1 study was planned in which 48 healthy Brazilian adults who had been treated for hookworm infection within 3 months of study entry were to be enrolled into one of three dose cohorts and randomized to receive either *Na*-ASP-2 Hookworm Vaccine or recombinant hepatitis B vaccine. In June 2007, vaccinations were initiated for the first cohort, in which participants were randomized to receive either 10 µg *Na*-ASP-2 or hepatitis B vaccine. 9 participants were vaccinated on the first day of the study. Within 2 hours of vaccination, 3 of 7 volunteers injected with *Na*-ASP-2 developed generalized urticaria. The reactions were not associated with signs of anaphylaxis, bronchoconstriction or angioedema, and all responded to treatment with an antihistamine. Anti-*Na*-ASP-2 IgE antibody responses were measured by ELISA on sera collected pre-vaccination and revealed that the individuals who developed urticaria all had elevated levels of antigen-specific IgE compared to those who didn't develop urticaria. Other than the allergic reactions, the most common adverse events were mild-to-moderate injection site pain and swelling. The data so far demonstrate that the *Na*-ASP-2 vaccine can induce immediate-type hypersensitivity reactions in previously infected adults. Vaccinations with the *Na*-ASP-2 vaccine are currently on hold and further clinical testing of this vaccine will require development of assays to detect pre-existing anti-*Na*-ASP-2 IgE before vaccination so as to prevent hypersensitivity reactions.

## Immune recognition of *Schistosoma mansoni* venom allergen-like (*Sm*-VAL) proteins in infected humans and identification of novel VAL homologs across the Platyhelminthes

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Members of the Sperm-coating protein/Tpx-1/Ag5/PR-1/Sc7 (SCP/TAPS) superfamily have been identified in several important parasitic nematode species such as *Necator americanus* (*Na*-ASP-2), *Brugia malayi* (*Bm*-VAL-1) and *Onchocerca volvulus* (*Ov*-ASP-1). Importantly, characterization has shown several of these SCP/TAPS superfamily members to have roles in immunomodulation (*Ov*-ASP-1 & *Ac*-NIF), the establishment of infection (*Na*-ASP-2) and the induction of host antibody responses (*Ss*-NIE).

In the trematode parasite *Schistosoma mansoni*, previous work in our group has led to the identification of 28 SCP/TAPS family members named *Schistosoma mansoni* venom allergen-like (*Sm*VAL1-28). Phylogenetic, genomic and protein structure analyses provided strong evidence for the division of the *Sm*VAL family into two distinct subfamilies; the group 1 proteins which are likely to be secreted/excreted from the parasite and the group 2 proteins which are likely to function intracellularly. Quantitative lifecycle transcription profiling demonstrated several highly restricted *Sm*VAL expression patterns, including transcripts specifically associated with lifestages involved in definitive host invasion (*Sm*VAL1, *Sm*VAL4 & *Sm*VAL10) and transcripts restricted to lifestages involved in the invasion of the intermediate host (*Sm*VAL2, *Sm*VAL3, *Sm*VAL5 & *Sm*VAL9). These expression patterns and supporting proteomic data suggest that the *Sm*-VAL proteins may be important in the establishment of *S. mansoni* infections as well as in the developmental biology of the parasite. Therefore, our current research is focused on discovering the function(s) of the *Sm*-VALs during schistosome lifecycle progression and determining whether the host mounts an immune response against members of this protein family.

During our investigation of the *Sm*VALs, we additionally examined the diversity of SCP/TAPS family members across the superkingdom Eukaryota. Here, we present data on the identification, protein features and evolutionary relationships of the VAL homologs within the phylum Platyhelminthes. These analyses highlight the existence of both group 1 and group 2 members in a range of parasitic and non-parasitic species. Within the schistosomes, highly conserved *Sm*VAL orthologs exist in both *S. japonicum* and *S. haematobium*, suggesting a common function. However, our analysis also suggests that species-specific homologs exist within the genera, signifying that some VALs may have evolved to perform additional roles in host/parasite interactions or developmental biology, particular to the specialized lifestyle exhibited by each species.

Finally, we report on the recombinant expression of selected *Sm*-VAL family members using both *Spodoptera frugiperda* (*Sm*-VAL-4 & *Sm*-VAL-6) and *Pichia pastoris* systems (*Sm*-VAL-1). Serological studies using these recombinant proteins provide evidence for specific IgE responses against *Sm*-VAL proteins in *S. mansoni* infected individuals. Current studies aim to extend this research by screening a larger cohort of infected human populations from endemic areas surrounding Lake Albert, Uganda and understanding the significance of this immune recognition.

### **Purine and pyrimidine metabolism in *Brugia malayi***

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Lymphatic filariasis, caused by parasitic filarial nematodes, *Brugia malayi* and *Wuchereria bancrofti* afflicts around 150 million people in 80 countries with more than a billion at risk of infection. Nucleotide metabolism represents an attractive drug target in these pathogens as purine and pyrimidine nucleotides are essential precursors of DNA and RNA and serve as an important energy source. The recent availability of genome sequence data from *B. malayi* and its obligate endosymbiotic bacteria, *Wolbachia* has allowed us to construct a model of the nucleotide metabolic pathways in *B. malayi* by integrating genomic and biochemical information. This analysis indicates that *B. malayi* lacks the complete *de novo* purine biosynthesis machinery and presumably depends on salvage of these important metabolites from the host and/or *Wolbachia*. Putative orthologs of several purine salvage genes representing potential drug targets have been identified in the parasite genome.

Genomic analysis indicates that *B. malayi* may possess a partial *de novo* pyrimidine biosynthesis pathway with only the final three enzymes of the pathway (dihydro-orotate dehydrogenase, orotate phosphoribosyltransferase and OMP decarboxylase) present in the parasite. Biochemical assays of enzymatic activity in extracts of *B. malayi* and *Wolbachia* (purified from insect cells) further support the absence of the first three enzymes (Carbamoyl-phosphate-synthase, aspartate transcarbamylase and dihydroorotase) in *B. malayi*. This suggests that the parasite may rely completely on salvage of pyrimidines, mediated by a number of pyrimidine salvage enzymes encoded by the *B. malayi* genome. Interestingly, some of the *Wolbachia* pyrimidine biosynthesis genes have been laterally transferred to the *B. malayi* genome, however, none seem functional. The putative absence of purine and pyrimidine biosynthesis in *B. malayi* is in contrast to mammals, which have the ability to both synthesize and salvage these metabolites. Further characterization of the *B. malayi* nucleotide biosynthesis and salvage pathways including drug challenge studies with cytotoxic purine and pyrimidine analogs is in progress. Analysis of the contribution of nucleotides from *Wolbachia* to the nucleotide economy of *B. malayi* and potential implications of these findings to *B. malayi* drug discovery will be presented.

### **The proteome of the core matrix of the *Schistosoma mansoni* eggshell**

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Schistosomiasis is an important parasitic disease affecting over 200 million people worldwide. Adult schistosomes are able to maintain themselves for decades in the veins of their mammalian host. Despite their abundant exposure to the immune system of the host, this parasite apparently prevents an adequate immune response. In contrast, schistosome eggs and their secretions are very immunogenic. They skew the host immune response towards a Th2 response and initiate granuloma formation. Pathology due to schistosome infection is mainly caused by the inflammatory response directed against the parasite's eggs trapped in the host tissue.

We previously unraveled the proteome of the tegument of the adult worms. We now investigated the core matrix of the eggshell and identified its proteins by mass spectrometry. *Schistosoma mansoni* eggs were isolated from livers of infected hamsters. After hatching of the eggs, the eggshells were collected and crushed into small fragments in a microdismembrator. Attached cellular material was removed in five consecutive steps, after which the remaining core matrix material of the eggshell was purified. These purified eggshell fragments were used for protein identification by mass spectrometry.

In contrast to previous reports, this study identified a relatively small number of proteins, 28 in total, to be part of the core matrix of the schistosomal eggshell. Among the identified eggshell proteins, expected schistosomal egg antigens were identified, such as the major egg antigen p40.

The identity of the other detected eggshell proteins and their possible function in host-parasite interaction will be discussed.

### **RNAi - its usefulness as a tool for schistosomiasis drug discovery**

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Treatment of schistosomiasis relies on just one drug, praziquantel. Accordingly, there is a need for continued research to identify and develop alternative therapies based the complementary strategies of gene-discovery and target validation. However, genetic manipulation of schistosomes is in its infancy and technologies typically used to explore gene function in other eukaryotes remain undeveloped.

RNA interference (RNAi) has been shown to knock down a number of genes in *Schistosoma mansoni* and we have employed the tool to investigate genes of interest that may be useful drug targets. We considered several methodological aspects: different developmental stages, tissue localization of the gene transcripts, dsRNA delivery, concentration of dsRNA and type of cultivation media. Fluorescent dsRNA and proteins provided observational tools as to how the parasite gut contributes to the RNAi effect. The data generated point to remarkable differences among the gene targets with respect to the efficiency of knock down. Research is ongoing to identify sets of conditions applicable to future RNAi studies.

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### ***Trichinella spiralis* secreted proteins modulate immune cell effector functions induced by extracellular nucleotides**

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Extracellular nucleotides are signalling molecules whose purinergic receptor-mediated effects are known to be key regulators of inflammatory and other immune responses, and they are considered archetypal activators of the innate immune system. We have recently shown that infective larvae of *Trichinella spiralis* secrete enzymes which catalyse the degradation of extracellular nucleotides, and thus potentially interfere with host purinergic receptor signalling. Bone marrow-derived mast cells of a mucosal phenotype (mBMMC), bone marrow-derived macrophages (mBMMΦ), either classically or alternatively activated, and the murine macrophage cell line RAW264.7 were used as models to study the effects of *T. spiralis* secreted proteins on host purinergic signalling. Via RT-PCR and pharmacological analyses we have identified the families and subtypes of functional purinergic receptors in all of the above cell lines. In mBMMC, nucleotides induced intracellular calcium mobilisation and calcium influx from the extracellular milieu. In addition, nucleotides induced mBMMC exocytosis, resulting in the release of mouse mast cell protease-1 (mMCP-1). We have identified the receptors responsible for these effects and characterised the intracellular pathways activated. We show that these effects are inhibited by *T. spiralis* secreted proteins and have identified the parasite enzymes responsible. In mBMMΦ and RAW264.7 we observed differential up-regulation of purinergic receptors depending on whether cells were activated classically or alternatively. LPS-induced NO production and LPS- induced cytokine release were further increased by extracellular nucleotides and the effects were reversed by *T. spiralis* secreted proteins.

**A-WOL drug discovery – from target discovery to *in vitro* drug screening of novel drugs with efficacy against *Wolbachia***

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Filarial nematodes are an important group of human pathogens infecting around 150 million people throughout the tropics with more than 1.5 billion at risk of infection. Control of filariasis currently relies on mass drug administration (MDA) programmes using drugs which principally target the microfilarial life-cycle stage. These control programmes are facing major challenges including the absence of a drug with macrofilaricidal or permanent sterilizing activity, and the possibility of the development of drug-resistance against the drugs available. Developing treatments based on the anti-symbiotic targeting of filarial *Wolbachia*, which are essential for worm development, fertility and survival, and are an important component of inflammatory disease pathogenesis, could provide a novel treatment. The Anti-*Wolbachia* Consortium (A-WOL) utilises post-genomic and computational bioinformatics to identify potential drug targets, and *in vitro* screening assays, to screen compound libraries and potential drug candidates for efficacy against *Wolbachia*. *In vitro* screening activities employ both traditional filarial nematode screening systems and a multi-well format *Wolbachia* cell-based assay we have developed for HTS, in which the reduction in numbers of *Wolbachia* are determined using quantitative real-time PCR (qPCR) and expressed as *Wolbachia* 16S:*Aedes* 18S ratios. The log drop in the ratio of 16S:18S gives a quantitative measure of the effect of the drug on *Wolbachia in vitro*. Using this strategy we are currently screening a chemical library of novel tetracyclines and novel drugs of targets identified through bioinformatic analysis, and have shown that we can identify compounds, which have improved efficacy over doxycycline against *Wolbachia in vitro*. Hit criteria together with pharmacological indices are currently being defined to select drugs for further screening against nematode screening systems. One novel drug identified with improved efficacy over doxycycline, a signal peptidase II (LspA) inhibitor, globomycin, shown to have potent anti-bacterial activity against Gram-negative bacteria will be discussed. We have recently identified *Wolbachia* diacylated lipoproteins as a virulence factor candidate for inflammatory mediated filarial pathogenesis. The lipoprotein biosynthetic pathway, which is absent in humans/mammals, is essential for bacterial growth and virulence. Two genes (LspA and Lgt) involved in this pathway have been identified from the *Wolbachia* genome, therefore enzymes involved in *Wolbachia* lipoprotein biosynthesis are promising chemotherapeutic targets. Screening of globomycin using the *in vitro Wolbachia* cell assay has demonstrated, for the first time, that a drug other than the tetracyclines or rifampicin, can deplete *Wolbachia* numbers. Therefore validating LspA as a drug target and identifying globomycin as a novel class of antibiotic with anti-*Wolbachia* activity. Preliminary data suggests that globomycin is as effective as doxycycline *in vitro* against adult *Brugia malayi*.

## **Bacteriophage $\phi$ C31 integrase-mediated transgenesis of *Schistosoma mansoni***

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Within the last few years, notable advances have been made in our ability to manipulate gene expression of the helminth parasite, *Schistosoma mansoni*. For example, the knockdown of endogenous gene expression through RNA interference, transgene expression from electroporated message, and most recently transgene expression from electroporated DNA constructs have been shown to be particularly successful approaches. However, transgene expression in schistosomes using these methods was limited to within several days after introduction. Here, a novel method is described in an attempt to develop a technique for stable, integrated transgene expression in *S. mansoni*. We have exploited the bacteriophage  $\phi$ C31 system used for integrating the phage DNA into the host's genome by co-electroporating: (1) message coding for the  $\phi$ C31 integrase protein and (2) plasmid constructs containing a reporter gene driven by an endogenous schistosome promoter as well as recognition sites used by the integrase for mediating recombination. Preliminary real-time PCR data indicate electroporation of the plasmid construct with the integrase message increases the presence of transgene DNA in the worm by approximately two fold 14 days later, versus electroporation with plasmid alone. Furthermore, co-electroporation of plasmid and integrase message also increases expression of the transgene at the transcript level by nearly 4 fold two weeks after introduction. Lastly, three days following electroporation, protein expression from the reporter is increased 4 fold when the integrase message is included. While these results are encouraging, we have yet to determine the extent of transgene integration in the schistosome genome. A strategy for examining the integration potential will be discussed. The ultimate goal of this work is to establish a straightforward method for stable transgene expression in *S. mansoni* for use in studying both parasite biology and the host response through the expression of model antigens.

## **pWormgateMulti: Tissue-specific RNAi using Gateway® hpRNAi vectors**

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There are many applications in which the induction of RNAi in specific tissues and at particular time points would be advantageous for dissecting gene function. One way this is achievable in *C. elegans* is using hairpin RNAi transgenes that express dsRNA endogenously within the cell. RNAi is performed in select tissues by using tissue-specific promoters to drive expression of the hairpin transgenes. The greatest limitation to this approach is the molecular construction of these transgenes; a process that is very problematic and poorly efficient. Building on a previous Gateway<sup>®</sup> destination plasmid, pWormgate (Johnson *et al* 2005 *Gene* **359**: 26-34), we have developed a new Gateway<sup>®</sup> Multisite destination plasmid, pWGMulti that, using promoter clones from the Promoterome library, and ORFs from the ORFeome library, creates promoter::hairpin transgenes in single *in vitro* reactions and with high efficiency. We demonstrate the use of pWGMulti by targeting various genes in a tissue-specific manner and, as proof-of-concept, investigate the ability of the system to dissect pleiotropic phenotypes. In addition, with the advent of transgenesis and RNAi in some parasitic nematodes, pWGMulti could be modified to allow the *in vivo* expression of hairpin RNAs in these organisms.

### **The cellular basis for reproductive regression in female schistosomes following separation from their male partners**

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The eggs laid by adult female schistosomes within their human hosts are key for both transmission and pathogenesis. In order to initiate and maintain sexual maturity, female schistosomes must pair with an adult male schistosome. The mechanism by which the male schistosome stimulates proliferation and differentiation of the female reproductive organs, and the mechanism of female reproductive regression upon separation are not well understood. We are investigating the cellular processes involved in regression of the female vitellaria upon removal of the male. We hypothesize that tissue regression is due either to cell death by apoptosis, cell loss through a cessation of cellular proliferation terminal process of egg production, or cell shrinkage by autophagy. We have determined that both apoptosis and autophagy are likely to take place in schistosomes, since they possess homologues of the core machinery of both pathways. Preliminary studies show that tissue regression in separated females is marked by a dramatic decline in the expression of eggshell precursor protein-encoding genes accompanied by the significant loss of cells. Cell loss does not occur in separated males. Moreover, cellular proliferation in the presumptive vitelline tissue ceases in female parasite shortly following separation, whereas it continues in paired females. Taken together, the data support the view that vitelline regression is the net consequence of a cessation in proliferation of vitelline cells, followed by their loss, without replacement, presumably due to their packaging into eggs for release from the worm.

### **Comparative biology of Hsp90 in *C. elegans* and *Brugia***

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Hsp90 is essential in all eukaryotes and functions as an ATP dependant chaperone in the cytoplasm, folding nascent polypeptides into their functional conformation. Geldanamycin (GA), a known inhibitor of Hsp90, kills all life cycle stages of the filarial parasite *Brugia pahangi* at nanomolar concentrations but has a lesser effect on the free-living *C. elegans*. Pull-down assays substantiate these data with GA binding to *Brugia* Hsp90 but not to *C. elegans* Hsp90. To further analyze the properties of *Brugia* Hsp90 we attempted to rescue a *C. elegans daf-21 (hsp90)* null mutant with *Brugia hsp90* to determine whether this confers increased GA susceptibility on the free-living species. Despite 87% amino acid homology between *Ce*-Hsp90 and *Brugia*-Hsp90, rescue has been unsuccessful. Both transcription and translation of the *Brugia* gene are observed and re-introducing the endogenous *C. elegans* gene into the mutant results in rescue indicating that *Brugia hsp90* may be too divergent to rescue. As most interspecies mutant rescue experiments reported to date have come from work carried out using *H. contortus*, a strongyloid nematode parasite of sheep, we are now in the process of creating an *H. contortus hsp90* transgene to ascertain whether rescue is possible using *hsp90* from a more closely related species.

### **Identification of Transcriptional Activators in *Schistosoma mansoni***

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Understanding the transcriptional program in yeast and mammalian systems has been extensive, but identification of activators in schistosome worms has been minimal. We describe the functional identification of activators of schistosome transcription based on the ability to activate gene expression in a heterologous system. We also describe a genetic screen based on a variation of the yeast two-hybrid to identify functional transcriptional activators in schistosomes. This identification of new transcription activators in schistosome will generate more knowledge in the basic understanding of plathyhelminth programs of gene expression.

### **Role of macrophages in the cystatin-induced modulation of inflammatory responses**

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Chronic helminth infections are shown to be negatively associated with allergic disorders in humans and animal models. Recently we could show that application of the single recombinant protein Av17, a cysteine protease inhibitor (cystatin) secreted by *Acanthocheilium viteae*, effectively abolishes the OVA-induced allergic airway responsiveness in a mouse model of asthma (Schnoeller et al., 2008). The reduction of OVA-induced airway hyperreactivity was characterized by a reduced eosinophil infiltration into the lung, decreased OVA specific and total IgE antibody level and a significant reduction of IL-4. The cystatin effect in this Th2 driven immune hyperreactivity response was blocked by the application of anti-IL-10 receptor antibodies. Since depletion of macrophages by liposomes containing clodronate also abrogated the cystatin effect we concluded that cystatin induced a special type of "immunosuppressive" macrophage. In order to reveal and characterize this type of macrophage we stimulated primary macrophages with Av17 in combination with various co-stimuli. Analysis of cytokine profiles and macrophage type specific markers by conventional and quantitative real time PCR suggest a role of type 2 macrophages. Hence, immunosuppressive macrophages might be interesting target cells to develop novel therapeutic strategies.

### **VHA-19, a protein predicted to associate with the vacuolar ATPase, has a crucial role in *C. elegans* reproduction**

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The vacuolar ATPase enzyme (v-ATPase) is an ATP-driven proton pump that contributes to pH gradients in the cell and is involved in many crucial processes. *vha-19* is a *C. elegans* gene of unknown function with similarities to predicted proteins in nematode parasites. VHA-19 has been predicted to associate with v-ATPase in *C. elegans*.

Previously we showed that RNA interference (RNAi) mediated knockdown of *vha-19* had a severe effect on development and reproduction in *C. elegans*. Feeding worms *vha-19* dsRNA from the fourth larval stage caused *vha-19*(RNAi) adults to produce progeny that arrested and died as either embryos or early stage larvae. We now show that by 24h post RNAi *vha-19*(RNAi) adult worms produce squashed, inviable eggs lacking an impenetrable eggshell and vitellogenin, a crucial egg yolk protein. This phenotype is evident even when RNAi is active in the germline alone. The implications of these results for the overall role of *vha-19* in *C. elegans* will be discussed.

### **A novel and divergent role of granzyme A and B in resistance to filariasis**

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Granzyme (gzm) A and B are the most abundant cytotoxic serine proteases of lymphocytes. They are released by CD4+ or CD8+ T cells, NK cells, B cells and regulatory T cells to induce cell death of infected or tumor cells, but also of effector cells. They can also facilitate leukocyte migration by degrading extracellular matrix. Thus, granzymes help to fight infections. We investigated, whether they play a role in helminth infection, since CD4+ T, NK and B cells promote local defence against filaria together with granulocytes, promoted by Th1/Th2 cytokines, while regulatory T cells suppress it. This immunosuppression enhances with worm burden in most onchocerciasis patients and in susceptible mice. The underlying immune mechanisms are not completely understood. Therefore, we examined the role of granzyme (gzm) A and B in murine infection with *Litomosoides sigmodontis* and in humans infected with *Onchocerca volvulus*. In resistant C57Bl/6 (B6) mice, worms are degraded in the pleural cavity before reaching maturity in contrast to susceptible BALB/c mice. In both strains, pleural and splenic cells up-regulated gzmA (mainly NK and B cells) and gzmB (mainly B cells) expression with early infection. GzmB expression decreased in B6 mice at the adult worm stage, but was sustained in BALB/c mice in parallel with Foxp3+/gzmB- Treg cell numbers. GzmA deficiency increased the worm load threefold in gzmA knockout (ko) B6 compared to wt B6 mice. In contrast, GzmAxB knock-out (ko) B6 mice had significantly lower worm loads than wt B6 mice. This hyperresistance was associated with an early reduction of total pleural leukocytes, NK cells and lower IFN-gamma production, and in the late phase, a stronger defence-promoting Th2 cytokine and antibody response. A defect in IL-2 release and proliferation in naïve gzmAxB ko mice was restored until day 9 p.i., but might have contributed to the weaker initial NK/Th1 response. D7 post infection microarray analysis indicated significant alterations of several inflammatory markers, which were different in the ko strains compared to the wt strain. Therefore, we conclude, that gzmA and B differentially affect the early immune response to filarial infection: gzmB induces an early and sustained Th1 shift, possibly via Th2 activation induced cell death, protecting the worms from a stronger Th2-mediated immune attack. This is counterbalanced by gzmA, promoting early defence, but the gzmB influence predominates.

In the human infection, we immunohistochemically examined both granzymes in subcutaneous nodules harbouring adult *O. volvulus*. GzmA+ or B+ cells were abundant in nodules with microfilariae-producing or dead adult worms from patients with the immunosuppressed form of onchocerciasis, but scarce in hyperreactive ones. Gzm+ cells were strongly induced six and eleven months after endobacterial depletion by doxycycline, closely associated with increased Foxp3+/CD4+ T cells.

In summary, we show a novel and, in mice, a divergent role of granzymes in the immune response of humans and mice to helminths: a new association of gzmB with immunosuppression in an infection in contrast to defence-promoting gzmA.

### **Nematode latrophilin repertoire: mapping and comparative analysis of latrotoxin binding sites**

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Latrophilins are G protein-coupled receptors of the secretin subfamily (GPCR2 subfamily) expressed predominantly in the nervous system. Vertebrate latrophilins have been described as Ca<sup>2+</sup>-independent receptors for  $\alpha$ -latrotoxin ( $\alpha$ -LTX), a major toxin of black widow spider venom. Although receptor/toxin interactions and intracellular signaling pathways have been extensively studied, the physiological functions of latrophilins remain unknown. In nematodes, latrophilins have been implicated in the action of the new anthelmintic emodepside. Transfection of *Haemonchus contortus* latrophilin-1 (*HcLAT-1*) into mammalian cells confers  $\alpha$ -LTX sensitivity to these cells and emodepside is able to antagonize the action of  $\alpha$ -LTX. Moreover, *Caenorhabditis elegans* with a *lat-1* null mutant are partially emodepside resistant.

Nematode LAT-1 proteins have two domains characteristic for GPCR2, *i.e.* a motif of unknown function termed HormR and the region containing seven transmembrane domains (7TM). In addition, there is a galactose-binding lectin domain (Gal) close to the NH<sub>2</sub>-terminus and a GPS proteolytic domain immediately before the 7TM domain. The latter domains are present in all latrophilins but can also be found in unrelated proteins.

In nematodes, there is another latrophilin-like protein, called LAT-2, containing a C-type lectin in the NH<sub>2</sub>-terminus flanked by two EGF domains. A weakly conserved second C-type lectin domain can be detected between the Gal and HormR domains.

In contrast, arthropods have only one latrophilin protein whose domain architecture closely resembles nematode LAT-1. Vertebrates have three closely related latrophilin genes that all contain an additional olfactomedin-like domain in their extracellular region. Remarkably, there is no sequence homology between these four different latrophilin families in their intracellular COOH-terminal regions.

Phylogenetic analyses reveal that the two nematode latrophilin subfamilies are not more closely related to one another than to the vertebrate and insect latrophilins. Moreover, different latrophilin domains show markedly distinct evolutionary rates. For instance, the GPCR2 7TM domains show a high degree of diversity, while the HormR and GPS domains are more closely related. For Gal, a higher diversity of LAT-2 proteins can be noted.

Overlay and pulldown assays demonstrated direct interaction of the NH<sub>2</sub>-terminal 54 kDa fragment of *HcLAT-1* with  $\alpha$ -LTX. Mapping of  $\alpha$ -LTX-binding regions with phage display identified two polypeptides, *HcLAT-1*<sub>161-261</sub> and *HcLAT-1*<sub>401-501</sub>, which increased binding of phagemids to  $\alpha$ -LTX by about 8- and 13-fold, respectively. These putative LTX-binding regions encompass the HormR domain and the region before the GPS domain.

$\alpha$ -LTX and latroinsectotoxins in black widow spider venom vary highly in their activity against nematodes, insects, and vertebrates. Using multiple sequence alignments we analyzed whether this variability correlates with sequence variability in putative LTX-binding regions of latrophilins. Conspicuously, in insects the first  $\alpha$ -LTX-binding region contains a small (honey bee and red flour beetle) or large (flies) insertion. Within the second  $\alpha$ -LTX-binding region a large block without any sequence similarity between the different latrophilin families can be identified. Remarkably, the HormR domain within the first LTX-binding region has been split in two parts in diptera and is highly divergent from that of other insects suggesting that spider toxins might exert a high selective pressure on this sequence.

### **Digestive proteases of the carcinogenic liver fluke, *Opisthorchis viverrini***

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The liver fluke *Opisthorchis viverrini* is an important human pathogen distributed throughout Southeast Asia, predominantly in Northeast Thailand. Infection with this parasite causes opisthorchiasis, which is associated with a number of hepatobiliary abnormalities, including cholangitis, obstructive jaundice, hepatomegaly, cholecystitis and cholelithiasis. More problematic, though, is that *O. viverrini* is the cause of cholangiocarcinoma, cancer of the bile ducts, in many people who have had long-termed infections with the parasite. Indeed, in regard to infection associated cancers, there is no stronger link between cancer and a parasite than between *O. viverrini* and cholangiocarcinoma. Moreover, excretory-secretory (ES) proteins of *O. viverrini* have been implicated in unchecked proliferation of bile duct cells, predisposing biliary cells to metastatic changes. Proteolytic enzymes are major components of helminth ES products, where they play essential roles in digestion of food proteins, immunomodulation and host tissue invasion. Given the link between *O. viverrini* and metastasis in the bile duct epithelia, we are interested in worm proteins that interact with surrounding host tissues. Proteases are predominant in ES products and are essential in the digestion of host tissue as a food source, so we are characterizing the major proteases found in the digestive tract of adult *O. viverrini*. Three full length cDNAs including transcripts encoding asparaginyl endopeptidase (AEP-1), cathepsin B3 and cathepsin B5 have been characterized and produced in recombinant form. *Ov*-AEP-1 is characteristic of the C13 cysteine protease family. *Ov-aep-1* transcripts were detected in adult and juvenile worms, eggs and metacercariae. Phylogenetic analysis indicated that *Ov*-AEP-1 was closely related to orthologous enzymes of other trematodes. Recombinant *Ov*-AEP-1 was expressed in bacteria and refolded to a soluble form. Excretory-secretory (ES) products derived from adult *O. viverrini* and refolded recombinant *Ov*-AEP-1 both displayed catalytic activity against the diagnostic legumain peptide, Ala-Ala-Asn-aminomethylcoumarin. Antiserum raised to *Ov*-AEP-1 identified native AEP-1 in somatic extract and ES products of adult worms, and within the gut of the fluke, implying a physiological role in digestion of food or activation of other digestive enzymes. Recombinant *Ov*-AEP-1 was recognized by serum antibodies from patients with opisthorchiasis but not other helminth infections, with a sensitivity and specificity of 85% and 100% respectively. The positive and negative predictive values are 100% and 67% respectively. Recombinant *Ov*-AEP-1, cathepsin B3 and cathepsin B5 also show potential for immunodiagnosis of human opisthorchiasis. Moreover, we are investigating the activity of these and other proteases of *O. viverrini* as mitogens for cultured biliary epithelial cells.

## The investigation of ivermectin metabolism by nematodes using *Caenorhabditis elegans* as a model organism

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Resistance to anthelmintics is a major problem in cattle and sheep and is of increasing concern in human helminths. A full understanding of molecular mechanisms of anthelmintic resistance is necessary to develop sensitive diagnostic tests that can be used to study the factors that influence its development and evaluate measures to limit its impact and spread. Ivermectin is an example of a macrocyclic lactone drug that has been marketed to treat gastrointestinal parasites of ruminants since the early 1980s. Resistance to ivermectin is now widespread and, in spite of a significant amount of research, no single definitive mechanism has been found. However, despite a well established precedent in insecticide resistance, the role of drug metabolism by parasites has received little attention as a possible mechanism of anthelmintic resistance. The transcription of genes encoding xenobiotic metabolising enzymes is often induced in the presence of their substrates. Therefore, we reasoned that the use of microarray technology to compare the transcriptomes of nematodes exposed to ivermectin with unexposed controls could identify candidate genes that may be involved in biotransformation of anthelmintics by nematodes. Since a truly whole genome approach is not yet possible in parasitic nematodes, the free-living nematode *C. elegans* offers an alternative powerful experimental system to explore this approach.

We have undertaken a series of experiments investigating the effects of ivermectin and albendazole exposure on *C. elegans* using Affymetrix whole genome microarrays. In the case of ivermectin we have identified 82 genes that show significant changes in expression levels following exposure to ivermectin and used real time PCR to confirm the results of selected genes. Several genes show dramatic increases in expression (10 to 40-fold), including a cytochrome P450 (CYP). For this gene a GFP reporter fusion has been produced that is responsive to ivermectin exposure in transgenic *C. elegans*. This represents an important finding as over-expression of CYPs are accepted as a major mechanism of insecticide resistance in *Drosophila melanogaster*, and CYP 3A4 is the major metabolising enzyme of ivermectin in the human liver. The microarray results represent several other genes with metabolism roles, but many of the genes code for uncharacterised proteins whose possible interactions with ivermectin are as yet unknown. For albendazole, several different CYP genes show changes in expression, along with members of the glutathione-s-transferase and UDP-glucuronosyl transferase families. Using a variety of techniques these genes are currently being further characterised and their ability to transfer ivermectin resistance to *C. elegans* following over-expression will be assessed.

## **A Comparative Phenotypic Analysis of Regulatory and Effector T cells induced during Chronic Helminth Infection**

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Our initial studies have demonstrated that regulatory T cells activated during a chronic infection with the helminth *Schistosoma mansoni* control antigen-specific T cell responses and immunopathology. Interestingly, they are simultaneously beneficial for the parasite indicating that these cells control a finely tuned state of immune-responses supporting host and parasite survival. Therefore, our current research focuses upon the phenotype of those regulatory and effector T cells that develop during the infection and whether it is possible to discriminate new genes involved in the processes of immunehomeostasis. Populations of CD4<sup>+</sup>CD25<sup>hi</sup> (Treg) and CD4<sup>+</sup>CD25<sup>-</sup> (Teff) were isolated using the MoFlo cell-sorter from naïve and schistosome-infected mice. RNA from those cells was subjected to microarray analysis using Affymetrix MOE 430A2.0 gene-chips. Statistical analysis revealed that compared to naïve cells, 91 genes were significantly ( $p < 0.01$ ) upregulated in the infected Treg whereas 396 genes were downregulated. Amongst the genes most strongly upregulated were classical Treg markers such as Foxp3, Itgae, GITR, OX40, CTLA-4 as well as Gzmb. In addition, transcription factors (Gata3, Tox, Egr2, Batf) and cell growth-related genes (Cyclin D2, Socs1/2, Rbm5, Cish) were also elevated. The genes most strongly downregulated were metabolism-related genes (Mmp8/9, Pygl, Mgst1, Apoe, Gstl1, Chi3l1), and cell-cycle-related genes (Anxa1, Rhou, GOs2, App, Csf1r, Fes, Rgs2) as well as transcription factors like C/EBP-delta, Spib, Hhex, Tal1, Klf1 and Tcf4. Furthermore Ear1/2/3/12, Ccl6/3, Cxcl4/7 were also strongly downregulated.

In the effector T cell population the pattern was different: 128 genes were significantly upregulated in samples from infected mice whereas only 45 genes were downregulated. Examples of strongly upregulated genes are Chi3l3/3l4, IL-4, IFN-gamma, Arg1, IL-21, Socs2, CXCR6, Gzmb and Gzma, many classical markers of chronic infections. We are currently analysing functional properties of selected genes as such studies may contribute to elucidating mechanisms adopted by Tregs during parasitic infections.

## Transcriptomes and pathways associated with infectivity, survival and immunogenicity in *Brugia malayi* L3

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This study was designed to explore changes in gene expression associated with the transition of infective *Brugia malayi* L3 from mosquitoes into mammalian hosts (genes associated with establishment of infection) and effects of radiation on L3 gene expression. Radiation effects are especially interesting, because irradiated L3 induce partial immunity to filarial infections. We compared expression profiles for three types of BmL3, namely infective L3 freshly dissected from mosquitoes (L3i), L3 cultured for 2 days under conditions that mimic the mammalian environment and permit molting (L3c), and L3 that were irradiated and then cultured as for L3c (L3ir). Expression profiles were obtained using a new, Version 2 filarial microarray. This array contains 18,104 65-mer oligo elements that represent the genes from *B. malayi* (15,412), *Onchocerca volvulus* (1,016), *Wuchereria bancrofti* (872) and *Wolbachia* endosymbiont (804). 771 genes were identified as differentially expressed (with expression ratios  $\geq 2$  and  $P < 0.01$ ) in two-way comparative analyses (L3i vs. L3c, L3ir vs. L3c, L3c vs. L3i and L3c vs. L3ir). 46% (353/774) of these genes were up-regulated in L3i relative to L3c. These genes may be important for invasion and establishment of infections in mammals. Other genes (33%, 256/774) were up-regulated in L3c relative to L3i (234) or L3ir (22). These culture-induced transcripts include key molecules required for growth and development. 21% (165/774) of differentially expressed genes were up-regulated in L3ir relative to L3c; these genes encode a number of proteins that are highly immunogenic and the proteins that are involved in repairing damages induced by radiation. Persistent expression of highly immunogenic proteins may explain the special immunogenicity of L3ir. *C. elegans* homologues of differentially expressed *B. malayi* L3 genes were much more likely to have RNAi phenotypes than the average *C. elegans* genes tested to date. The most common phenotypes associated with these genes included developmental delay (slow growth, larval arrest and lethal), embryo lethal, and sterility. Gene Ontology and KEGG analyses also revealed important differences between L3 types. For example, energy supply and survival appear to be priorities for L3i, while growth and development are priorities for L3c. We also compared differences in gene expression associated with the L3i to L3c transition in *B. malayi* with reported differences in gene expression associated with parallel transitions in *A. caninum* and *C. elegans*. This study has improved our understanding of genes involved in parasite invasion and immune evasion, potential targets of protective immunity, and molecules required for parasite growth and development. Data generated in this study may be useful for identifying targets for new anthelmintic drugs and vaccines.

### The SMART transposons of *Strongyloides stercoralis* and *S. ratti*

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In our search for tools for genetic study of the parasitic nematode *Strongyloides stercoralis*, we have characterized a *mariner*-like element (MLE) dispersed in the genome of this worm which we name SMART for *Strongyloides mariner* transposon. We first found this sequence in the expressed sequence tags (EST) database and expanded it into genomic flanking sequences by inverse PCR. Sequencing of flanking sequences confirms that this transposon resides at multiple sites in the *S. stercoralis* genome. The consensus sequence of seven copies of the transposon consists of 1291 bp, includes an open reading frame of 1017 bp encoding a deduced 339 amino acid residue protein and two inverted terminal repeats (ITRs). The 5' and 3' ITRs consist of perfect inverted repeats 29 bp long (TATTAGGTCTTTACATATGAAATGGGTTT, reading inward from the 5' end) followed by non-identical insertions of 6 and 19 bp near the 5' and 3' ends, respectively, and an additional 8 base identical inverted repeat which is itself a palindrome (ATGCGCAT). A presumptive TA insertion site duplication flanks the transposon at both ends. The deduced transposase (SMARTase) amino acid sequence contains specific and non-specific DNA binding domains and a catalytic domain containing the DD(34)D motif that characterize SMART as a *cecropia* clade MLE. Phylogenetic analysis shows closest relationship of SMART to the recently reported *bandit* transposon of *Ancylostoma caninum*, human *hsmar1* and primate SETMAR transposons. Highly degenerate sequences of a closely related MLE have also been found in the genome survey sequence database of *Strongyloides ratti*. To distinguish these elements, the *S. ratti* element is designated SMART<sup>r</sup>, and the *S. stercoralis* element is called SMART<sup>st</sup>. Whereas the presence of SMART ESTs in the databases of both species indicates that some copies of the transposon are transcriptionally active, there is no indication that transposition is currently taking place. The degeneracy of the genomic sequences indicates that many if not all copies of these elements may have become mutationally inactivated. By aligning multiple sequences of the SMART<sup>st</sup> element, we have established a consensus sequence that should approximate the sequence of the original active transposon. Our goal is to resurrect active copies of SMART from both species in this way and to develop them into tools for genetic manipulation of these two nematode parasites. Despite their close similarity, SMART<sup>st</sup> and SMART<sup>r</sup> have related but distinct ITR sequences, suggesting that the two transposases may show species specific recognition.

**ABSTRACTS  
FOR POSTER PRESENTATIONS  
SESSION 2**

### **Altered expression of specific acetylcholine receptor subunit genes in pyrantel-resistant hookworm**

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High-level resistance to the nicotinic anthelmintic pyrantel has now been well documented in isolates of the canine hookworm, *Ancylostoma caninum*. While the emergence of resistance in this companion animal parasite is worrisome, it also serves as both a warning for drug resistance in the human hookworms *Ancylostoma duodenale* and *Necator americanus*, as well as an opportunity for its investigation. Given that mass drug administration programs now underway in humans are not dissimilar to those used in companion animals, it is reasonable to expect that anthelmintic resistance might also occur in *A. duodenale* and *N. americanus*. An understanding of the molecular basis of resistance might permit the development of a rapid assay for the detection of hookworm isolates resistant to pyrantel, and could allow continued use of the drug in the face of resistance if the mechanism is able to be overcome (e.g. through co-administration of other drugs). We investigated the molecular basis of resistance to pyrantel in *A. caninum* by comparing a strain of highly resistant parasite (PR) with a strain expressing low levels of resistance (PS). We cloned 3 key orthologues of the pyrantel-sensitive nicotinic acetylcholine receptor in *C. elegans*. Analysis of mRNA levels by quantitative PCR revealed no significant polymorphisms between the two *A. caninum* strains. However, quantitative analysis of transcription revealed significantly lower expression of the three putative pyrantel receptor subunits in the PR *A. caninum* strain compared to the PS strain. In contrast, expression of the 3 subunits thought not to constitute the pyrantel receptor was never lower in the PR strain, and in some cases was significantly higher. These data suggest that alterations to nicotinic acetylcholine receptor subunit expression may mediate pyrantel resistance in *A. caninum*, and that a compensatory increase in the expression of receptor subunits not involved in the response to pyrantel may occur as a means of subverting the biological cost associated with resistance. Further work is needed to determine whether these observations correlate with further isolates resistant to pyrantel.

### **Serine peptidases in cercariae of the neuropathogenic schistosome *Trichobilharzia regenti* and its intermediate snail host *Radix peregra***

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Schistosome cercariae belonging to the species *Schistosoma mansoni* and *S. haematobium* employ a serine class peptidase generally called elastase, for histolysis. On the contrary, *S. japonicum* and the bird schistosome *Trichobilharzia regenti* have been shown to possess a high level of cysteine peptidase activity of cathepsin B type. It has been previously suggested that cysteine peptidases represent an archetypal tool for tissue invasion among primitive metazoa and the use of serine peptidases arose later in schistosome evolution. Using Western blots and appropriate antibodies we confirmed the presence of both the elastase and cathepsin B2 in cercarial extracts of *S. mansoni*. As cathepsin B2 occurs in postacetabular penetration glands of *T. regenti*, we employed biochemical, molecular and immunological approaches to reveal if an elastase orthologue is present in cercariae of this species, too. In our experiments we showed that the activity of serine peptidases in *T. regenti* is negligible. No proteins from cercarial extracts reacted with antibodies against *S. mansoni* elastase. Using degenerated primers based on selected elastase peptides, PCR and RACE methods and a mixed cDNA library from parasite larvae in snail hepatopancreas, we obtained two sequences coding for serine peptidases of snail origin but none of parasite origin. Zymography of cercarial extracts revealed a sporadically appearing 28 kDa trypsin-like serine peptidase band which was of snail origin. Its mass corresponded to one of the obtained snail serine peptidase genes. The contamination originated from snail faeces as confirmed by measurements of activity and zymographs of snail-treated water. A 50 kDa gelatinolytic band in cercarial zymographs is of parasite origin, different from previously detected cathepsin B and not of serine class. Isolation and further characterization of this peptidase is in progress. Recent results indicate it is a metallopeptidase. Thus, we conclude that cercariae of *T. regenti* do not use serine peptidases for penetration of the skin of their hosts and cathepsin B2 seem to be the main (or the only) histolytic enzyme of this species.

### **Functional analysis of the interaction of *Brugia malayi* VAL-1 with peripheral blood leukocytes**

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Like many parasitic nematodes, *Brugia malayi* secretes several Venom allergen homologues/ASP-Like proteins (VALs). Despite extensive work and interest in VALs as vaccine candidates in a range of nematode species, little has been done to investigate their function. VALs are representatives of the PR-1 subfamily of the Pathogenesis-Related (PR) protein superfamily, of which 2 members expressed by *Ancylostoma caninum* have been described to act as integrin antagonists. Moreover, analysis of the structure of a hookworm VAL (*Na*-ASP-2) revealed a putative binding cavity, suggesting that it may interact with an unidentified host ligand, and also highlighted similarities in structure and charge distribution to chemokines. We therefore investigated the functional properties and interactions of *B. malayi* VAL-1 with a variety of leukocytes isolated from human peripheral blood. VAL-1 was expressed as a secreted protein in *Pichia pastoris*. The purified protein was not directly chemotactic for granulocytes or mononuclear cells, and furthermore did not influence chemotaxis induced by IL-8, leukotriene B4 or C5a in vitro. In contrast, native secreted proteins from *B. malayi* microfilariae inhibited C5a-induced chemotaxis, and this was shown to be due to direct cleavage of C5a by a serine protease. VAL-1 also had no effect on platelet aggregation induced by a variety of agonists or binding of platelets to collagen and fibrinogen, although as previously reported, microfilarial extracts inhibited platelet aggregation. Preliminary data suggested that VAL-1 inhibited binding of both granulocytes and mononuclear cells to primary human umbilical vein endothelial cells (HUVECS), however subsequent analysis indicated that this was likely due to a minor contaminant secreted by *P. pastoris* which bound CD11b. In the absence of this contaminant, VAL-1 bound only weakly to neutrophils, monocytes and basophils, and not at all to T, B or NK lymphocytes. Possible functions and/or binding partners of VALs thus remain elusive.

### **Characterising the host-parasite interface using novel fractionation techniques and tandem mass spectroscopy**

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Helminths have intimately coevolved with their mammalian hosts, surviving for many years, often without eliciting much pathology. Much of the success of parasitic helminths can be attributed to the proteins they secrete into their environment or display on their surfaces. Characterisation of these parasite secretomes has, for the most part, relied on traditional proteomics methods; complex protein mixtures have been fractionated on 1D or 2D gels and excised bands or spots subjected to in-gel tryptic digestion prior to protein identification with tandem mass spectrometry (MS/MS). While this has proven to be a useful approach, such studies are labour intensive and relatively insensitive due to the extensive processing of gel fragments during in-gel digestions. Recent advances in mass spectrometry and associated technologies have now made possible the rapid and sensitive determination of the protein content of biological fluids. One such technique is OFFGEL electrophoresis (OGE), a recently developed technology that fractionates peptide or protein fragments in solution according to their isoelectric points. The ability to fractionate proteins in solution allows for relatively large-scale fractionations and reduces the processing required before MS/MS by removing the need for in-gel digests to obtain protein identifications. It is possible using this approach to rapidly identify proteins in complex mixtures using either a shotgun approach with pre-digested protein fragments or on a protein-by-protein basis by focusing the mixture prior to tryptic digestion. Using OGE we have characterised the protein composition of the excretory/secretory (ES) products of two distinct parasitic helminths - (1) *Ancylostoma caninum*, a model organism for the human hookworms *Necator americanus* and *Ancylostoma duodenale*, both important public health problems in developing countries; and (2) the human liver fluke *Opisthorchis viverrini*, a primary risk factor for the liver cancer cholangiocarcinoma throughout parts of SE Asia. Furthermore we have utilised OGE in coordination with selective labelling of host exposed tegument proteins from *O. viverrini* to characterise the membrane bound/associated proteins exposed to host tissues. The increased sensitivity afforded by OGE has enabled us to identify many more proteins in the ES products of these parasites or in the surface exposed tegument of *O. viverrini* than did traditional methods relying on gel digests. Many of these proteins likely interact with host tissues and are therefore valid targets for the development of novel therapeutics to control both helminth infections and autoimmune disorders.

## Investigation into the potential immune function of the *Bm-VAL-1* gene using a transgenic *Leishmania* system

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Proteins of the VAL (Venom allergen/*Ancylostoma* secreted protein-Like) family have been found in all species of nematodes, both parasitic and free-living, but as yet their structure and function in these systems has remained elusive. They are members of the larger Pathogenesis Related Protein super-family. The venom allergen homologues are notable by an SCP domain and fall into either single or double domain sub groups. Unfortunately, the known characteristics of different family members determined so far have not identified any common function.

In the filarial parasite *Brugia malayi*, the *Bm-VAL-1* gene is very highly expressed by the infective larval (L3) stage of the parasite. Here the hypothesis is that *Bm-VAL-1* may promote the invasion/infection process by down regulating host immunity, providing a more "parasite friendly" environment in which an otherwise hostile immune response is neutralised. In contrast, homologous proteins from the free-living nematodes *C. elegans* would not have needed to evolve with such effect on the immune system.

To address the question of whether this gene product can alter host responses an *in vitro* system was used as previously described by Gomez-Escobar et al. (BMC Biol. 2005 Mar 23;3:8.). *Leishmania mexicana* parasites were transfected with a construct carrying either *Bm-VAL-1* or a homologous gene from the free-living nematode *C.elegans*. These constructs enable homologous recombination and stable expression of the gene of interest. The immunological phenotype of transfected *Leishmania* was then assessed by *in vitro* infection of bone marrow derived macrophages. The growth of *L.mexicana in vitro* is well characterised and sensitive to quantitative changes in macrophage immune responsiveness. Cytokines were measured in culture supernatants of infected macrophages. Comparisons were then drawn between wild type promastigotes and parasite lines that contained either a parasitic or non-parasitic gene. Here we found that macrophages infected with the transgenic line for the *C.elegans* VAL homologue, actually secreted the type 2 cytokines IL-10 and IL-6 the latter being pro-inflammatory. Macrophages stimulated with the parasitic transgenic lines also secreted IL-10 but to a lesser degree and with no IL-6 being present.

As *Leishmania* infections in murine models have well characterised phenotypes, comparisons can be made between wild type and transgenic lines with regards to immune response *in vivo*. Experiments are now under way to assess the impact of expression of these genes on the course of infection *in vivo*.

## Characterization of *C. elegans glf-1* mutants reveals an important role for Galactofuranose metabolism in nematodes

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Galactofuranose (Gal<sub>f</sub>) is the furanoic form of D-Galactose. Unlike the pyranoic form of the sugar (Gal<sub>p</sub>), which is ubiquitously found across the tree of life, Gal<sub>f</sub> has a more sporadic distribution, and is present in protozoa and some lower eukaryotes, while notably absent in mammals. In bacteria, protozoans, and fungi where it has been detected, Gal<sub>f</sub> is often a crucial component of the cell wall/surface glyco-conjugates. Gal<sub>f</sub> is produced by UDP-galactopyranose mutases (UGMs/GLFs), which are flavin-binding coenzymes catalyzing the reversible conversion of UDP-Gal<sub>p</sub> into UDP-Gal<sub>f</sub>. The presence of the Gal<sub>f</sub> biosynthetic pathway in several eukaryotic and prokaryotic pathogens, the important role of the enzyme in the viability of these organisms and its absence in mammals have led to the proposal of UGM as major target for the development of wide spectrum drugs.

The existence of Gal<sub>f</sub> metabolism in nematodes is uncertain. While genes coding for UGM homologs are certainly present in Spirurid, Tylenchid and Rhabditid nematodes, a direct proof of the occurrence of synthesis of Gal<sub>f</sub> in a nematode species has not hitherto been reported.

*C. elegans* has a single gene coding for a UGM homolog, termed *glf-1*; we have characterized its function and determined that it is essential for viability and required for synthesis of the worm's surface coat glycans. Heterologous rescue experiments with a protozoan UGM homolog corroborate the hypothesis that *C. elegans* GLF-1 is a functional UGM, while the pattern of expression and subcellular localization are consistent with a role in synthesis of the surface coat and in modification of nucleotide sugars. Using HPLC and mass spectrometry, we have analysed the sugar nucleotide pools of wild-type *C. elegans* and confirmed the presence of UDP-Gal<sub>f</sub>. Our results reveal a new aspect of the glycomic repertoire of *C. elegans*, namely the occurrence of synthesis of Gal<sub>f</sub> and its important direct or indirect role in synthesis of surface coat glycans, pointing to nematode UGMs as novel potential targets for the development of nematicidal drugs.

### **Neutralising monoclonal antibodies to the hookworm aspartic protease *Na*-APR-1: implications for a multi-valent vaccine against hookworm infection and schistosomiasis**

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The major intestinal aspartic protease of the human hookworm, *Na*-APR-1, is an efficacious vaccine antigen that likely exerts its effect by generating antibodies which bind to the parasite gut and neutralize the hemoglobinolytic activity of the enzyme, impairing the worms' ability to feed. Vaccination with an active protease is problematic, so we made catalytically active (*Na*-APR-1<sub>wt</sub>) and inactive (*Na*-APR-1<sub>mut</sub>) forms of recombinant *Na*-APR-1 and showed that *Na*-APR-1<sub>mut</sub> protected dogs against a heterologous hookworm challenge, and anti-*Na*-APR-1<sub>mut</sub> antibodies neutralized the catalytic activity of *Na*-APR-1<sub>wt</sub>. In an effort to define the epitopes recognised by inhibitory antibodies, monoclonal antibodies were raised against *Na*-APR-1<sub>mut</sub> and tested for *in vitro* neutralisation of *Na*-APR-1<sub>wt</sub>. Three IgG mAbs exhibited various degrees of inhibition and were shown by preliminary mapping to bind to linear epitopes contained within a 57 amino acid fragment of *Na*-APR-1. Furthermore, localisation of these mAbs to the hookworm intestinal lumen confirmed the presence of these epitopes on the parasite-derived molecule. This enzyme fragment – termed Fragment 5A – has been expressed at high yield in soluble form with various fusion partners, including the schistosomiasis vaccine antigen *Sm*-TSP-2-EC2. Studies aimed at assessing the anti-hookworm vaccine efficacy of this fusion protein, and eventually the potential effectiveness of a chimeric protein vaccine for hookworm infection and schistosomiasis, are now underway.

### **The origin and evolution of nematode Wnt/beta-catenin signalling pathways**

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Wnt signalling is used throughout the metazoa to regulate cell fate during development. The canonical Wnt signalling pathway involves the binding of secreted Wnt ligands to their target membrane receptors, leading to accumulation of beta-catenin in the nucleus. Nuclear beta-catenin binds to and activates TCF/LEF-1 transcription factors, bringing about changes in gene expression in the target cell. In most animals the same beta-catenin is also a component of the cadherin-catenin cell adhesion complex, which means that one protein can potentially integrate cell adhesion and gene expression regulation. However, in *C. elegans* there are four different beta-catenin molecules, with one molecule dedicated to cell adhesion, and three others involved in Wnt signalling. Moreover, several key components conserved between insects and vertebrates are apparently missing in *C. elegans*.

To determine whether this unique situation represents the ancestral condition of the nematode phylum I have searched the draft genome sequences of *Trichinella spiralis* and *Brugia malayi* for Wnt signalling component genes. This analysis has revealed that *B. malayi* is much like *C. elegans*, having multiple, specialised beta-catenins, and lacking obvious homologues of the same Wnt signalling components that are also missing from *C. elegans*. In contrast *T. spiralis* possesses a much more conventional suite of Wnt signalling components. All the Wnt signalling components absent from *C. elegans* and *B. malayi* are present in *T. spiralis*. Strikingly, I have also found the *T. spiralis* homologues of vertebrate Wnt signalling components that are not encoded in any known arthropod genome. These are the first Ecdysozoan representatives of these genes to be identified. Taken together these results show that the Wnt signalling system in *T. spiralis* is much less derived than those found in *C. elegans* or *Drosophila*, and offers a window into the ancestral state of Wnt signalling in the nematode phylum.

Recent work from a number of systems suggests that Wnt/beta-catenin signalling has an ancestral role in the regulation of asymmetric cell division. We are now investigating ways of addressing the function of Wnt signalling components in *T. spiralis*, adapting RNA interference strategies used in *C. elegans* to knock-down gene function in developing *T. spiralis* embryos.

**Mitochondrial encoded genes are up-regulated in response to depletion of essential *Wolbachia* endobacteria from *Brugia malayi***

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The filarial nematodes *Wuchereria bancrofti*, *Brugia malayi* and *Onchocerca volvulus* infect humans and cause the debilitating diseases lymphatic filariasis and onchocerciasis (River blindness) in 150-200 million people. The past decade has seen several groups publishing results about the *Wolbachia* endobacteria found in these three nematodes. Much of the work has demonstrated that, in addition to being strictly vertically transmitted to the eggs, the endobacteria are known to be essential for oogenesis, embryogenesis, larval development and adult survival, making them mutualists. Antibiotic therapy, i.e. 4-6 weeks doxycycline, is approved as an antifilarial treatment for individuals and may be extended to include current community directed efforts to eliminate transmission. A first step to developing better anti-*Wolbachia* treatments is to understand their role in the symbiosis at the molecular level. Previously we had identified a phosphate permease that was up-regulated in the nematode concomitant with depletion of *Wolbachia* by differential display PCR. With completion of the sequencing of *B. malayi* and the *Wolbachia* of *B. malayi* (*wBm*) genomes a microarray containing oligos for the approximately 18,000 genes, including mitochondria and *wBm* genes was developed by the *Brugia malayi* Microarray Consortium. The microarray provides an easier and faster method of screening for regulated genes over differential display. The microarray was hybridized with probes made from RNA extracted from control and tetracycline worms collected on days 6, 15 and 36 after commencing treatment of IL-5 knockout mice with patent *Litomosoides sigmodontis* infections, a murine model of filarial infection. Setting the false discovery rate to <5% resulted in hundreds of genes as positive hits. More genes were regulated on days 6 and 36 than day 15 of treatment and down-regulated genes outnumbered up-regulated genes. Many of these genes are "house keeping" genes including ribosomal genes, actin, myosin, initiation and elongation factors, etc. Unexpectedly, several genes that encode subunits of cytochrome c oxidase and cytochrome bc1, complexes of the electron transport chain, were up-regulated concomitant with the depletion of *Wolbachia*. Both cytochrome c oxidase and cytochrome b require heme for their activity, a molecule that cannot be synthesized by *B. malayi* as none of the genes that encode the proteins for heme synthesis have been found in the genome. However, all of these genes are present in *wBm*. Our results further support the hypothesis that *Wolbachia* are a source of heme to their filarial nematode hosts, and supports targeting the heme synthesis pathway for anti-*Wolbachia* drug discovery in our current consortium efforts.

### **Intestinal granuloma formation and egg expulsion requires host bacterial gut flora**

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According to recent studies, Schistosome infections are the fourth most prevalent helminth infection worldwide and fatalities arise due to granulomatous, immune-mediated responses to eggs that become trapped in the host liver and gut. During *Schistosoma mansoni* infection the immune response characteristically switches from an initial Th1 immune response (IFN- $\gamma$ ) to a strong Th2 response (IL-4, IL-13), that is itself eventually controlled by strong regulatory mechanisms (e.g IL-10, Treg). In our previous *in vivo* studies, we demonstrated that MyD88, a central adaptor molecule for innate immune responses, is necessary to initiate an antigen-specific Th1 response. Interestingly, *in vitro*, various schistosomal antigens fail to stimulate dendritic cells to release pro-inflammatory cytokines, indicating that additional stimuli are required to initiate antigen-specific Th1 responses. Schistosome eggs penetrate into the lumen of the gut and we hypothesized that during this process the epithelial barrier might be disrupted to allow the commensal bacteria to enter into the Lamina propria. There, bacterial PAMPs like LPS could locally act as the necessary "adjuvants" to instigate a Th1 response. We therefore investigated whether *Schistosoma* parasites manipulate the hosts bacterial repertoire and analysed the parasitological, microbiological and immunological responses of infected mice that were simultaneously depleted of bacteria using broad spectrum antibiotics and antimycotics in the drinking water. The variety and amount of commensal aerobic and anaerobic bacteria did not change during 22 weeks of schistosomal infection. Confocal microscopy of intestinal samples revealed internalization of ZO-1 at the tight junction and local production of IFN- $\gamma$ , indicating destruction (leaking) of the epithelium. After bacterial depletion, we found significantly less inflammation in the intestine and this was accompanied by a significant decrease in egg load, intestinal granuloma frequency and development. Interestingly, mesenteric lymph-node Th1 cell responses were dampened in infected-depleted mice, however, liver granuloma development was normal indicating that the immune responses were only affected in bacteria-residing areas. Ongoing experiments are elucidating the activities of intestinal dendritic cells during schistosomal infection.

This study demonstrates for the first time, that commensal, host derived, bacteria are necessary to perpetuate the life-cycle of a helminth, by supporting egg-penetration into the lumen via Th1-induced immune responses.

### **Short read sequencing technology applied to studying the *Schistosoma mansoni* transcriptome: a new approach to gene modeling and quantitative gene expression analysis**

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*Schistosoma* spp. are helminth parasites responsible for causing schistosomiasis in approximately 200 million people in 74 developing countries and for claiming about 300,000 lives each year. *S. mansoni* is endemic in the sub-tropical region of Africa where it causes important levels of morbidity and economical losses. Praziquantel, active against the adult schistosome, is the front line drug for treatment of schistosomiasis. Although highly effective, it does not prevent against re-infection and the possibility of the emergence of resistant strains is a constant threat. Therefore, the search for new drug targets for intervention and vaccine candidates is vital.

As part of this, a combined effort has led to a draft genome sequence of *S. mansoni*, one of the most clinically relevant species. Other efforts regarding transcriptome analysis in this species include the generation of many ESTs and microarray data from different life stages of the parasite. Although these data have been successfully used in the identification of gene structures and the study of differentially expressed genes throughout the parasite's life cycle, the accurate prediction of exon-intron boundaries and different splice variants is still an enormous challenge.

Our work describes the use of new sequencing technologies (Solexa® (Illumina) and 454-pyrosequencing) to provide accurate gene predictions based on the sequencing of transcriptomic material from selected life stages of *S. mansoni*. This new approach has many advantages such as the high sensibility and reproducibility of the method and that relatively low quantities of RNA material are needed to perform one assay (a common limitation in helminth research). In addition, sample preparation is easy and fast and it does not include the sometimes-complicated ligation/cloning steps that are often laborious and can introduce certain biases.

With this approach we aim to gain a better understanding of gene structures by improving the accuracy of the current annotation of the *S. mansoni* genome. The possibility of using this technology for quantitative analysis of gene expression using sequencing data is also discussed.

### **Involvement of a Rho/Dia/Src signalling complex in gonad differentiation of *Schistosoma mansoni***

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Schistosomiasis is one of the most important parasitic infections worldwide affecting humans and animals. Parasitic helminths of the genus *Schistosoma* are the causative agents of this disease. *Schistosomatidae* represent the only bisexual family of the class *trematoda*. Paired females produce non-embryonated eggs, of which approximately 50% are swept into the circulation and are mainly deposited in spleen and liver causing the severe pathology. A continuous pairing-contact is essential for the development of the reproductive organs of the female. By pairing, mitogenic and differentiation processes of germ cells into oocytes and vitellocytes are initiated. Cytoplasmatic protein tyrosine kinases are well known members of signalling cascades regulating such processes.

In previous studies the Src-like cellular tyrosine kinase SmTK3 was identified, which may play a role in receptor tyrosine kinase-mediated signalling in schistosomes. Employing *in situ* hybridisation and immunolocalisation experiments the expression of SmTK3 was detected specifically in the ovary and vitellarium of females and in the testes of males.

Using yeast two-hybrid (YTH) analyses we identified a number of 43 clones, representing putative interaction partners of SmTK3, 8 of which encode the *Schistosoma* homologue of a „diaphanous-related“ formin, SmDia1. Results of differential interaction analyses will be presented that show the supportive role of the unique region of SmTK3 for SmDia1 binding. As shown in cell cultures of higher eukaryotes *in vitro*, signalling pathways containing Src-kinases and Rho GTPases lead to diaphanous proteins during cytoskeleton organisation processes associated with mitosis, cytokinesis, and cell proliferation. Therefore, we investigated the potential of SmDia1 also to interact with the GTPase SmRho1 from *S. mansoni*. This was done by further YTH analyses using constitutively active variants of SmRho1 generated by site-directed mutagenesis. Finally, *in situ* hybridisation experiments were performed to localise the transcripts of SmDia1, SmRho1 and SmTK3 in both male and female adults. Based on our results and on previous data from studies in *Drosophila*, mouse and human, we suggest the existence of a minimum of two conserved cooperative signal-transduction pathways, involving Rho and Src, that bridge at Dia to organise cytoskeletal events in the gonads of *S. mansoni* adults. To our knowledge this is not only the first report of the existence of these pathways in gonad tissues of a helminth, but also in gonad tissues of eukaryotes in general.

### **The *Haemonchus contortus* ACR-23 homologue of the acetylcholine receptor DEG-3 subfamily and its role in sensitivity to AADs**

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The emergence of drug resistance and multi-drug resistance to all known classes of anthelmintics is stressing the need for new drugs against gastro-intestinal nematodes. A novel chemical class of synthetic anthelmintics, the amino-acetonitrile derivatives (AADs), was discovered recently. In *Caenorhabditis elegans*, the AADs were found to act through the nicotinic acetylcholine receptor ACR-23, a member of the nematode-specific DEG-3 subfamily. Preliminary data indicated related receptors to be involved in AAD susceptibility also in the target parasite *Haemonchus contortus*. Using a novel *in vitro* selection procedure, we have developed mutant *H. contortus* worms populations that are over a thousand-fold resistant to AAD. A cDNA library from a AAD susceptible adult worms was constructed and the full-length sequence of the *H. contortus* *acr-23* homologue (*Hc-acr23H*) was determined. In order to investigate the role of *Hc-acr23H* in AAD sensitivity, we cloned and sequenced *Hc-acr23H* and related nAChR genes from AAD susceptible *H. contortus* and their AAD resistant progeny, at the level of genomic DNA as well as complementary DNA. Three different mutations were observed exclusively in AAD resistant worms, and they all resulted in miss-spliced *Hc-acr23H* transcripts that lacked particular exons. These smaller mRNA products of AAD-resistant worms either contained premature stop codons or lacked the sequence encoding for the N-terminal export signal. In another selected AAD resistant isolate, the *H. contortus* Howick, no miss-spliced was detected but a premature stop codon was discovered in the *Hc-acr23H* transcript. These results strongly indicate that *Hc-acr23H* is the most likely target for AAD action against *H. contortus* and that loss-of-function splice mutations in *Hc-acr23H* are responsible for loss of sensitivity to AADs.

### **Function and Regulation of *Haemonchus contortus* genes**

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*Haemonchus contortus* is a gastro-intestinal parasitic nematode of ruminants. Symptoms of infection range from anaemia to mortality, particularly in younger animals; thus infection is a contributing cause for productivity losses and significant economic damage. Current methods of control include the use of antihelmintic chemicals but this has led to the development of strains resistant to these chemicals. As a result there is an increasing need for immunological control of infection through molecular vaccines. Functional gene analysis through knock-out or other approaches would aid in identifying vaccine targets and also in understanding the biology of the parasite.

RNA interference is an extremely effective technique to knock out specific genes to examine their function. RNAi has been used extensively in *Caenorhabditis elegans* but has proven to be less effective in parasitic nematodes. However we have obtained successful knockdown for some *H. contortus* genes. We are currently testing different genes and dsRNA delivery methods. Initial analysis of *H. contortus* genome data suggests that some parts of the RNAi pathway are conserved whilst other parts may be missing/not conserved. We are currently testing components of this pathway to confirm the knockdown is an RNAi effect and to improve on current methods.

Genes involved in essential functions such as development, reproduction and feeding in parasitic nematodes are likely to be conserved in all nematode species. This has led to the recent use of the free-living nematode *C. elegans* as a model system for examining the function and regulation of *H. contortus* genes. At present, little is known of the spatial and temporal regulation of parasitic nematode genes. We are examining the regulation of *H. contortus* genes by two main methods; analysing sequence data to identify promoter regions and regulatory motifs, and by promoter reporter constructs to examine expression patterns of specific genes in transgenic *C. elegans*.

### **Differential transcriptome analysis of free-living and parasitic L3 of *Ancylostoma caninum***

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The canine hookworm *Ancylostoma caninum* is an important parasite of dogs in tropical climates with zoonotic potential. Infective L3 are able to penetrate the skin of their host and reach their final destination, the small intestine, via the bloodstream. While penetrating the skin the L3 become parasitic. Specific transcripts were compared between free-living L3 and parasitic L3 after in-vitro migration through isolated dog skin in a specifically designed incubation chamber. To identify specific transcripts Suppression Subtractive Hybridization (SSH) was used and subtracted libraries were produced. 2000 clones of each library were spotted on high density arrays to verify stage-specific transcription via differential screening using subtracted and unsubtracted probes. Clones identified to be differentially transcribed were sequenced, processed, as well as aligned and clustered. Transcripts were analysed and annotated by gene ontology search, domain/motif search and mapping to respective pathways in *Caenorhabditis elegans*. Predicted peptides were also compared with sequences published in the NCBI database, WormBase as well as the Parasite genome WU-BLAST2 Nematode database. Results of the differential analysis will be presented.

### **Omega-1, a glycoprotein secreted by *Schistosoma mansoni* eggs, drives Th2 responses**

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The complex mixture of soluble egg antigens of the parasitic helminth *Schistosoma mansoni* (SmEA) induces strong Th2 responses both *in vitro* and *in vivo*. However, the specific molecules that prime the development of Th2-polarized T cell responses have not been identified yet. Here we report that omega-1, a glycoprotein secreted by *S. mansoni* eggs and present in SmEA, modulates human monocyte-derived dendritic cell (DC) maturation and cytokine production and conditions DCs to drive Th2 polarization with similar characteristics as complete SmEA. Furthermore, immunization of IL-4 dual reporter mice demonstrated that omega-1 alone is sufficient to drive robust Th2 responses by induction of IL-4 *in vivo*. Identical studies in IL-4R $\alpha$ -deficient IL-4 dual reporter mice revealed that this factor can initiate Th2 responses in the absence of IL-4R signalling. Lastly, recombinant omega-1 also induced Th2 responses in these assays confirming the Th2-polarizing capacity of native omega-1. Taken together, our data identify omega-1 as a single component from SmEA that is able to prime Th2 responses.

### **Proteomic analysis of non-activated and activated *Taenia solium* oncospheres**

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Human cysticercosis caused by *Taenia solium* is a major cause of seizures and epilepsy in the world. In the gastrointestinal tract of infected individuals, taeniid eggs release the oncospheres, which are then activated by intestinal stimuli, getting ready to penetrate the gut wall and reach distant locations where they transform in cysticerci. Information about oncospherical molecules is scarce, and elucidation of the oncosphere proteome could help in the understanding of the host-parasite relationship during the first steps of infection. In this study, using a mild, mainly surface-associated, trypsin digestion, followed by liquid chromatography and tandem mass spectrometry (LC-MS/MS) analysis, we could identify a set of proteins involved in several signaling and metabolic pathways, as well as in immune evasion mechanisms. Both qualitative and quantitative differences in the proteome composition were found between activated and non-activated parasites. The availability of this subset of oncospherical proteins constitutes the first step to elucidate their specific roles in the biology of the parasite.

### **Transcriptome changes in the bovine lungworm *Dictyocaulus viviparus* during the transition from L1 to L3 stage**

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The bovine lungworm *Dictyocaulus viviparus* is one of the most important parasites in cattle farming in temperate areas. This parasite causes high economic losses due to parasitic bronchopneumonia or even death of susceptible cattle. The free-living L1 lungworm larvae develop via L2 to L3 stage larvae. This development occurs within 4 to 8 days, depending on climate conditions. Since the larvae feed during this time on their reserve granules only, larval development is supposed to be accompanied with changes in larval metabolism. To identify transcripts specific for either of these stages, Suppression Subtractive Hybridization (SSH) was performed to create subtracted libraries. 2000 clones of each library were spotted on high density arrays to verify stage-specific transcription via differential screening using subtracted and unsubtracted probes. Clones identified to be differentially transcribed were sequenced, processed, as well as aligned and clustered, respectively. Transcripts were analysed and annotated by gene ontology search, domain/motif search and mapping to respective pathways in *Caenorhabditis elegans*. Predicted peptides were also compared with sequences published in the NCBI database, WormBase as well as the Parasite genome WU-BLAST2 Nematoda database and results will be presented.

**The identification of novel Th2 inducing proteins in the secreted products of L3 stage of *Nippostrongylus brasiliensis***

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Infection of mammalian hosts by nematode parasites is characterised by the initiation and development of CD4+Th2 immune responses. This is characterised by the specific induction of IL-4, IL-5, IL-13 producing Th2 cells mediating eosinophils, basophils, mucus production and IgE production by B cells. It has been long proposed that the nematode parasites achieve this by actively excreting/secreted a wide range of potentially antigenic products during their migration through several tissues site, and this may directly influence the cytokine and cellular milieu. *Nippostrongylus brasiliensis* has long been known to be a potent activator of both systemic and mucosal Th2 immune responses. This preference toward a Th2 response can be reproduced when the excreted/secreted protein products are collected from *in vitro* culture of *N. brasiliensis*. This has been shown in both the larval (L3) and adult (L5) stages of *N. brasiliensis* (Marsland *et al* 2004, Holland *et al* 2000). The host's encounter with the parasite begins with L3 infective larvae; further study of the products this larval stage releases may provide some insight into this Th2 preference. We have recently developed a novel assay of Th2 induction involving G4 mice, which have the eGFP reporter construct, inserted into the IL-4 locus. Using this assay we were able to verify that the excreted/secreted products (NES) collected from the infective L3 *N. brasiliensis* larvae induce a strong Th2 response. Furthermore, fractionation of this L3 *N. brasiliensis* NES by high performance liquid chromatography (HPLC) reveals that only a minor species of the L3 NES proteins are responsible for the induction of Th2 response and the responses were enhanced when compared to the unfractionated L3 NES. We will present data on the characterisation of this unique entity and its relationship with the excreted/secreted products of other stages of *N. brasiliensis* and nematode worm species.

**What does it take to be a parasitic nematode – the *Strongyloides* view?**

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Parasitism is an evolved trait. Nematodes seem to have been particularly good at this, apparently evolving parasitism on at least three separate occasions. Key questions are therefore how does parasitism evolve, and why are nematodes so good at it?

We have undertaken extensive microarray-based analyses of gene expression through the *S. ratti* life-cycle. Complete analyses of these gene-expression data now show that there are remarkably few differences in gene expression between most life-cycle stages, with two notable exceptions. Firstly, the gene expression of the infective larval stage seems to be most different from that of other life-cycle stages. Secondly, there is a significant difference in the expression of ribosomal protein coding genes between adult free-living and parasitic stages. These data are consistent with observations in *C. elegans* that alteration in the levels of protein synthesis can alter lifespan. For *S. ratti*, this therefore suggests that this difference in the expression of ribosomal protein coding genes may underlie the 80-fold difference in lifespan between the genetically identical free-living and parasitic adult morphs of this species. Together the results of these gene expression studies can be used to consider the key questions of the evolution of nematode parasitism. We have also used these expression and other data to prioritise genes for further study.

## Identification and localization of emodepside receptors of parasitic nematodes in *Caenorhabditis elegans*

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The mode of action of the new anthelmintic compound emodepside is not clarified in detail yet. The G-protein-coupled receptor Hc 110-R from the sheep nematode *Haemonchus contortus* was shown to be a target protein for emodepside. Its ortholog in *C. elegans* is the latrophilin like protein 1 (LAT-1). The paralyzing effect of emodepside on the nematode pharynx was decreased in *lat-1* knockout mutants. As a second target protein LAT-2 is discussed to be involved in the mode of action. A *lat-2* gene deletion mutant and dsRNA targeted at *lat-2* and were both resistant to the effects of emodepside on locomotion. Recently it was shown that the calcium activated potassium channel SLO-1 of *C. elegans* plays a key role in the mode of action of emodepside. Knockout mutants, which lack this channel, are no longer sensitive to the effect of emodepside on locomotion. Within this study, orthologs for LAT-1, LAT-2 and SLO-1 were identified in the parasitic nematodes *Ancylostoma caninum* (canine hookworm) and *Cooperia oncophora* (cattle strongyle), respectively. To examine where these proteins are located in the nematode, *C. elegans* was chosen as a model organism. Putative promoter regions (approx. 3 kb in length) of the respective LAT1, LAT2 and SLO-1 proteins of *A. caninum* and *C. oncophora*, respectively, were amplified using Genome Walker technology (Clontech) and cloned into a GFP-Reporter vector. *C. elegans* hermaphrodites were transfected and the offspring were analyzed for GFP-expression driven by the heterologous promoter. Expression occurred in the pharynx and in the tail region of transgenic worms. Expression could be detected as well in neurones as in muscle cells.

## Parallel studies of developmentally essential protein folding enzymes in the model nematode *Caenorhabditis elegans* and the filarial parasite *Brugia malayi*

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The enzymes prolyl 4-hydroxylase (P4H) and protein disulphide isomerase (PDI) are developmentally essential protein folding catalysts. Both reside in the endoplasmic reticulum (ER) where the folding of secreted and cell surface proteins occurs. P4H catalyses the conversion of peptide bound proline residues to 4-hydroxyproline, a residue found predominantly in collagens. In the majority of organisms P4H enzymes are hetero-oligomeric complexes composed of PHY and PDI subunits. Only the PHY subunits contribute directly in the hydroxylation of proline residues, PDI is therefore often termed the inactive subunit. Importantly PDI is also found abundantly in a non-P4H associated form where it acts independently to catalyse additional protein folding events in the ER. Disulphide bond formation, catalysed by PDI, is a key step in the stability and function of many proteins in the secretory pathway. We are looking at the roles played by these enzymes in nematodes, particularly with respect to the folding of the collagens which form the specialised extra-cellular matrix of the cuticle.

We are using the full potential of the genetic, transgenic and molecular techniques available in the model organism *Caenorhabditis elegans* to define the functions of P4H and PDI enzymes. Specialised biochemical techniques, required to express these multi-subunit enzymes, has enabled enzymatic activity and subunit association to be analysed *in vitro*. This demonstrates that the P4H subunits of *C. elegans* assemble in a unique manner. Genetic mutants of the P4H subunit encoding genes, *phy-1*, *phy-2* and *pdi-2*, reveal that these genes are essential for development and nematode body morphology. The developmental timing and cell specificity of gene expression, along with the malformation of the cuticle in genetic mutants at the level of EM and of specific collagens, are consistent with a role in collagen folding for these genes.

Due to the essential nature of these enzymes in formation the nematode cuticle we are, in parallel, studying the homologous genes from the human infective filarial parasite *Brugia malayi*. We are using transgenic expression of *B. malayi* genes in *C. elegans* mutants to define function and have successfully rescued the *pdi-2* mutant with the homologous parasite gene. Tissue specific expression for *B. malayi phy-1* is similar to the *C. elegans* enzymes, as shown by parasite gene promoter driven expression of reporters in transgenic *C. elegans*. Co-expression is being used to determine enzyme activities and subunit associations which have revealed some parasite specific characteristics, such as PDI independent solubility of the PHY subunits and low level P4H activity from a *B. malayi* PHY-1 homotetramer. The *B. malayi* genome database is critical to this work in defining any additional members of the parasite PHY and PDI gene families which may be involved in collagen P4H complex formation.

## Hydra Meeting Delegate List

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