

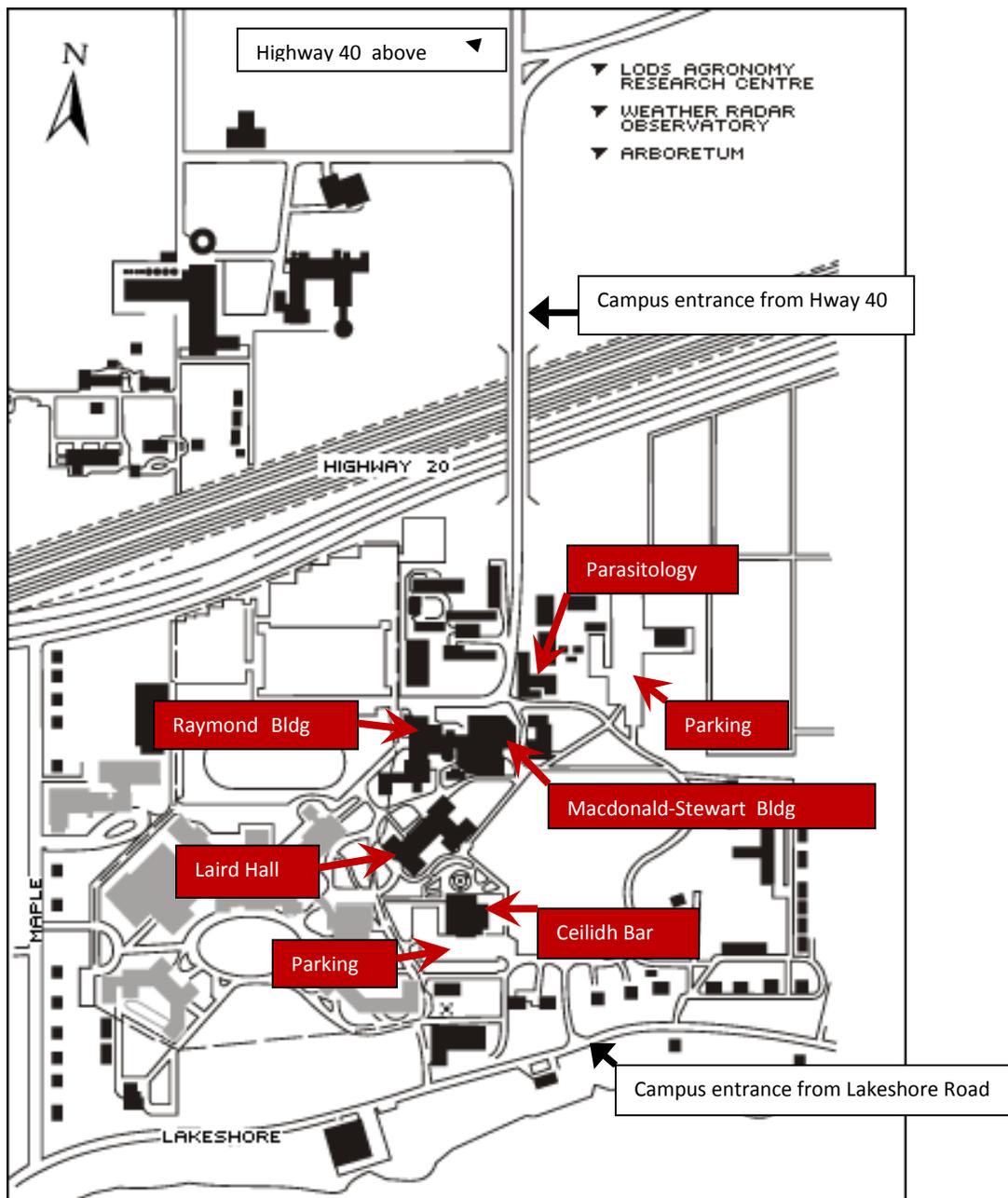
Eleventh Annual Quebec Molecular Parasitology Symposium

11ième Symposium Annuel de Parasitologie Moléculaire du Québec

June 9-10, 2011
Macdonald Stewart Building,
Macdonald Campus of McGill University
21,111 Lakeshore Road
St. Anne De Bellevue
Québec, Canada H9X 3V9



Macdonald Campus Map



Access by Highway 40 West from Montreal:

Take exit #44 Blvd Morgan (#41 for Ste Anne de Bellevue is closed); continue on service road until you have to turn right at Chemin Ste-Marie; go up the hill and turn right at stop sign; you will cross Hwy 40 and the fields of the Macdonald Farm; at the stop sign turn left, go over the overpass and at the bottom, stop sign, you will see the Institute of Parasitology on your left and the Macdonald-Stewart Bldg across the street.

Access by Highway 20 West from Montreal:

Take exit #39 Ste Anne de Bellevue; at lights turn left and continue on St-Pierre Street until Blvd Ste-Anne (one set of lights and 3 stop signs); at 3rd stop sign turn left and continue on this road (you are on Lakeshore Road) until you reach a large green sign indicating Macdonald Campus; turn left; at stop sign you can go left for the Ceilidh Bar or right to reach Macdonald-Stewart and the main buildings as well as the main parking lot.



CHPI 2011 Program

Thursday, June 9th

09:00-09:15 Opening Remarks by **Dr. Armando Jardim**, Director of the CHPI. (**Raymond Bldg, R2-045**)

9:15-10:15 Keynote Speaker:

Prof. Dennis Kyle (University of South Florida, Tampa, FL.)
“Mechanisms of recrudescence and resistance to Artemisinin in Malaria”

10:15-10:30 COFFEE BREAK (Faculty Lounge, Macdonald-Stewart Bldg, MS2-022)

Session I: Parasite Molecular Biology (Chair: Reza Salavati)

10:30-10:50 **Rona Strasser**, and Armando Jardim.

Leishmania donovani glycosomal proteins form complexes in the cytosol for trafficking to the organelle surface.

10:50-11:10 **Normand Cyr**, Jean-Louis Schwartz and Armando Jardim

A hydrophobic domain of the *Leishmania donovani* peroxin 14 is required for membrane association

11:10-11:30 **Colin Du**, Nakita Haynes, Hamed Shateri Najafabadi, Reza Salavati, and Janet Yee

Characterization of p105: A Putative Transcription Factor for the Core Histone Genes in *Giardia lamblia*

11:30-11:50 **Daniel Feingold**, Laura Nilson and Joseph A. Dent

Characterization of three ligand gated ion channel subunits – Potential pesticide targets?

11:50-1:30 LUNCH (Faculty Lounge, Macdonald-Stewart Bldg, MS2-022)

Session II: Drugs and Drug Targets (Chair: Elias Georges)

1:45-2:05 **Sonia EDAYE**, Scott BOHLE, Dagobert Tazoo, Elias Georges.

Characterization of Antimalarial Activity of Two Novel Chloroquine Derivatives

2:05-2:25 Victoria Muise, **Tita N. Walter**, Eliane Ubalijoro, Timothy G. Geary.

Screening for novel anthelmintics that act on nematode neuropeptide receptors

2:25-2:45 **Claudia M. Wever**, Jin-Kyung Chang, Danielle Farrington, Patrick Janukavicius, Igor Putrenko and Joseph A. Dent.

Validating Acetylcholine-Gated Chloride Channels as Novel Nematocide Targets.

2:45-3:05 **Nelly El-Sakkary**, F. El-Shehabi; A. Taman and P. Ribeiro.

Characterization of the Novel Schistosomal Receptor SmGPR-3

3:05-3:30 **COFFEE BREAK (Faculty Lounge, Macdonald-Stewart Bldg, MS2-022)**

Session III: Biochemistry and Physiology (Chair: Paula Ribeiro)

3:30-3:50 **Serge Cloutier**, Mukesh Samant, Conan Chow, Barbara Papadopoulou

Regulation of the translation initiation factor 2-alpha subunit throughout *Leishmania's* life cycle and under stress.

3:50-4:10 **Amber Olson**, Kin Chan, Chi-Yip Ho, and Janet Yee

Gene expression profiling of *Giardia lamblia* isolates from two different genetic assemblages.

4:10-4:30 **Laura-Isobel McCall**, Greg Matlashewski.

Involvement of the *Leishmania donovani* virulence factor A2 in the parasite stress response: protection against heat shock and oxidative stress.

4:30-4:50 **Carolina P.Gómez**, Marina Shio, Martin Olivier, Albert Descoteaux.

Role of the Protein SHP-1 in the Biogenesis of the Phagolysosome.

5:00-7:30 **POSTER SESSION & HAPPY HOUR (Ceilidh Bar and Lobby, Centennial Centre)**



Friday, June 10th

09:15-10:15 Keynote Speaker: (Raymond Bldg, R2-045)

Prof. Miguel Stadecker (Tufts University, Boston, MA)

“Induction and regulation of the pathogenic Th17 response in murine schistosomiasis”

10:15-10:30 COFFEE BREAK (Faculty Lounge, Macdonald-Stewart Bldg, MS-022)

Session IV: Immunity (Chair: Florence Dzierszinski)

10:30-10:50 Floriana Berretta, Ciriaco A Piccirillo, and Mary M Stevenson.

Regulatory CD4⁺Foxp3⁺ T cells up-regulate CXCR3 expression less efficiently than effector CD4⁺Foxp3⁻ T cells during blood-stage *Plasmodium chabaudi* AS infection.

10:50-11:10 Esther Dalko, Nilson Quintana, Mégane Lefebvre, Jaime Sanchez-Dardon, Robert Moreau & Tatiana Scorza.

Unraveling a possible implication for free heme in skewed Th1 responses in malaria.

11:10-11:30 Fikregabrail A. Kassa, Marina T. Shio, Momar Ndao and Martin Olivier.

Serum proteins alter the host innate immune response against hemozoin.

11:30-11:50 Louis-Philippe Leroux, Manami Nishi, Sandy El Hage, and Florence S. Dzierszinski.

Subversion of MHC-II antigen presentation by *Toxoplasma gondii* is mediated by the upregulation of the MHC-II associated invariant chain.

11:50-1:30 LUNCH (Faculty Lounge, Macdonald-Stewart Bldg, MS2-022)

Session V: Parasite resistance (Chair: Catherine Bourguinat)

1:45-2:05 **Felipe Dargent**, Marilyn Scott, Andrew Hendry, Gregor Fussmann
Evolution of resistance in *Poecilia reticulata* released from predators and parasites.

2:05-2:25 **Hugues Nana-Djeunga**, Catherine Bourguinat, Sébastien D.S. Pion, Joseph Kamgno, Jacques Gardon, Flobert Njiokou, Michel Boussinesq, Roger K. Prichard.
Resistance of *Onchocerca volvulus* to ivermectin: a prospect at time of uncertainty

2:25-2:45 **Patrick Janukavicius**, Laura Tiseo, Ludmel Urdaneta, Roger Prichard, Joseph Dent
The mapping of ivermectin resistance genes

2:45-3:05 **Pablo Godoy**, Jing Lian, Hua Che, Robin N. Beech, Roger K. Prichard.
Interaction of macrocyclic lactone endectocides with *Haemonchus contortus* P-glycoproteins

3:05-3:30 **COFFEE BREAK (Faculty Lounge, Macdonald-Stewart Bldg, MS2-022)**

Session VI: Host-parasite interaction (Chair: Timothy Geary)

3:30-3:50 **Felipe Perez-Jvostov**, Andrew P. Hendry, Gregor Fussmann and Marilyn E. Scott.
The importance of host-parasite co-evolution on life history traits: A test with guppies and *Gyrodactylus*.

3:50-4:10 **Neda Moradin**, Diana Matheoud, Mitsunori Fukuda, Michel Desjardins, Albert Descoteaux.
Alteration of macrophage membrane fusion regulators during infection with *Leishmania* promastigotes

4:10-4:30 **Felix Hugentober**, Karen K. Yam, Raya Mahbuba, Martin Olivier and Benoit Cousineau.
Development of a live vaccine against *Leishmania major* using the Gram-positive bacterium *Lactococcus lactis*

4:30-4:50 Michaela Müller, Prasad K. Padmanabhan, Hiva Azizi, **Barbara Papadopoulou**:
Retroposon-mediated post-transcriptional control in the parasitic protozoan *Leishmania*

4:50 – 5:15 **CLOSING REMARKS**

5:30 – 8:00

DINNER & PRIZES

(Ceilidh Bar, Centennial Centre)

8:00 – Late

ENTERTAINMENT with the Band “Papacool” (cash bar)

POSTERS

- 1 Samiah Alam, Janet Yee and Steven Rafferty: Expression & characterization of a cytochrome-*b*₅ from *Giardia lamblia*.
- 2 Héctor Álvarez de Celis, Albert Descoteaux & Pascale Duplay : Degradation of Dok Proteins During Phagocytosis of *Leishmania* by Macrophages.
- 3 Guillermo Arango Duque, Mitsunori Fukuda, Albert Descoteaux: Characterization of Synaptotagmin XI in macrophages.
- 4 Dayal Dasanayake, Louis-Philippe Leroux, Armando Jardim, Florence S. Dzierszinski: Identification of *Toxoplasma gondii* factors that are responsible for the inhibition of IFN- γ mediated up-regulation of MHC Class II in macrophages.
- 5 Corine Demanga Galamo, Lena Juliette Daher, Eric Prieur, Catherine Blanc, Jean-Louis Pérignon, Pierre Druilhe: Malaria vaccine rational design: example of MSP3 family-based vaccine.
- 6 Nilson Quintana, Véronique Gaudreault, Diane Tshikudi Malu, Olha Kevorkova, Jaime Sanchez Dardon, Martin Olivier, Robert Moreau & Tatiana Scorza : Alterations in bone status in malaria: effect of hemozoin and heme on the differentiation, viability and function of bone remodeling cells *in vitro*.
- 7 Doris González Fernández, Odalis Sinisterra, Kristine G. Koski, Marilyn E. Scott: Combined Impact of Nutritional Deficiencies and Infectious Diseases on Pregnancy Outcomes and Infant Health in a Rural Community in Panama.
- 8 Stephanie Goyette, Momar Ndao, Brian J. Ward, and Florence S. Dzierszinski: Insights into *Toxoplasma gondii* reactivation through biomarker discovery.
- 9 Kasra Hassani and Martin Olivier: Immunomodulatory impact of *Leishmania*-induced macrophage exosomes.
- 10 Hamed Hojjat and Armando Jardim: Dissection of the *Leishmania donovani* LdPEX14-LdPEX5 Interacting Domains.
- 11 Amandine Isnard, Martin Olivier: *Leishmania* protease GP63 affects Nuclear Pore Complex (NPC) to enter into host macrophage nucleus.

- 12 Kala Smriti, Najafabadi Hamed Shateri, Salavati Reza: The role of Tb09.211.2960, a putative RNA-binding protein involved in post-transcriptional regulation of gene expression in *Trypanosoma brucei*.
- 13 Marie-Claude Laffitte, Danielle Légaré and Marc Ouellette : *Mre11* involvement in DNA double strand break repair, generation of extrachromosomal DNA amplicons and drug resistance.
- 14 Christine Matte & Albert Descoteaux: Disruption of Akt downstream events by *Leishmania major* promastigotes.
- 15 James McLean and Armando Jardim: Characterization of the *Leishmania major* glycosomal PTS2 receptor, Peroxin 7.
- 16 Jenny Miu, Rebecca Ing, and Mary M. Stevenson: Dendritic cells regulate CD4⁺ T cell responses to blood-stage malaria.
- 17 Layla S. Mofid, Theresa W. Gyorkos, Quentin Bickle: Lessons learned during MSc thesis field research on soil-transmitted helminths in rural China.
- 18 Chase Reaume, Christine Ouellet, and Janet Yee: The application of counterflow centrifugal elutriation and quantitative RT-PCR in the cell cycle analysis of *Giardia lamblia*.
- 19 Ruiz-Lancheros Elizabeth, Viau Charles, Tita Walter, Geary Timothy: Cut worms: A useful tool to study anthelmintic activities in *Caenorhabditis elegans*.
- 20 Mukesh Samant, Prasad Padmanabhan, and Barbara Papadopoulou: Disruption of the ATP-dependent RNA-helicase-67 gene impairs amastigote-specific growth and differentiation in *Leishmania*.
- 21 Hamed Shateri Najafabadi, Chad Macpherson, Reza Salavati: A global functional atlas of *Trypanosoma brucei* genome.
- 22 Lisa Starr, Maurice Odiere, Marilyn E. Scott, Johanne Naylor, Marie-Pierre Lardeau, Kristine G. Koski, Odalis Sinisterra : Vitamin D, Growth, Cytokines and Infection Status in Panamanian Mothers and their Preschool Children.
- 23 Guillaume St-Pierre, Pampa Bhaumik, Christian St-Pierre, Sachiko Sato: Galectin-3 implication in the neutrophils recruitment during cutaneous leishmaniasis.
- 24 Christina Tadiri, Felipe Dargent, Marilyn Scott: The impacts of food availability on epidemic dynamics: A *Poecilia reticulata-Gyrodactylus turnbulli* host-parasite model.
- 25 Rajesh M. Valanparambil, Yovany Moreno, Mariela Segura, Mifong Tam, Pierre-Paul Gros, Timothy G. Geary and Mary M. Stevenson: Proteomic analysis of excretory-secretory products of *Heligmosomoides polygyrus* assessed with next-generation sequence transcriptomic information.

ABSTRACTS

Samiah Alam¹, Janet Yee^{1,2} and Steven Rafferty^{1,2} ¹Environmental and Life Sciences Graduate Program, ²Biochemistry Program, Trent University

Expression & characterization of a cytochrome-*b*₅ from *Giardia lamblia*

Most eukaryotes obtain their energy from reduced carbon compounds with the mitochondria and oxidative phosphorylation having central roles in this process. A key component of oxidative phosphorylation is the respiratory chain, which uses heme-containing cytochromes as electron shuttles. Although it is a eukaryote, the parasitic protist *Giardia lamblia* lacks mitochondria and the components of oxidative phosphorylation, and cannot synthesize heme. However, *Giardia* encodes a gene with sequence similarity to cytochrome *b*₅, a potential heme-binding protein. This gene was PCR amplified from *Giardia* genomic DNA, cloned into a pET-14b expression vector and expressed in *E. coli*. The protein was isolated with heme bound to it, and its oxidized and reduced UV-visible spectra are consistent with those of known *b*-type cytochromes with two histidine residues as axial ligands to the heme. Furthermore, the heme is non-covalently bound to the protein as it can be extracted by acid butanone treatment. While such cytochromes serve as electron shuttles with well-established roles in electron transfer pathways, the role of the *Giardia* cytochrome *b* and mechanism by which it acquires heme are not yet known. This work is supported by NSERC Discovery Grants (JY and SR) and a NSERC USRA (SA).

Héctor Álvarez de Celis, Albert Descoteaux & Pascale Duplay, Institut National de la Recherche Scientifique-Institut Armand-Frappier, Université du Québec, Laval, Canada.

Degradation of Dok Proteins During Phagocytosis of *Leishmania* by Macrophages.

Dok proteins are adaptor proteins that share structural similarities characterized by an amino-terminal PH domain, a central PTB domain and a carboxy-terminal domain rich in proline and tyrosine residues. Three members of this family, Dok-1, Dok-2 and Dok-3 are expressed in macrophages. In macrophages, Dok proteins are implicated in the negative regulation of signaling in response to LPS and various cytokines and growth factors. We investigated the role and the fate of Dok proteins during the phagocytosis of *Leishmania* major promastigotes. We compared NO and TNF- α secretion in response to *Leishmania* infection in BMMs from WT and Dok1/Dok2 deficient mice. Our results show that Dok proteins positively regulate these responses. By confocal microscopy, we showed that Dok1 is recruited to phagosomes containing latex beads and that Dok remained associated to phagosomes during the maturation process. By contrast, Dok1 is absent from phagosomes containing *Leishmania* promastigotes. Further biochemical analysis revealed that Dok1 is rapidly degraded upon infection and that this degradation is mediated by the surface metalloprotease gp63. Similarly, Dok2 is a substrate of *Leishmania* gp63. In the absence of gp63, Dok1 is recruited to phagosomes containing *L. major* promastigotes. These results suggest that Dok proteins may be important regulator of macrophage responses to *Leishmania* infection.

Guillermo Arango Duque¹, Mitsunori Fukuda², Albert Descoteaux¹. ¹INRS-Institut Armand-Frappier and centre for Host-Parasite interactions. ²Department of Developmental Biology and Neurosciences, Tohoku University.

Characterization of Synaptotagmin XI in macrophages.

Synaptotagmins (Syts) are a group of type-I membrane proteins that regulate vesicle docking and fusion in processes such as the exocytosis of synaptic vesicles and hormones, and phagocytosis. All Syts possess a conserved transmembrane domain, and two tandem Ca²⁺-binding C2 domains. Syts IV and XI, however, possess a conserved serine in their C2A domain that precludes them from binding to Ca²⁺ and phospholipids. SytXI is thought to inhibit vesicle fusion or to mediate vesicle trafficking processes independent of Ca²⁺. As of today, a function has not been ascribed to SytXI. We show that, in murine macrophages, SytXI localizes to transferrin receptor 1-containing recycling endosomes. SytXI was found to be recruited early to phagosomes. Nonetheless, SytXI recruitment was absent in *Leishmania* promastigote phagosomes. We demonstrate that SytXI was degraded by *L. donovani* and *L. major* promastigotes, but not amastigotes. Degradation occurred shortly after infection, and it was dependant on the gp63 metalloprotease in the case of *L. major*. Real-time quantitative reverse transcription PCR (QPCR) data showed that *Leishmania* promastigotes lowered SytXI mRNA levels rapidly but temporarily. Also, SytXI coimmunoprecipitated with Syntaxin 13, a soluble NSF-attachment protein receptor (SNARE) involved in the early stages of phagocytosis and in protein and cholesterol trafficking. Altogether, our data point to a possible role for SytXI in phagocytosis, vesicle trafficking and in *Leishmania* infection. Presently, siRNA-mediated knockdown of SytXI is being used to study the role of SytXI in cytokine secretion.

Floriana Berretta^{1,2}, Ciriaco A Piccirillo^{1,3,4}, and Mary M Stevenson^{1,2,4}. ¹Centre for the Study of Host Resistance, ²Centre for Host Parasite Interactions, ³Department of Microbiology and Immunology, and ⁴Department of Medicine, The Research Institute of the McGill University Health Centre and McGill University, Montreal, Quebec, Canada.

Regulatory CD4⁺Foxp3⁺ T cells up-regulate CXCR3 expression less efficiently than effector CD4⁺Foxp3⁻ T cells during blood-stage *Plasmodium chabaudi* AS infection.

The chemokine receptor CXCR3 and its ligands CXCL9 and CXCL10 are critical for trafficking of pathogenic CD8⁺ T cells to the brain during experimental cerebral malaria in *Plasmodium berghei* ANKA infected mice. We investigated the role of chemokines and their receptors during blood-stage *P. chabaudi* AS infection in C57BL/6 (B6) mice which control and resolve the infection with a peak of parasitemia at days 8-9 post infection (p.i.). Real-time PCR analysis revealed significantly increased CXCL9 mRNA expression in spleen cells of infected mice at days 6, 8 and 11 p.i. while FACS analysis showed significantly increased CXCR3 expression on splenic CD4⁺ T cells at days 8 and 11 p.i. compared to naïve mice. To determine if CD4⁺ T cell populations differentially expressed CXCR3 during *P. chabaudi* AS infection, we examined CXCR3 expression on splenic CD4⁺Foxp3⁻ T cells (Teffs) compared to CD4⁺Foxp3⁺ T cells (Tregs). In naïve mice, 15% of Tregs and 15% of Teffs were CXCR3⁺, at day 8 p.i., 30% of Tregs vs. 45% of Teffs were CXCR3⁺, and at day 11 p.i., CXCR3⁺ Tregs were still 30% while CXCR3⁺ Teffs were 55%. Increased CXCR3 expression on Teffs correlated with increased protective CD4⁺T-bet⁺IFN- γ Th1 cells in the spleen. These findings indicate that CXCR3 expression is up-regulated on both Teffs and Tregs during blood-stage malaria. Higher expression of CXCR3 on Teffs might allow these cells to migrate more efficiently to the spleen, the critical tissue for immune control and resolution of the infection. Supported by FQRNT (Quebec) and CIHR (MOP14663 and MOP67211).

Hugues Nana-Djeunga^{1,2}, Catherine Bourguinat², Sébastien D.S. Pion³, Joseph Kamgno⁴, Jacques Gardon⁵, Flobert Njiokou¹, Michel Boussinesq³, Roger K. Prichard², ¹University of Yaoundé 1, Yaoundé, Cameroon; ²Institute of Parasitology, McGill University, Quebec, Canada; ³UMI 233, IRD and University of Montpellier 1, Montpellier, France; ⁴Filariasis and Other Tropical Diseases Research Centre, Yaoundé, Cameroon; ⁵UMR 5569, IRD, CNRS and Universities Montpellier 1 and 2, Montpellier, France

Resistance of *Onchocerca volvulus* to ivermectin: a prospect at time of uncertainty

Remarkable success has been achieved in the control of onchocerciasis since the introduction of ivermectin (IVM) in human therapy in 1988. However, the reliance on a single drug during decades might become problematic in the future due to the possible development of anthelmintic resistance. Sub-optimal responses and genetic changes have been reported in *O. volvulus* populations under high IVM pressure but unequivocal evidence of resistance has yet to be established. In this perspective, the present study aimed at comparing the genotypic frequencies of four SNPs occurring in the β -tubulin gene of *O. volvulus* macrofilariae collected before and after 4 annual or 13 three-monthly IVM treatments (150 μ g/kg or 800 μ g/kg) and to test their relationship with the reproductive status of the female worms. Multiple nucleotide changes associated with the dose and the frequency of IVM were observed. Three SNPs showed a high level of selection and non-random allelic associations after treatments. Therefore, they may be relevant markers to follow selection for IVM resistance in the field. A significantly higher proportion of non-fertile female worm was also observed only in heterozygotes three months after 13 IVM treatments. These results strengthen the warning that selection for IVM resistance could occur in some parasite populations. *This study was supported by The River Blindness Foundation, OCP, APOC, TDR and CHPI. We pay tribute to the late Brian O. Duke and we thank the Mbam population for his participation in the trial.*

Serge Cloutier, Mukesh Samant, Conan Chow, Barbara Papadopoulou. Research Centre in Infectious Disease, CHUL Research Centre (CHUQ), Laval University, Quebec, Canada.

Regulation of the translation initiation factor 2-alpha subunit throughout *Leishmania*'s life cycle and under stress.

In higher eukaryotes, eIF2 α is a 36kDa protein that together with eIF2 β and eIF2 γ , a GTP molecule and the initiator methionine Met-tRNA_i forms the ternary complex, which is a key component for translation initiation. The specific phosphorylation of eIF2 α at serine 51 upon various stress conditions by stress-responsive kinases is a major mechanism to downregulate general translation in eukaryotes. We showed that the *Leishmania* eIF2 α homolog is phosphorylated at threonine 166 during the parasite's differentiation from promastigote to amastigote forms and also under endoplasmic reticulum (ER) stress. We also found that the ER PERK eIF2 α kinase homolog in

Leishmania is critical for the intracellular development of the parasite as an N-truncated form of PERK acting as a dominant-negative allele prevented eIF2 α phosphorylation and markedly delayed promastigote to amastigote differentiation within macrophages. Furthermore, we report that the eIF2 α protein in *Leishmania*, which possesses an extension of 11kDa in comparison to its eukaryotic counterparts, is regulated at the post-translational level during the parasite's differentiation. Post-translational regulation of eIF2 α in promastigotes yields different eIF2 α isoforms, most likely through proteolytic cleavage, whereas only the 47kDa protein is present in amastigotes. Several mutants have been generated in order to gain a better insight into the mechanism of eIF2 α post-translational control and its putative role during the parasite's differentiation process.

Normand Cyr¹, Jean-Louis Schwartz² and Armando Jardim¹, ¹Institute of Parasitology, McGill University and ²Department of Physiology, Université de Montréal

A hydrophobic domain of the *Leishmania donovani* peroxin 14 is required for membrane association

Leishmania compartmentalizes several vital metabolic pathways the glycosome, a microbody organelle that is distantly related to peroxisomes found in plant, yeast, and mammalian cells. Trafficking of proteins into the glycosome is dependent on the specialized cytosolic receptors peroxin 5 (PEX5) for C-terminal PTS1 signals or peroxin 7 (PEX7) that recognize proteins containing peroxisomal targeting signal (PTS) 1 and 2, respectively. These cargo-receptor complexes dock to the peroxin 14 (PEX14) containing platform on the glycosome surface. This binding is critical for the translocation of cargoes across the glycosomal membrane. Previous genetic experiments have demonstrated that this glycosome biogenesis machinery is vital for the parasite survival. Structure studies have established that *Leishmania donovani* PEX14 (LdPEX14) is a peripheral membrane protein and that, in solution, forms a large homomeric complex. Binding of the receptor LdPEX5 to this complex triggers a dramatic conformational change that results in the reorganization and exposure of a hydrophobic segment on LdPEX14. Here we show that LdPEX14 exhibits membrane binding activity and that this interaction with the lipid bilayer is dependent on the presence of anionic phospholipids. Binding to lipid bilayer alter the LdPEX14 structure which confers protease protection possibly due to insertion of this protein into the lipid bilayer. Furthermore, deletion of the hydrophobic region of LdPEX14 disrupts its capacity to bind membranes. Biophysical analyses show that this hydrophobic region was membrane active and is capable of disrupting the bilayer integrity possibly through the formation of a transmembrane pore. This may be functionally important for translocation of proteins into the glycosome matrix.

Esther Dalko, Nilson Quintana, Mégane Lefebvre, Jaime Sanchez-Dardon, Robert Moreau & Tatiana Scorza.
Département des sciences biologiques, Université du Québec à Montréal, Montreal Canada.

Unravelling a possible implication for free heme in skewed Th1 responses in malaria.

There is compelling evidence that a polarized T helper (Th)-1 response, involving IFN- γ secreting CD4⁺ T cells, cytophilic antibodies and M1 macrophages, is pivotal for the control of blood stage malaria. Considering that antigen presenting cells subjected to oxidative stress favour the induction of Th2 responses and that elevated levels of methaemoglobin and free heme are found in subjects with malaria, we hypothesize that heme, a potent oxidant, may interfere with the induction of protective Th1 responses in malaria. As a first approach to unravel a plausible role for heme on the skewing of protective anti-malaria immunity, we examined the effects of oxidised heme (hemin, HE) on resting CD4⁺ T cells and on CD4⁺ T cells stimulated with anti-TCR antibodies under different conditions of culture. In addition, we assessed the effect of parental administration of HE on the functional state of CD4⁺ T cells and on their ulterior responses to *in vitro* stimulation. Our data demonstrates that HE induces polyclonal activation of CD4⁺ T cells and B cells and secretion of IFN- γ and IL-4 by total splenic cell populations, effects that are significantly abrogated in splenic cell cultures depleted of B cells. Interestingly, higher percentages of activated CD4⁺ T cells and B cells are also measured in mice conditioned with HE *in vivo* which associated to enhanced production of IL-4 and decreased production of IFN- γ in response to TCR stimulation. Our preliminary results indicate that HE may interfere with the induction of protective Th1 responses and may be also involved in the polyclonal activation of B cells in malaria. This work has been partially funded by NSERC and CIHR.

Felipe Dargent¹, Marilyn Scott², Andrew Hendry¹, Gregor Fussmann¹, 1- Depart of Biology, McGill Univ., 2- Institute of Parasitology, McGill Univ.

Evolution of resistance in *Poecilia reticulata* released from predators and parasites

Although parasites are ubiquitous and presumably a major agent of selection on their hosts, we see variation in the ability of hosts to defend themselves against disease among and within populations. We explore this variation

as a function of optimal strategies of resistance under trade-off with other traits that enhance fitness, variation in the costs of defence expression and local adaptation. As a model we use *Poecilia reticulata* (guppies) and their monogenean ectoparasite *Gyrodactylus turnbulli*. Wild guppies from the island of Trinidad where collected at a site with high prevalence of *Gyrodactylus spp.*, high levels of predation pressure and high food availability (source site). Collected fish were introduced into two streams where *Gyrodactylus spp.* is absent and predation pressure is low. Both introduction sites further differ in that one has lower food availability (Introduction 1) than the source and other introduction site (Introduction 2). After three years fish were collected and reared in common garden for two generations. We individually infected these descendants with two lab reared *G. turnbulli* and monitored infection every two days until death or recovery of the host. Guppies from the source population had higher peak burdens ($p < 0.0001$) and higher total number of parasites ($p < 0.0001$) than guppies from the introduction sites. The introduction guppies did not significantly differ from each other. Our results indicate that release from predation and parasitism, but not decrease in food availability, can lead to rapid evolution of resistance. Acknowledgements: Financial support was provided by NSERC-Vanier CGS and Richard H. Tomlinson Fellowship awarded to FD, NSERC-SRO grant awarded to GF, AH, MS and Paul Bentzen. I will like to thank D. Reznick and C. Ghalambor for advice and providing the fish.

Dayal Dasanayake, Louis-Philippe Leroux, Armando Jardim, Florence S. Dzierszinski, Institute of Parasitology, McGill University, Ste-Anne-de-Bellevue, Quebec, H9X 3V9.

Identification of *Toxoplasma gondii* factors that are responsible for the inhibition of IFN- γ mediated up-regulation of MHC Class II in macrophages

Toxoplasma gondii owes much of its success to its ability to establish chronic infections in its intermediate hosts before complete immune elimination. MHC class II –restricted antigen presentation to CD4⁺ T cells is a crucial step in the development of an effective cellular immune response required to control intracellular pathogens such as *T. gondii*. Therefore, it is no surprise that most intracellular pathogens have developed mechanisms to interfere with antigen presentation in the context of MHC-II. We and others have shown that *T. gondii* is able to inhibit maturation-mediated (LPS, IFN-gamma, TNF-alpha and CD40L) activation and maturation of various professional antigen presenting cells (dendritic cells, macrophages, B cells, astrocytes, microglia). This effect is differentially observed with both live infections and parasite lysates and is not strain dependant. Through various steps of sub cellular fractionation of *T. gondii* parasites, we have localized this MHC-II regulatory activity to the fractions containing secretory organelles. Further resolution was carried out using sucrose gradients, and fractions with inhibitory activity were analyzed by mass spectrometry.

Corine Demanga Galamo, Lena Juliette Daher, Eric Prieur, Catherine Blanc, Jean-Louis Pérignon, Pierre Druilhe. Biomedical Parasitology Unit, Institut Pasteur, Paris, France.

MALARIA VACCINE RATIONAL DESIGN: EXAMPLE OF MSP3 FAMILY-BASED VACCINE.

Plasmodium falciparum merozoite surface protein-3 (MSP3), identified from clinical observations as a main target of protective immunity against malaria, belongs to a multi-gene family of six proteins with similar structural organisation particularly in their highly conserved C-terminal regions. To design a vaccine construction combining carefully chosen regions from these proteins, immuno-epidemiological studies were performed on recombinant antigens and a series of peptides designed from the C-terminal part of each MSP3 protein. Further more the biological anti-parasite activity of their corresponding antibodies was assessed. A majority of malaria exposed individuals harbour antibodies to each MSP3 antigen, with a dominance of cytophilic IgGs. The designed peptides were almost all antigenic, defining epitope relevant of native proteins, with distinct IgG isotype pattern for each and an overall predominance of the IgG3 subclass. Human antibodies affinity-purified upon each peptides exerted a *P.falciparum* killing effect in cooperation with blood monocytes (ADCI) in most cases as strong as that of IgG from protected African adults. Two regions “b” and “d” showing sequence homologies were found to generate in humans a broad network of cross reactive antibodies with various affinities. These findings suggest that all the six members of MSP3 family complement each other and play collectively an important role in protection. A first MSP3 multigenic construct designed according to these findings and those from immunogenicity studies in mice was shown to have valuable immunological properties. These findings pave the way towards tailoring vaccine constructs based on knowledge.

Colin Du¹, Nakita Haynes², Hamed Shateri Najafabadi³, Reza Salavati³, and Janet Yee¹

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Characterization of p105: A Putative Transcription Factor for the Core Histone Genes in *Giardia lamblia*

Giardia lamblia is a unicellular, eukaryotic, intestinal parasite found in freshwater systems worldwide. This parasite is of medical importance because it infects humans, domestic and wild mammals causing giardiasis, a disease manifested by severe diarrhea and abdominal cramping. A 15 bp conserved DNA sequence called *him* for the histone motif, is found to be unique to the promoters of *Giardia* core histone genes and is essential for their maximal expression. We speculate that this motif may be involved in the coordinated expression of *Giardia* core histone genes during the cell cycle by acting as a binding site for regulatory proteins. Previous work in our laboratory identified several potential *him*-binding proteins from *Giardia* nuclear extracts including p105, a putative 105 kDa transcription factor. INTERLOG analysis of the *Giardia* genome database yielded a protein-protein interaction network that predicts a direct interaction of p105 with a N-terminal acetyltransferase (NAT) and an indirect interaction with G2/Mitotic-specific cyclin B. Our results from gel-shift assays showed that p105 binds preferentially to single-stranded DNA. Moreover, p105 binds specifically to singlestranded DNA containing *him* and another 15 bp sequence, called *her* – both motifs are found within the promoters of the *Giardia* core histone genes. Results from these and other studies of p105 will be discussed. This work is supported by a NSERC Discovery Grant (JY) and an Ontario Graduate Scholarship in Science and Technology (CD).

Sonia EDAYE, Scott BOHLE, Dagobert Tazoo, Elias GEORGES, Institute of Parasitology, Chemistry Department, McGill University.

Characterization of Antimalarial Activity of Two Novel Chloroquine Derivatives

Malaria affects millions of people around the world and is a serious public health concern given the development and the spread of antimalarial drug resistance. The antimalarial drug artemisinin has been effective against *Plasmodium falciparum* malaria for many years now, however the recent rise of drug resistance to this drug has once again elevated the urgent need to identify and develop new antimalarial drugs. Chloroquine, the first effective antimalarial drug, is currently near absolute due to the spread of chloroquine resistant *P. falciparum*. However, given the present understanding of Chloroquine drug action and its mechanism(s) of drug resistance, it is likely that novel derivatives of this drug may be viable antimalarials drugs. The aim of this study was to evaluate the antimalarial potential of two novel chloroquine derivatives (e.g., 3-BrCQ and 3-ICQ, CQBr and CQI respectively) *in vitro* against chloroquine susceptible and resistant strains of *P. falciparum*. Using Sybr GreenI cell viability assay, we examined the effects of increasing concentrations of CQ, CQBr and CQI on the viability of CQ-susceptible (3D7) and –resistant (FCR3 and 7G8) strains. Our results show the IC₅₀ for CQ are 26 nM, 96 nM, and 82 nM for 3D7, FCR3, and 7G8, respectively. By contrast, the IC₅₀ for CQI are 408 nM, 417 nM, and 369 nM for 3D7, FCR3 and 7G8, respectively; while the IC₅₀ for CQBr are 760 nM, 748 nM, and 533 nM for 3D7, FCR3 and 7G8, respectively. Although CQ is more toxic to the susceptible strain, CQI and CQBr are equally toxic to both susceptible and resistant strains. Moreover, both CQI and CQBr show the same toxicity profile to human cell lines with no toxicity at 10 µM. Although studies are ongoing to further characterize the antimalarial action of CQBr and CQI on susceptible and resistant *P. falciparum* strains, the current findings suggest that the latter drugs are no subject to CQ drug resistance mechanisms.

Nelly El-Sakkary, F. El-Shehabi; A. Taman and P. Ribeiro, Institute of Parasitology, McGill University,

Characterization of the Novel Schistosomal Receptor SmGPR-3

The main causative agent of schistosomiasis, a disease which infects over 200 million people worldwide, is the parasite *Schistosoma mansoni*. Treatment of the disease is primarily with praziquantel (PZQ). There is an increasing fear that with the widespread use of the drug, and the lack of an available alternative, PZQ will lose its effectiveness. We are currently researching the schistosome nervous system to gain insight into this area. The nervous system coordinates many vital functions in the worm and is considered to be an excellent target for anti-schistosomal drugs. Recently, we have discovered a new group of schistosomal biogenic amine (BA) G-Protein Coupled Receptors (GPCRs), the smGPRs, which likely have a neuronal function. SmGPR-1, -2 and -3 have been cloned, and their pharmacological profiles determined by our lab. These receptors differ from the BA GPCRs of the human host in both sequence and function. SmGPR-3 was immunolocalized in the adult worm and shown to be expressed abundantly in the central and peripheral nervous system, including peripheral neurons which innervate

the worm musculature, indicating a neuromuscular role. To further characterize smGPR-3, a predicted binding site (D3.32) was mutated and functional expression studies were performed, to assess receptor activity. The wild-type and mutant plasmid constructs were individually expressed in yeast cells which, upon successful interaction with ligand and receptor activation, express a reporter gene, allowing for the cells to be selectively grown in media. Cell growth is then quantitatively assayed, using the Alamar Blue fluorescence assay, as a measure of receptor activation. The mutants showed varied levels of responsiveness to ligand, indicating the site's importance. RNAi knock-down of smGPR-1, -2, and -3 in schistosomulae, followed by video analysis was performed. Targeting of smGPR-3 results in a decrease in worm motility by approximately half, as compared to the control, indicating a role in motility for the receptor.

Daniel Feingold, Laura Nilson and Joseph A. Dent Dept of Biology, McGill University, Qc

Characterization of three ligand gated ion channel subunits – Potential pesticide targets?

Cys-loop ligand gated ion channels (LGIC) are pentameric neurotransmitter receptors that are ubiquitous in both vertebrate and invertebrate nervous systems. Their large diversity as well as their central role in mediating rapid synaptic transmission has made these channels attractive molecular targets for various pesticides. Despite the widespread use of such pesticides, issues regarding drug specificity and resistance continue to pose serious problems in regions that rely on pesticides for crop protection and prevention against disease. We are characterizing three novel Cys-loop LGIC subunits; CG7589, CG6927 and CG11340 in *Drosophila melanogaster*. These genes are of particular interest because they exhibit little homology among invertebrate species and do not possess any orthologs in vertebrate systems (Dent, 2006). Consequently, pesticides that target channels formed by these genes are predicted to be safe and have low risk for cross-resistance with current pesticides. We generated putative knockouts of all three genes via P-element excision mutagenesis or FRT mediated recombination. Data suggest that mutations in CG7589 and CG11340 are semi-lethal. Gene expression profiles have been examined via in situ hybridization. CG7589 and CG11340 are expressed in the midgut and Malpighian tubules; tissues involved in ion regulation and renal function, while CG6927 appears to be expressed in tracheal tissue and the salivary glands. Preliminary data indicate that CG11340 mutants are sensitive to osmotic stress. Electrophysiological tests indicate that CG11340 can form a functional homomeric channel while CG7589 and CG6927 form a heteromeric channel. Based on the relative divergence of these genes from other Cys-loop LGIC subunits as well as the lethal phenotypes associated with the corresponding mutants, these putative subunits may provide a promising molecular target for the development of a novel class of highly selective and efficient pesticides.

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Alterations in bone status in malaria: effect of hemozoin and heme on the differentiation, viability and function of bone remodeling cells *in vitro*.

Osteoporosis is frequently associated to *B*-hemoglobinopathies such as thalassemia and sickle cell disease but its etiology in these disorders remains unknown. We hypothesize that the important release of heme induced by concurrent hemolytic episodes affects the physiology of osteoclasts and osteoblasts, cells involved in bone remodeling. We also propose that heme may exert a rapid effect on these cells as short-term acute haemolysis induced by infection with *Plasmodium chabaudi adami* or by treatment with phenylhydrazine promotes a rapid and important loss of bone mass and altered bone metabolism markers in the serum. In order to elucidate the plausible role for hemin or its derived metabolite hemozoin in the alteration of bone status in malaria, we assessed the effects of both compounds on the *in vitro* differentiation of osteoclasts and on the viability and alkaline phosphatase responses of murine and human osteoblasts. Hemin, in contrast with hemozoin, significantly inhibited osteoclast differentiation in response to M-CSF and RANKL, decreasing also CD11b (Mac-1) expression, an integrin playing a relevant role in the responses of osteoclast precursors to RANKL. In the osteoblast cell lines, both compounds diminished the production of alkaline phosphatase (required for bone mineralization) in a dose-dependent manner whereas hemin was the sole compound inducing heme oxygenase-1 expression, an enzyme induced by oxidative stress. Taken together, these results suggest that hemin is a potent inhibitor of osteoclast differentiation whereas it shares inhibitory effects with hemozoin on osteoblasts but does so most probably through a distinct mechanism. This work was supported by a New Initiative Grant (CHPI, FQRNT).

Godoy, Pablo ; Lian, Jing ; Che, Hua ; Beech, Robin N. and Prichard, R.K. Institute of Parasitology, McGill University.

Interaction of macrocyclic lactone endectocides with *Haemonchus contortus* P-glycoproteins

Haemonchus contortus is a nematode of small ruminants that has developed resistance to Macrocyclic lactone (ML) anthelmintics, such as ivermectin (IVM) and moxidectin (MOX). There is accumulating evidence that *H. contortus* has developed ML resistance due, in part, to the involvement of constitutive over expression of ABC transporters such as P-glycoproteins (P-gp), which may influencing the concentration of MLs that reaching the target inside the worm. The goal of this work was to clone and express *H. contortus* P-glycoproteins in mammalian cells and establish the interaction of these transporters with IVM and MOX. After the characterization and stable expression of these *H. contortus* P-gps, the transport function was assessed by measuring the accumulation of the fluorophore Rhodamine123 (Rho123). HcP-gps showed at least a 50% less fluorescence accumulation in comparison with untransfected parental cells. Further, was tested the effect of the MLs in the inhibition of the Rho123 transport by HcP-gps. IVM produced a much more effective inhibitor of nematode P-gp transport than was MOX. The results indicate that HcP-gps may play an important role in regulating the concentration of IVM that reaches the mode of action receptors in the parasite, and thus the development of resistance, whereas for MOX, HcP-gps appear to play much less of a role in regulating its availability at the receptors and development of resistance. Research supported by CONICYT-Chile, NSERC, Canada and the FQRNT Centre for Host-Parasite Interactions, Quebec.

Doris González Fernández, Odalis Sinisterra, Kristine G. Koski, Marilyn E. Scott. Institute of Parasitology, School of Dietetics and Human Nutrition, McGill University and MINSAs Panama.

Combined Impact of Nutritional Deficiencies and Infectious Diseases on Pregnancy Outcomes and Infant Health in a Rural Community in Panama.

Infections and micronutrient deficiencies are considered as independent causes for adverse pregnancy outcomes. We are studying how iron deficiency anemia, vitamin D deficiency and maternal underweight when combined with oral, skin, gastrointestinal and urogenital infections are influencing maternal and fetal health in a population of indigenous pregnant women in Panama. Our cross-sectional study included 213 pregnant women. The incidence of anemia using standard red blood cell analysis was 52%; severe vitamin D deficiency was present in 7% and vitamin D inadequacy in 57%. Additionally, 20% of the women had skin infections (scabies, bacterial and fungal skin infections); 23% had oral infections (caries or gingivitis); 87% had one or more urogenital infection (urinary tract infection, bacterial vaginosis, vaginal candidiasis, vaginal trichomoniasis, or diplococcus infection); and the presence of intestinal parasites (*Ascaris*, hookworm, *Trichuris*, *E.coli* or *Giardia*) was positive in 72% of the 120 stool samples collected. Infections (diplococcus, hookworm and *Trichuris*) and anemia resulted in lower maternal weight; as well, higher intensity of hookworm and *Trichuris* was associated with lower maternal weight. On the other hand, bacterial vaginosis was associated with maternal anemia. Finally, fetal health evaluated through fundal height, fetal movements and fetal cardiac rate was affected by vitamin D inadequacy or deficiency and specific maternal infections including bacterial skin infections and *Trichomona vaginalis*. This project is funded by SENACYT – Panama and the McGill Vitamin Fund.

Stephanie Goyette^{1,2}, Momar Ndao², Brian J. Ward², and Florence S. Dzierszynski*^{1,1} Institute of Parasitology, McGill University, ²MUHC, McGill University.

Insights into *Toxoplasma gondii* reactivation through biomarker discovery.

Upon infection with *Toxoplasma gondii*, tachyzoites multiply and spread throughout the host to reach target organs, where they differentiate into dormant encysted bradyzoites. Bradyzoites sustain the chronic phase of infection, are drug resistant, and persist for the lifetime of the host. Although *T. gondii* infection typically causes mild disease in healthy adults, toxoplasmosis can be a debilitating and/or life-threatening disease in the congenitally-infected fetus or immunocompromised host. Much of the morbidity and mortality associated with this organism occurs as a result of reactivation, but the mechanisms that lead to cyst rupture and recrudescent infection are poorly understood. In order to investigate the mechanisms that trigger tissue cyst reactivation, we used a murine model of reactivation, in which chronically infected mice are depleted of interferon-gamma (IFN- γ) with a specific antibody or treated with dexamethasone until they show signs of disease. Simultaneously, we are analyzing sera and cerebrospinal fluid from experimentally infected mice over the course of disease (acute, chronic, reactivation) using sequential high-throughput (SELDI TOF MS) and targeted proteomics platforms (MALDI TOF TOF MS). Parasite and host biomarkers associated with different disease stages will be presented, focusing on

proteins that give insight into the host-parasite interaction. SG holds a CIHR doctoral scholarship. This work is partly supported by a CRC award to FD, and a CIHR award to BW and MN.

Kasra Hassani and Martin Olivier, Department of Microbiology and Immunology, McGill University

Immunomodulatory impact of *Leishmania*-induced macrophage exosomes.

Exosomes are small secretory vesicles that are about 50-100nm in size and are released by various cells types. These vesicles are formed via invagination of the membrane into designated compartments named multivesicular bodies (MVB). Fusion of the MVB with the plasma membrane results in release of exosomes to the environment. During the recent years, exosomes released from immune cells have shown to possess immunomodulatory effects. Interestingly, exosomes released from antigen-presenting cells (APCs) especially macrophages infected with bacteria have been shown to be pro-inflammatory and immunostimulatory to other immune cells. However, the exosomes released from macrophages infected with immunomodulatory protozoan parasites such as *Leishmania* remain uncharacterized. We performed a comparative proteomic analysis of the exosomes produced by untreated macrophages, LPS-stimulated macrophages and *Leishmania mexicana*-infected macrophages. Our preliminary results reveal interesting differences among the protein content of these three groups of exosomes especially with regards to apoptosis related proteins such as Programmed Cell Death Interacting protein (Alix) and 14-3-3 protein as well as Rab GTPases. In addition, we show by electromobility shift assay (EMSA) that exosomes from *Leishmania*-infected macrophage induce translocation of NF- κ B in naïve macrophages, although to a lesser extent compared to exosomes from LPS-stimulated macrophages. Translocation of the other prominent pro-inflammatory transcription factor AP-1 was not induced. Nevertheless, these modulations did not result in significant nitric oxide production in the treated macrophages. We are currently undertaking deeper proteomic analyses of these exosomes as well as their potential immunomodulatory effects on other immune cells. This work has been supported by an operating grant from CIHR.

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Dissection of the *Leishmania donovani* LdPEX14-LdPEX5 Interacting Domains

Glycosomes are unique organelles that compartmentalize many important metabolic and biosynthetic pathways including glycolysis, fatty acid β -oxidation and purine salvage in kinetoplastids. Glycosomal proteins are synthesized in cytosol and targeted to the glycosome post-translationally by the aid of two major topogenic signal sequence designated Peroxisomal Targeting Signal 1 and 2 (PTS-1 and PTS-2). In *Leishmania donovani* parasites, nascent glycosomal proteins containing a PTS-1 or PTS-2 are bound by the cytosolic receptor proteins LdPEX5 and LdPEX7, respectively. These cargo-loaded receptors complexes traffic to the glycosome surface and dock to LdPEX14, an interaction that is crucial for import of protein across the glycosomal membrane. Previous studies showed that PEX5, PEX7, and PEX14 are crucial for kinetoplastid parasite viability. Among these protein-protein interactions the association between LdPEX5 and LdPEX14 is crucial for targeting PTS-1 proteins glycosome. We previously showed that LdPEX5 bound to a region on LdPEX14 situated within the first 120 residues. To more precisely define the critical residues required for contact LdPEX5 we exploited the bacterial two-hybrid system to screen an Ldpex14 random mutagenesis library to identify key amino acid substitutions that abrogate LdPEX5 binding. These results coupled with deletion mutagenesis data have a critical 45 amino acid segment and more specifically at 10 amino acids that are important for making contacts with LdPEX5. A panel of LdPEX14 mutant proteins will be used to assess the contribution of each of these residues to stabilizing the LdPEX5-LdPEX14 association will be assessed ELISA and isothermal titration microcalorimetry.

Felix Hugentober, Karen K. Yam, Raya Mahbuba, Martin Olivier and Benoit Cousineau, Department of Microbiology & Immunology, McGill University, Montreal, QC

Development of a live vaccine against *Leishmania major* using the Gram-positive bacterium

Lactococcus lactis

Lactococcus lactis is a non-pathogenic, non-colonizing Gram-positive lactic acid bacterium commonly used in the dairy industry. *L. lactis* was given Generally Recognized As Safe (GRAS) status by the American Food and Drug Administration. *Leishmania* is a human parasite affecting over 12 million individuals worldwide, yet no vaccine is currently available. Protective immunity in mice against *Leishmania major* depends on the activation of T-helper (Th) type 1 cells induced by the essential cytokine interleukin 12 (IL-12). To generate *L. major* live vaccines, we engineered *L. lactis* strains expressing the protective *Leishmania* antigen, LACK, anchored to the cell wall or secreting mouse IL-12. We found that subcutaneous immunization with these strains induced antigen-specific

multifunctional Th1 CD4⁺ and CD8⁺ T cells and a systemic Th1-driven immune response. This correlated with protection against *L. major* infection in immunized animals. Furthermore, animals immunized with LACK-expressing *L. lactis* live vaccines displayed antigen-specific humoral immune responses before and after challenge with *L. major*. In addition, protected animals displayed *Leishmania*-specific Th1 immune responses in draining lymph nodes shortly after challenge and in spleen and draining lymph nodes at the end of the study. Similar to the subcutaneous study, mice were also immunized orally with the same *L. lactis* live vaccines which lead to a significant delay in footpad swelling.

Amandine Isnard, Martin Olivier, Department of Microbio and Immuno, McGill Univ.

***Leishmania* protease GP63 affects Nuclear Pore Complex (NPC) to enter into host macrophage nucleus**

The parasite surface protease GP63 is an important virulence factor in *Leishmania* (*L.*) infection. GP63 is highly involved in the inhibition of the microbicidal functions of the host macrophages (MΦ), thus enabling the parasite to survive inside these cells. During infection, GP63 has been suggested to enter the host MΦ partly via lipid raft microdomains, independently of parasite internalization. We recently demonstrated that GP63 can reach the nucleus of the host MΦ. This is the first report that a parasite protease can reach the nucleus of a mammalian cell, where it could directly affect transcription factors and gene expression related to immune response. We present here preliminary results about the mechanisms whereby GP63 enters into the nucleus of host MΦ. We tested if GP63 required a Nuclear Localisation Sequence (NLS) to be targeted to the NPC via an importin carrier protein. *L. major* mutants for a NLS-like sequence in GP63 were obtained. We compared the nuclear translocation of GP63 in murine MΦ infected with *L. major* WT, GP63^{-/-}, with add-back GP63, or NLS mutants, by western blot and confocal microscopy. No difference was observed. We also analyzed if GP63 could directly target the NPC. We demonstrated that infection of MΦ with *L. mexicana* leads to disappearance of NPC and is time-dependent. Infection with *L. major* WT or GP63^{-/-} showed that the NPC disappearance is GP63-dependent. Then, results from a co-immunoprecipitation assay of NPC after infection of MΦ with *L. mexicana* tend towards a direct interaction between GP63 and NPC. Collectively, these results show that GP63 affects NPC to enter the nucleus of the host macrophage. This is the first time that such a mechanism is observed for a parasite protease.

Patrick Janukavicius, Laura Tiseo, Ludmel Urdaneta, Roger Prichard, Joseph Dent, Institute of Parasitology and Department of Biology, McGill Univ. **The mapping of ivermectin resistance genes**

Ivermectin is a drug used to kill parasitic nematodes. However, parasites are evolving resistance to ivermectin. We are studying the mechanisms of resistance in the model organism *C. elegans* in order to elucidate good targets for future drugs, to which parasites would have more difficulty developing resistance. Typical methods for finding ivermectin resistant *C. elegans* involve using the mutagen ethyl methane sulfonate. James and Davey (2008) pointed out that parasites develop resistance by being exposed to low concentrations of ivermectin which selects for spontaneous mutations. They propagated *C. elegans* on low concentrations of ivermectin. After many generations, they created an ivermectin resistant strain, IVR10, which shows ten-fold resistance to ivermectin. James and Davey claim that the increased resistance is “largely epigenetic” and provide evidence for the increased expression of ABC transporter proteins which are believed to pump ivermectin. To determine if mutations lead to the increased expression of these pumps we performed mapping experiments on the strain IVR10 and found two regions linked to resistance. One region is located on chromosome II and the other region is on the X-chromosome. We believe that the observed linkage on the X-chromosome is due to a 2 nucleotide deletion in the gene *dyf-7*. The gene, *dyf-7*, was first characterized by Starich *et al.* (1995) for its DYE-Filling defective (*dyf*) phenotype. When wild-type worms are exposed to dye, the amphid neurons will fill with dye. However, the amphids of *dyf* mutants do not fill with dye. This phenotype is due to decreased permeability of the dye by the loss of sensory pores in the cuticle of the worm. We believe that increased resistance in *dyf* mutants is associated with a decrease in the permeability of ivermectin.

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The role of Tb09.211.2960, a putative RNA-binding protein involved in post-transcriptional regulation of gene expression in *Trypanosoma brucei*.

Regulation of gene expression in trypanosomatids is mainly post-transcriptional, mostly through interaction of *cis* and *trans*-acting elements that affect mRNA maturation, localization, stability, and translation rate. These regulatory elements are, however, largely unknown. We have prepared the complete computational and

experimental framework required for characterization of such regulatory elements in *T. brucei*. Here we characterize an RNA-binding protein from *T. brucei*, Tb09.211.2960 (abbreviated as 2960); containing a putative K-homology (KH)-domain which is differentially up-regulated in the procyclic form (PF) of *T. brucei*. We hypothesized that 2960 plays an essential role in developmental regulation of gene expression by binding to a novel motif and, consequently, affecting the pathways that are potentially regulated by this protein. PF cells in which 2960 was down-regulated by tetracycline induced RNAi showed a much slower growth rate compared to the wild type *T. brucei*. Microarray based expression profiling of genes from the 2960 RNAi samples showed a highly significant de-regulation of a large number of *T. brucei* genes indicating an essential role for a potential *trans*-regulatory factor, the KH domain-containing 2960 in *T. brucei*.

Fikregabrail A. Kassa, Marina T. Shio, Momar Ndao and Martin Olivier, Department of Microbiology and Immunology; Center for the Study of Host Resistance and The Research Institute of McGill University Health Center, McGill University, Montreal, Quebec

Serum proteins alter the host innate immune response against hemozoin

Malaria still remains to be one of the most important infectious diseases worldwide and hemozoin, which is produced during the intraerythrocytic stage of the malaria parasite *Plasmodium*, remains to be the focal point of malaria research. Upon its release from infected red blood cells, hemozoin interacts with serum proteins. The role of hemozoin-serum protein complexes remained elusive. Here we show that hemozoin specifically binds to serum proteins such as LPS binding protein (LBP), serum amyloid A (SAA), and Apolipoprotein E (ApoE). LBP and SAA are acute phase proteins whose levels dramatically increase during infection where as ApoE regulates the lipid levels by forming molecules called lipoproteins. PMA-differentiated THP-1 cells were stimulated or not with hemozoin alone or hemozoin with increasing concentrations of LBP, SAA or ApoE. Our results show that SAA bound to hemozoin induces the production of IL-1 β in a dose dependant manner, where as ApoE abrogates this concentration-dependent production of IL-1 β . Furthermore, SAA also induces the phosphorylation of Lyn. Apart from IL-1 β production, hemozoin bound serum proteins also modulate ROS production and phagocytosis in PMA-differentiated THP-1 cells. While hemozoin alone induces ROS production in a dose dependant manner, when bound to LBP, there is a significant increase in ROS production. However, when hemozoin is bound to increasing concentration of ApoE, ROS production is substantially abrogated. Interestingly, LBP enhances the recognition and phagocytosis of hemozoin by THP-1 cells. Overall, our results show that serum proteins alter the recognition, phagocytosis, IL-1 β and ROS production by THP-1 cells upon stimulation with hemozoin.

Marie-Claude Laffitte, Danielle Légaré and Marc Ouellette. Centre de recherche en Infectiologie, CHUL, Université Laval.

***Mre11* involvement in DNA double strand break repair, generation of extrachromosomal DNA amplicons and drug resistance.**

The protozoan parasite *Leishmania* is known to modulate gene expression through the formation of extrachromosomal DNA amplicons as a mechanism of drug resistance. During the gene amplification process, rearrangements occurring between direct or inverted DNA repeated sequences (RS) lead to the formation of circular and/or linear amplicons containing genes implicated in resistance. We hypothesize that this phenomenon involves the formation of a DNA double strand break (DSB) near to the RS, followed by its repair by homologous recombination between the RS and the formation of amplicons. Here we investigated the role of the gene *Mre11*, which codes for a putative DNA-binding protein involved in DSB repair through homologous recombination. Our hypothesis is that *Mre11* should have a role in the formation of amplicons, and hence in drug resistance. We performed the knock-out of the *Mre11* gene in *Leishmania infantum*, using hygromycin and neomycin as selecting markers. We observed a growth delay in the *Mre11*^{-/-} null mutants, compared to the wild-type cells. This phenotype was partially reverted when the *Mre11*^{-/-} null mutants were complemented with an episomal *Mre11* construct. The role of *Mre11* in DNA-damage repair was further studied by inducing DSB in our panel of strains using the DNA-damaging agent methylmethane sulfonate (MMS). Interestingly, the *Mre11*^{-/-} null mutants were more sensitive to MMS than both the wild-type and the genetically-complemented strains, which suggested the involvement of *Mre11* in DSB repair in *Leishmania infantum*. We are currently assessing the role of *Mre11* in the formation of extrachromosomal DNA amplicons by selecting for resistance against the model drug methotrexate and comparing the level of *dhfr-ts* amplicons, the target of methotrexate, between *L. infantum* wild-type and *Mre11*^{-/-} null mutants.

Louis-Philippe Leroux, Manami Nishi, Sandy El Hage, & Florence S. Dzierszinski, Institute of Parasitology, McGill Univ. **Subversion of MHC-II antigen presentation by *Toxoplasma gondii* is mediated by the upregulation of the MHC-II associated invariant chain**

Like many intracellular pathogens, *Toxoplasma gondii* interferes with MHC-II antigen presentation to dampen CD4 T cell responses, and gain a head start on the adaptive immune response. Transcriptional downregulation of MHC-II genes by *T. gondii* is well known, but precise molecular mechanisms remain elusive. Here, we show that the first layer of transcriptional regulation has to be complemented by a second layer of interference in the host cell endocytic pathway. This involves the MHC-II associated invariant chain (Ii), which chaperones MHC-II throughout the endocytic pathway and protects the MHC-II groove from unspecific peptides, until it is proteolysed and the remaining CLIP peptide is removed by the editor H2-DM. The absence of Ii restored the ability of infected DCs to present the parasite antigen in the context of MHC-II. This novel layer of regulation was found necessary to achieve not only low levels of surface MHC-II, but also inhibition of MHC-II antigen presentation in *T. gondii*-infected cells. Although the expression of MHC-II genes is typically coordinated, Ii mRNA and protein levels were surprisingly induced in *T. gondii*-infected murine macrophages (MP) and dendritic cells (DCs), both *in vitro* and *in vivo*. Ii accumulated specifically in infected cells until host cell lysis, mainly in the ER, and did not traffic to the cell surface. This process did not require active parasite replication, but was specific of live *T. gondii* infection. Not only the p31 and p41 Ii isoforms, but also the p10 cleavage product, accumulated in FACS-sorted infected compared to non-infected macrophages, suggesting a bias in Ii proteolytic processing during infection. Cathepsins S, L, and legumain activities were indeed modulated in either WT or Ii knockout infected DCs and MPs, pointing toward a wide manipulation of the host endocytic pathway by *T. gondii* to subvert MHC-II antigen presentation.

Christine Matte¹ & Albert Descoteaux¹, ¹INRS-Institut Armand Frappier, Université du Québec

Disruption of Akt downstream events by *Leishmania major* promastigotes

Signaling through the Akt/mammalian target of rapamycin (mTOR) pathway plays a pivotal role in the regulation of multiple cellular processes, including protein synthesis, cytokine secretion, autophagy and apoptosis. It is therefore a major target of microbial infections and tumors. Protozoa of the *Leishmania* genus cause a wide spectrum of diseases in humans, termed leishmaniasis, with clinical manifestations ranging from self-healing skin ulcers to life-threatening visceral disease. These parasites primarily infect macrophages and are renowned for their ability to sabotage host-cell signal transduction pathways. Here, we report that infection of Balb/c bone marrow-derived macrophages (BMDM) with the promastigote stage of *Leishmania major* causes rapid, time-dependent degradation of key components of the Akt/mTOR axis, including Akt, mTOR and the tuberous sclerosis complex-2 (TSC-2). Results show that disruption of the Akt/mTOR pathway by *L. major* is dependent on the surface metalloprotease gp63, an important virulence factor of the parasite, and appears to be species- and strain-specific. Analysis of IL-12 secretion by infected BMDM indicated that *L. major* promastigotes promote production of this cytokine, although the precise mechanism underlying this effect has yet to be identified. The impact of *L. major* on the autophagic system and on apoptosis is currently being investigated. These studies highlight a novel mechanism by which *L. major* interferes with macrophage functions and responses and will provide a better understanding of *Leishmania* pathogenesis.

Laura-Isobel McCall, Greg Matlashewski, Dept. of Microbiology and Immunology, McGill Univ.

Involvement of the *Leishmania donovani* virulence factor A2 in the parasite stress response: protection against heat shock and oxidative stress

Leishmaniasis is associated with a broad variety of clinical manifestations, from fatal visceral disease to self-healing cutaneous lesions. A2 proteins are expressed by visceral *Leishmania donovani* but not by cutaneous *Leishmania major*, and A2 has been shown to play a role in visceral infection: *L. donovani* parasites with knocked down A2 expression display decreased virulence. Moreover, introducing A2 into *L. major* increases *L. major* visceral survival. Here, we examine the molecular basis of these observations, focusing on the involvement of A2 in the *Leishmania* stress response. A2 is expressed in the amastigote stage of *L. donovani*, but it can also be induced in promastigotes by several stress signals, including heat-shock, ethanol and misfolded protein stress. *L. major* promastigotes ectopically expressing A2 survive heat-shock significantly better than control-transfected *L. major*. Conversely, *L. donovani* promastigotes and amastigotes with decreased A2 expression are more sensitive to heat shock than control-transfected cells. A2-deficient *L. donovani* axenic amastigotes are also more sensitive to reactive oxygen and nitrogen species, exhibiting increased internal oxidant levels and decreased survival following treatment with hydrogen peroxide or with a nitric oxide donor, SNAP. These results suggest that A2 may play a role in the parasite stress response and help *L. donovani* survive host defense mechanisms, in particular oxidants

and fever. Our results therefore provide valuable insight into the mechanisms of *L. donovani* virulence and into the *L. major*-*L. donovani* dichotomy.

James McLean and Armando Jardim, Institute of Parasitology, McGill Univ.

Characterization of the *Leishmania major* glycosomal PTS2 receptor, Peroxin 7

Leishmania are Trypanosomatid parasites that infect roughly 12 million people in tropical countries. This neglected tropical disease causes debilitating and often fatal consequences in the absence of chemotherapeutic intervention. Consequently, there is a need to identify new drug targets to combat the increasing incidence of resistance to current treatments. An attractive drug target in these parasites is the glycosome, a unique microbody organelle that compartmentalizes several essential enzymatic pathways behind an impermeable membrane. The *Leishmania major* Peroxin 7 (LmPEX7) is a receptor protein that recognizes glycosomal matrix proteins containing an N-terminal peroxisomal targeting signal 2 (PTS-2) and facilitates the trafficking of these protein to the glycosome. Genetic studies in the related kinetoplastid parasite, *Trypanosoma brucei*, have demonstrated that PEX7 is essential for parasite viability. LmPEX7 is predicted to have a hydrophobic outer surface which has made production of the recombinant protein in *E. coli* heterologous system challenging. To investigate the biophysical role of LmPEX7 in the trafficking and import of proteins into the glycosome, we have developed a strategy to express and purify recombinant *Leishmania* PEX7. Subsequent, biochemical studies have confirmed that the recombinant LmPEX7 is functionally active and, like the native protein, binds LdPEX5, LdPEX14, and PTS-2 ligand proteins.

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Dendritic cells regulate CD4⁺ T cell responses to blood-stage malaria.

Dendritic cells (DCs) play a pivotal role as antigen presenting cells to drive adaptive CD4⁺ Th cell responses to infections, including malaria, a disease that remains a significant health burden worldwide. Studies in mice infected with rodent *Plasmodium* species which differ in virulence suggest that deficiencies in the host immune response, including DC function, correlate with a lethal outcome of blood-stage malaria. Here, we investigated if deficiencies in DC function contribute to the difference in the outcome of *P. chabaudi* AS infection in susceptible A/J mice compared to resistant C57BL/6 (B6) or H-2 congenic B10.A mice. Analysis of DC uptake of CFSE-labeled pRBC by flow cytometry demonstrated that during early infection splenic CD11c⁺ DCs from A/J mice ingested pRBC and expressed co-stimulatory molecules (CD40, CD80, CD86) at higher levels than DCs from B6 or B10.A mice. DCs from infected B6 and B10.A mice stimulated *in vitro* with pRBC produced high levels of the Th1-polarizing cytokine IL-12 and low levels of IL-2. Furthermore, DCs from resistant B6 or B10.A mice induced moderate proliferation of syngeneic CD4⁺ T cells and secretion of the Th1 cytokine IFN- γ . In contrast, DCs from A/J mice produced significantly lower levels of IL-12 but higher IL-2 levels and induced high CD4⁺ T cell proliferation and secretion of the regulatory cytokine, IL-10. Thus, these data suggest that CD11c⁺ DCs from *P. chabaudi* AS-susceptible A/J mice are fully functional antigen presenting cells but differ from resistant mice in their ability to elicit a Th1 immune response required to control the infection.

Supported by FRSQ, CIHR, and the FQRNT-Centre for Host-Parasite Interactions

Layla S. Mofid, Theresa W. Gyorkos, Department of Epidemiology, Biostatistics and Occupational Health, McGill University; Quentin Bickle, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine. **Lessons learned during MSc thesis field research on soil-transmitted helminths in rural China** A field survey was undertaken in 2008 to investigate risk factors for soil-transmitted helminthiasis in school-age children in rural China, as part of my MSc thesis research at the London School of Hygiene and Tropical Medicine (LSHTM). **Lessons Learned #1:** Assess research topics completed by former students. In 2007, a baseline soil-transmitted helminth (STH) survey had been conducted in Yunnan, China and so I arranged to meet with the student's supervisor, Dr. Quentin Bickle, to discuss the desirability of expanding this survey and adding an epidemiological component. An expanded follow-up study was warranted based on the high prevalence of STH infection (60.6%), and lack of deworming activities in the area. **Lessons Learned #2:** Find an experienced supervisor in your field of research. Dr. Bickle agreed to supervise the project and provide suggestions on study procedures. Dr. Bickle is a consultant to the World Health Organization (WHO) and was familiar with research gaps. He also provided feedback to WHO once data were analyzed. **Lessons Learned #3:** Collaborate with local health professionals. To ensure that cultural norms and expressions were clear and concise, the questionnaire required

iterative revisions until a final version was acceptable to all research partners. **Lessons Learned #4:** Experience in primary data collection was invaluable at such an early stage of academic training and prepared me for both a job and PhD studies. This experience gave me insight into the challenges associated with working in developing countries and language barriers in research. I obtained concrete knowledge and skills that I am now using in a doctoral program in Epidemiology. A rigorous epidemiological research study is planned involving STH infections in rural Peru, under the supervision of Dr. Theresa Gyorkos.

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University. **Alteration of macrophage membrane fusion regulators during infection with *Leishmania* promastigotes**

Some pathogens like *Leishmania* promastigotes inhibit phagosome maturation. This inhibition is mediated by the different virulence factors such the promastigote surface glycolipid lipophosphoglycan, which causes exclusion of Synaptotagmin V from the phagosome membrane. As a consequence, promastigotes establish infection in a phagosome from which the vesicular proton-ATPase is excluded and which fails to acidify. Synaptotagmins constitute a large family of membrane-trafficking proteins which are in close interaction with SNAREs (soluble N-ethylmaleimide-sensitive-factor attachment protein receptor) proteins. To investigate the impact of some key SNAREs on the control of *Leishmania*, we infected macrophages with different species and strains of *Leishmania* for different periods and we discovered that upon infection, there was degradation of VAMP8, VAMP3 and Syntaxin4 by some species and strains of *Leishmania*. This degradation is strictly dependent on the presence of the *Leishmania* surface zinc-metalloprotease gp63. In fact, using a gp63^{-/-} mutant, we observed no degradation of those SNAREs. Also, by using macrophages and dendritic cells from VAMP8 knock-out mice, we showed that VAMP8 is essential for the cross presentation pathway which we confirmed the same results after infection of wild-type macrophages with *Leishmania*. Taken together, by discovering new virulence mechanism of *Leishmania*, there is a chance to target some components through invading pathway and also, prevention of phagosome maturation to control this parasitic disease in a near future.

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Gene expression profiling of *Giardia lamblia* isolates from two different genetic assemblages

Giardia lamblia is one of the most common causes of parasitic infections worldwide. Molecular genotyping has shown that *Giardia lamblia* isolates can be classified into seven different assemblages named A through G. *Giardia* belonging to assemblages C to G are host specific (*i.e.* assemblages C and D infect only dogs, and assemblage E infects only cattle). In contrast, *Giardia* in assemblages A and B have an extended host range that includes humans, live stock, wildlife, and companion animals; and thus, have a potential for zoonosis. In addition, *Giardia* in assemblages A and B show differences in experimental infections, their growth rate in laboratory cultures, and ability to encyst & excyst *in vitro*. In this study microarray analysis was used to compare the gene expression profiles between isolates of assemblages A and B. The WB isolate originated from a human patient and belongs in assemblage A. The GS isolate also originated from a human patient but belongs in assemblage B. The third isolate originated from a beaver and belongs in assemblage A. Fluorescent-labelled cDNAs were generated from RNA extracted from laboratory cultures of the three *Giardia* isolates, and used in hybridization experiments with microarray slides containing oligonucleotides corresponding to 9115 genes and ORFs within the *Giardia* WB genome. A subset of genes that were identified as differentially expressed among these isolates in the microarray experiments were also analyzed by RT-PCR. We will present the results of our analysis of the microarray data and discuss their relevance to the biological differences between *Giardia* in assemblages A and B, and potential adaptation of assemblage A isolates to infection of different hosts. This work is supported by a NSERC Discovery Grant (JY) and a Graduate Scholarship from the Walkerton Clean Water Centre (AO). The microarray slides are provided by PFGRC.

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Retroposon-mediated post-transcriptional control in the parasitic protozoan *Leishmania*

Leishmania are evolutionary early-branched unicellular protozoa causing a diverse spectrum of diseases in humans. Control of transcription initiation is absent in these parasites and regulation of gene expression occurs mainly posttranscriptionally. We have previously shown that *Leishmania* possess two widespread families of formerly active retroposons, namely SIDER1 and SIDER2 (Short Interspersed DEgenerate Retroposons) that are preponderantly located within 3'UTRs and participate in distinct RNA networks. Members of the SIDER1 subfamily regulate translation, whereas members of the SIDER2 class are involved in mRNA degradation. Further studies on the mechanism by which SIDER2 promotes degradation of short-lived mRNAs in *Leishmania* demonstrated that these transcripts are degraded by endonucleolytic cleavage without prior deadenylation. Cleavage was mapped within the second 79-nt signature sequence, which is highly conserved amongst SIDER2 retroposons. Selective inactivation of SIDER2 retroposons causes the onset of distinct mRNA decay pathways resulting in stage- and species-specific gene expression. We further showed that overexpression of SIDER2 antisense RNA can increase sense transcript stability and that complementarity to the cleavage region is required for protecting transcripts from degradation. These findings are consistent with a broad role for SIDER retroposons in dynamically controlling gene expression throughout the parasite's development and between different *Leishmania* species.

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The importance of host-parasite co-evolution on life history traits: A test with guppies and *Gyrodactylus*.

It is generally acknowledged that specialist parasites have a higher fitness on their sympatric host due to the intimate relation between host defense and parasite counter-adaptations. However, it is still unclear how the local environment, where co-evolution occurs, may affect the evolution of virulence and resistance, resulting in ecological differences between host populations exposed to different parasite strains. The monogenean *Gyrodactylus* are ubiquitous ecto-parasites of the Trinidadian guppy (*Poecilia reticulata*). In Trinidad, guppies inhabit different environments, distinguished mainly by the presence or absence of predatory piscivorous fish. Here, we test if the local environment (high or low predation) affects guppy-*Gyrodactylus* co-evolution by indirectly selecting for higher or lower virulence, due to guppy mortality. We followed parasite population dynamics and host phenotypic response in experimentally infected fish collected from four guppy populations (two high and two low predation) with *Gyrodactylus* strains from each of these populations, using artificial streams that replicate wild conditions. We report our findings on parasite population dynamics and their effect on the phenotypic response of the host. This work was supported by NSERC -Special Research Opportunity, le Fonds Québécois de la Recherche sur la Nature et les Technologies and the Consejo Nacional de Ciencia y Tecnologia (CONACYT).

[Carolina P.Gómez](#)¹, Marina Shio², Martin Olivier², Albert Descoteaux¹, ¹Institut National de la Recherche Scientifique- Institut Armand Frappier, ²McGill University.

Role of the Protein SHP-1 in the Biogenesis of the Phagolysosome

The processes of phagocytosis and phagosomal maturation involve the recruitment of effector proteins that participate in phagosome formation and in the acidification and/or fusion with various endocytic vesicles. Among these proteins, phosphatases are known to be involved in such processes (e.g. PTP-MEG-2), and in some cases, are used by intracellular parasites to delay the maturation process to their advantage (e.g. *M. tuberculosis*). The role of the Src homology 2 domain-containing phosphatase 1 (SHP-1) in this process has yet to be determined. Using murine bone-marrow derived macrophages (BMMs) we showed that SHP-1 is indeed recruited to the nascent phagosome and colocalizes with the Lysosomal-associated membrane protein 1 (LAMP1), a marker of phagosomal maturation. To evaluate the impact of SHP-1 on phagosome biology, we used macrophage cell lines deficient for SHP-1 (motheaten [*me SHP-1^{-/-}*]), and their wild type littermate (LM) as a control. We studied the kinetics of the recruitment of LAMP1 and Cathepsin D (CathD) in presence or absence of SHP-1. Using confocal microscopy and Western blotting analyses, we noticed a delayed recruitment in SHP-1^{-/-} cells for LAMP-1, as well as for other markers such as Cath D and Flotillin-2. Also, we obtained evidence that in the absence of SHP-1, phagosomal acidification was impaired. Consistently, acquisition of the proton-vacuolar ATPase (v-ATPase), in our preliminary results, was shown to be impaired in SHP-1^{-/-} cells. Taken together, these results are consistent with a role for SHP-1 in the regulation of signaling or membrane fusion events involved in phagolysosome biogenesis.

Chase Reaume¹, Christine Ouellet², and Janet Yee^{1,2} ¹Environmental and Life Sciences Graduate Program, ²Biochemistry Program, Trent University. **The application of counterflow centrifugal elutriation and quantitative RT-PCR in the cell cycle analysis of *Giardia lamblia***

Giardia lamblia, a protist infecting freshwater systems worldwide, is the causative agent of giardiasis or “Beaver Fever”, a condition characterized by severe diarrhea and abdominal pain. Proliferation of the trophozoite form of this parasite in the intestinal tract of a host contributes to the onset of these symptoms, making the cell cycle an essential area of research. In order to examine gene expression during the cell cycle, a cell culture must be synchronized so that the cells progress through the cell cycle starting from the same stage. Previous attempts at synchronizing *Giardia* trophozoite cultures have used the drugs nocodazole combined with aphidicolin and aphidicolin alone in “block-and-release” experiments. Although both methods were effective in obtaining semi-synchronous *Giardia* cultures, these treatments also damaged the cells’ DNA, which could potentially affect subsequent gene expression analysis of these cells. We are developing counterflow centrifugal elutriation (CCE) as a method to obtain samples of *Giardia* cultures that are enriched in cells from different stages of the cell cycle. As cells progress through the cell cycle from G1, S to G2, they undergo an increase in overall size due to the replication of genetic material and other cellular components. CCE uses these differences in cell size and density to effectively separate asynchronous cell cultures into the individual phases of the cell cycle. Initial experiments showed that fractions enriched in the stages of G1, S, and G2/M can be achieved. Preliminary quantitative PCR (qPCR) analysis showed that the RNA levels of the *Giardia* core histone genes increase up to 5-fold in CCE fractions containing a majority of S-phase cells. Further results from our CCE experiments and quantitative RT-PCR analysis of the cell cycle fractions obtained from this procedure will be presented and discussed. This work is supported by a NSERC Discovery Grant (JY) and a NSERC Alexander Graham Bell Canada Graduate Scholarship (CR).

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Cut worms: A useful tool to study anthelmintic activities in *Caenorhabditis elegans*.

Caenorhabditis elegans has been extensively used for the study of anthelmintic effects in worms, screening for new drugs and identification of drug targets. However its soil-based free living life style may influence its response to xenobiotics in comparison with parasitic nematodes. For example, the novel anthelmintic derquantel (2-desoxoparasherquamide, 2-DOPH) has weak activity in *C. elegans* even though it has potent effects in gastrointestinal parasitic worms. To determine whether the cuticle permeability of *C. elegans* is a barrier to the entry of 2-DOPH, we adapted the cut-worm model introduced by Lewis et al (1980). Cutting the worms at approximately one-third of their length between the anterior end and mid-point increased the respond to nAChR (nicotine acetylcholine receptor) agonists compared to intact specimens. The nAChR antagonist mecamylamine also has effects in cut worms and antagonized the paralytic effects of the nAChR agonists pyrantel, levamisole bephenium and nicotine. Using the cut worm model, we observed that 2-DOPH rapidly paralyzed cut preparations of *C. elegans* at concentrations as low as 10^{-5} M, and antagonized the different agonists, especially bephenium. In contrast, 2-DOPH in concentrations as high as 10^{-4} M failed to delay the onset of paralysis caused by low concentrations of the agonist AAD (monopantel) in cut worms. These results suggest that 2-DOPH is a nAChR agonist in nematodes, including free-living nematodes, and this drug and monopantel may not suffer from a negative interaction if used together in the treatment of nematodiasis. We also show that cut worm bioassays are useful to test physiological effects of new anthelmintics or molecules with low accessibility through the cuticle. This work was supported by NSERC, Canada Research Chairs and a Tomlinson Fellowship from McGill.

Mukesh Samant, Prasad Padmanabhan, and Barbara Papadopoulou. Research Centre in Infectious Disease, CHUL Research Centre (CHUQ), Laval University, Quebec, Canada. **Disruption of the ATP-dependent RNA-helicase-67 gene impairs amastigote-specific growth and differentiation in *Leishmania*.**

RNA helicases are enzymes that use ATP to unwind double-stranded RNA (dsRNA) molecules or to remodel RNA or ribonucleoprotein complexes (RNPs). One of the largest protein classes in RNA metabolism, RNA helicases are present in almost all organisms, ranging from bacteria and viruses to humans. To explore the role of RNA-helicases in the protozoan parasite *Leishmania*, we engineered *Leishmania infantum* deficient in the helicase-67 gene (LdiHEL-67). Remarkably, helicase-67 null mutants (LdiHEL-67^{-/-}) were not able to differentiate into axenic amastigote forms and showed slow growth rate in promastigotes. Furthermore, LdiHEL-67^{-/-} mutants were unable to infect macrophages *in vitro*. We also evaluated this growth impairment using pH and temperature stress separately and observed that promastigotes of LdiHEL-67^{-/-} mutants were able to grow in acidic pH (5.5) but not under temperature stress (37°C). Primer extension studies revealed that temperature-stressed LdiHEL-67^{-/-} mutants showed a significant degradation of the ribosomal RNA in comparison to wild type. To explore the

mechanism by which LdiHEL-67^{-/-} mutant becomes sensitive to temperature stress, we overexpressed the LdiHEL-67 gene fused to an HA-tag in wild type *L. infantum* and performed immunoprecipitation (IP) studies followed by mass spectrometry (MS/MS) analysis. One of the major immunoprecipitated proteins identified was HSP70, suggesting that the RNA helicase-67 and HSP70-related proteins may form a complex during heat stress, which is necessary for the parasite's survival in elevated temperature (37°C). The high sensitivity of *Leishmania* to temperature stress in the absence of the RNA helicase-67 could explain the defect of LdiHEL-67^{-/-} mutant in differentiating into axenic amastigotes and growing in infected macrophages *in vitro*. Additional studies are currently under way to better understand the protective effect of the RNA helicase-67 under temperature stress. The lack of virulence in LdiHEL-67^{-/-} mutants could also be exploited as a potential live attenuated vaccine against leishmaniasis.

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A global functional atlas of *Trypanosoma brucei* genome.

The evolutionary distance between trypanosomatids and well-studied model organisms has imposed many obstacles on the challenging task of functional characterization of trypanosomatid genomes. About half of trypanosomatid genes have no obvious homologs in available non-trypanosomatid genomes, making it impossible to predict their functions based on homology. To address this issue, we have constructed a functional interaction network of *Trypanosoma brucei* genes using an integrated genomics approach. In the first step, we compiled a compendium of 70 gene expression profiling experiments, including 39 microarray experiments from our lab and 31 publicly available microarray/RNA-Seq datasets from other labs. Our microarray experiments included profiling of 23 different RNAi clones, 11 different chemical treatments, and five independent cultures of procyclic form *T. brucei* cells. This compendium was used to construct a co-expression network of *T. brucei* genes. By combining this co-expression network with the codon usage content and phylogenetic profiles of *T. brucei* genes, we have been able to create a high-quality functional interaction network which has led us to predict the functions of several hundred previously uncharacterized *T. brucei* genes. These predictions include assignment of uncharacterized genes to several essential biological processes and pathways. This great leap towards comprehensive functional annotation of *T. brucei* genome leads us to a better understanding of the biology of trypanosomatids, and also provides new potential targets for treatment of their respective diseases. This research is supported by CIHR and NSERC.

Lisa Starr, Maurice Odiere, Marilyn E. Scott, Institute of Parasitology, McGill Univ. Johanne Naylor, Marie-Pierre Lardeau, Kristine G. Koski, School of Dietetics and Human Nutrition, McGill Univ. Odalis Sinisterra, Ministry of Health- Nutrition Division, Panama. **Vitamin D, Growth, Cytokines and Infection Status in Panamanian Mothers and their Preschool Children.**

Background: Vitamin D (vitD) is emerging as an important regulator of immune function and growth.

Methods: This cross sectional study evaluated the vitD status of parasite-infected Panamanian mothers (n=68) and their preschool children (n=86) in order to determine associations between vitD and birth weight, stunting, growth factors and/or cytokines. Cytokines were measured using Luminex and 25-hydroxyvitD (25(OH)D) using chemiluminescence. *Results:* There was a high prevalence of short stature/stunting (47% and 62%), as well as infection (72% and 58%) in both mothers and children. Male children had significantly higher 25(OH)D than females (73.7 vs. 61.8 nmol/L, $P < 0.05$). Birth weight was 154 g lower ($P < 0.05$) and height-for-age Z-score was 0.95 lower ($P < 0.05$) in children of mothers with 25(OH)D < 75 nmol/L and < 50 nmol/L respectively. There was also a correlation between maternal and child 25(OH)D concentrations. In children, IGF-1 was positively correlated with 25(OH)D, while TNF- α was inversely correlated with IGF-1. Other cytokines were measured but were not associated with vitD, except MCP-1 in mothers. *Conclusion:* Despite year-round UV exposure, suboptimal vitD concentration is common in Panamanian mothers and their children. Our data suggest that lower maternal 25(OH)D negatively affects offspring growth. This work was supported by SENACYT (Panama) and the McGill Vitamin Fund.

Guillaume St-Pierre, Pampa Bhaumik, Christian St-Pierre, Sachiko Sato Research Centre for Infectious Diseases, CHUQ, Laval University, Quebec

Galactin-3 implication in the neutrophils recruitment during cutaneous leishmaniasis

Leishmania major is the causal agent of cutaneous leishmaniasis, which affects millions of people around the world, mostly in developing countries. During the immunological challenge, neutrophils are the first leukocytes to

be recruited to the infection site. These cells are important for the parasite killing and for the modulation of the immune response. In our lab, we study the role of galectins (soluble β -galactoside binding lectins) following different challenges. Galectins are known to have various roles in cellular adhesion and activation. Our previous *in vitro* studies demonstrate that Galectin-3 (Gal-3) recognizes *L. major*-specific phosphoglycan (PG). Using Gal-3 knock out mice, we next investigated the role of Gal-3 in cutaneous *L. major* infection. First, we observed that Gal-3 KO mice had a defect in neutrophil recruitment 6-24 h after a footpad infection, which was also observable in the corresponding draining lymph nodes. This reduction in the number of neutrophils recruited in Gal-3 KO mice correlated with a higher parasite load in the footpad 24h post infection. Then, we followed the evolution of the footpad lesion for a longer time period. In the first few weeks of the infection, the Gal-3 KO mice footpad volume was significantly higher than what we observed in WT mice. Finally, to further study the role of neutrophils in the cutaneous leishmaniasis, we pre-treated WT mice with anti-Ly6G antibody to specifically deplete neutrophils. At 24h post infection, footpad parasite load was higher in neutrophil-depleted mice compared to mice treated with the isotype control, indicating the significant role of neutrophils in the initial clearance of the parasites at the infection site. In the future, it is interesting to investigate further on the mechanism by which Gal-3 mediates neutrophil recruitment to the infection sites.

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***Leishmania donovani* glycosomal proteins form complexes in the cytosol for trafficking to the organelle surface.**

The glycosomes of *Leishmania donovani* are unique microbody organelles that compartmentalize a variety of metabolic pathways essential for parasite survival. Trafficking and import of newly synthesized proteins into the glycosome is dependent on the receptor proteins peroxin 5 (LdPEX5) and peroxin 7 (LdPEX7), and the docking protein peroxin 14 (LdPEX14), a peripheral membrane protein anchored to the surface of the glycosomal membrane. To understand the molecular events required for the sorting and trafficking of nascent polypeptides to the glycosome we employed a variety of techniques to examine the structure of native LdPEX5, LdPEX7, and LdPEX14. Biochemical analysis revealed that in the cytosol of *L. donovani*, LdPEX5 and LdPEX7 form heteromeric complexes loaded with various PTS1 and PTS2 cargo proteins in the cytosol. These structurally diverse arrays of heteromeric complexes are responsible for trafficking of proteins to the surface of the glycosome, into which they are trafficked via LdPEX14, the glycosomal docking protein. Investigation of the effects of receptor-cargo complex binding to LdPEX14 revealed that docking of receptor-cargo complexes causes LdPEX14 to transform from a peripheral to an integral membrane protein.

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The impacts of food availability on epidemic dynamics: A *Poecilia reticulata*-*Gyrodactylus turnbulli* host-parasite model

Understanding the links between environmental variables and disease transmission is important to resource management and human health, particularly in the context of global environmental change. One environmental variable of particular importance is food availability. Although the impacts of host nutritional status on individual disease experience have been well-studied, how this relationship may translate to the population level has not. This study was undertaken to determine what effect the amount of food allotted to a population might have on the spread of infectious disease throughout that population, using the *Poecilia reticulata*-*Gyrodactylus turnbulli* system as a model. 21 populations of ten guppies were assigned to low, medium or high food availability treatments and one fish from each tank was infected. Epidemics were monitored by examining each fish for parasites every other day for ten days. Individual infection variables for each fish and major epidemic variables for each population were calculated and analyzed using generalized linear models. At the population level, food availability was found to have a significant negative effect on peak tank burden and a moderate negative effect on parasite aggregation, despite a strong effect of replicate. At the individual level, a significant positive relationship between initial condition index and major infection variables was observed. Our findings suggest that high food availability has a moderate mitigating effect on the total burden of disease in the population and also to some extent on the distribution of that burden, but that other factors, particularly relating to the individual level, are important.

Rajesh M. Valanparambil¹, Yovany Moreno², Mariela Segura¹, Mifong Tam¹, Pierre-Paul Gros¹, Timothy G. Geary² and Mary M. Stevenson¹. ¹Research Institute of the McGill University Health Centre and ²Institute of Parasitology, McGill University. **Proteomic analysis of excretory-secretory products of *Heligmosomoides polygyrus* assessed with next-generation sequence transcriptomic information**

Heligmosomoides polygyrus (Hp) is a murine intestinal nematode convenient for the study of the biology and pathology associated with gastrointestinal helminth infections. Hp suppresses immune responses to unrelated antigens, secondary infections, and alleviates immune-mediated diseases. These effects may be mediated by the ability of excretory-secretory products (ESP) of this parasite to modulate dendritic cell function with down-stream effects on adaptive CD4⁺ T cell responses to bystander antigen. Studies on the cellular and molecular aspects of Hp-mediated immunoregulation are not feasible as the whole genome sequence of Hp is not available. To overcome this hurdle, a transcriptome next generation sequencing (RNA-seq) *de novo* assembly containing 33,641 transcripts was generated, annotated and employed to interrogate mass spectrometry (MS) data derived from 1D-SDS PAGE and LC-MS/MS analysis of ESP. Using the database generated from the 6 open reading frames deduced from the RNA-seq assembly, 209 proteins were identified in ESP. The identified proteins include homologues of vitellogenins, retinol- and fatty acid-binding proteins, globin, and the allergen V5/Tpx-1-related family of proteins, as well as several potential immunomodulators, such as homologues of macrophage migration inhibitory factor, cysteine protease inhibitors, galectins, C-type lectins, peroxiredoxin, and glutathione S-transferase. These results provide data that will help to reveal molecular and biochemical aspects of host-*H. polygyrus* biology. In particular, the analysis identified proteins that are candidate effectors of parasite-induced immunomodulation. Supported by NSERC, CHIR and FQRNT-Centre for Host-Parasite Interactions (NIP).

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Screening for novel anthelmintics that act on nematode neuropeptide receptors

Neuropeptides in the FMRFamide family (FLPs) are essential components of nematode neuromuscular physiology. Multiple G protein-coupled receptors (GPCRs) in this family have been identified from the free-living nematode *Caenorhabditis elegans* as well as from many parasitic species. cDNAs encoding several FLP-GPCRs have been functionally expressed in yeast (*Saccharomyces cerevisiae*) in a format that allows facile high-throughput screening assays to identify small molecule, non-peptide ligands that act as agonists or antagonists of these GPCRs; such compounds are good candidates for development as novel anthelmintics. We adapted these recombinant yeast strains for screening a collection of synthetic and natural product chemicals held at McGill (HTS/HCS facility, Department of Biochemistry) as part of the Canadian Chemical Biology Network. We present here a description of the screening system and results from the initial assays of natural products held in this collection.

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Validating Acetylcholine-Gated Chloride Channels as Novel Nematocide Targets.

We have identified a novel class of ligand gated ion channels, the acetylcholine-gated chloride channels (ACCs), in *Caenorhabditis elegans* (*C. elegans*) that we believe would be a good targets for the design of new anthelmintic drugs. This ACC family is specific to nematodes (Dent, 2006) and is not a target of previously known nematocides. The class of ACCs comprises 8 subunit genes; a beneficial characteristic since a gene family that represents multiple targets of a single drug may slow the onset of resistance. To test the validity of these ACC subunits as targets for nematocides, we determined the expression pattern of the 8 ACC subunit genes using promoter::GFP fusion constructs and have been characterizing the electrophysiological properties of ACC channels formed by the different subunits. Most of the subunits are expressed in a non-overlapping subset of approximately 20 neurons in *C. elegans*. We have shown that the K10D6.1 subunit forms a functional homomeric channels that responds to acetylcholine (ACh) in oocytes and we have shown that it is a chloride channel. We also have evidence that ACC-1 and F47A4.1 subunits can form a heteromeric ACh-sensitive channel in oocytes. Due to the expression of ACCs in a significant fraction of the nervous system, a drug that targets these channels by over-activating them promises to have highly deleterious effects on nematode physiology. We have shown that chronically inhibiting ACC-1 expressing tissues is lethal to the worm and have begun experiments to validate the other ACC subunits as good drug targets as well. Because of this, and the fact that the ACCs are nematode-specific, we conclude that ACCs merit further investigation as anti-parasitic drug targets and we hypothesize that a drug that targets these channels would be a safe and effective nematocide. This work is supported by NSERC and Chemtura Co.