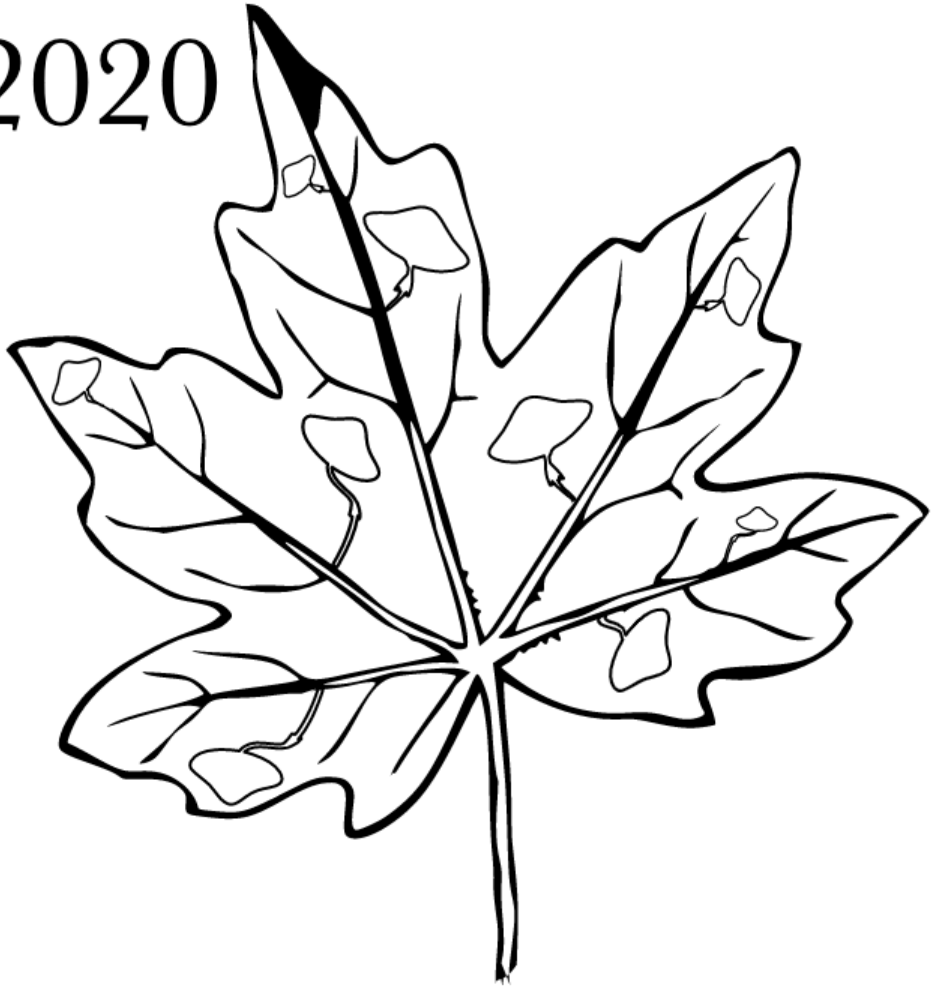
Canadian Fungal Research Network | CanFunNet 2020

Schedule Book

CanFunNet 2020



July 29-30, 2020

Online

Canadian Fungal Research Network | CanFunNet 2020

Welcome to CanFunNet 2020! We hope this is the first of many events to bring together the Canadian Fungal Research community into the same space.

The Canadian Fungal Research Network was first established at a small meeting in Winnipeg in 2019, with the aim of uniting the diverse group of fungal researchers across Canada. You can read more about that establishment meeting in our white paper (currently in press at the *Canadian Journal of Microbiology*).

If you're interested in becoming more involved in CanFunNet (or have an idea for a future event!) please get in touch! Email: canfunnet@gmail.ca.

Organizing committee
Aleeza Gerstein (University of Manitoba)
Emile Gluck-Thaler (University of Pennsylvania)
Steve Harris (University of Manitoba)
Rebecca Shapiro (University of Guelph)

Career development panel
Linda Horianopoulos (University of British Columbia)
Allison Walker (Acadia University)

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Michelle Agyare-Tabbi
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SCHEDULE OF EVENTS

WEDNESDAY, July 29		
8:30-9:50 PT / 10:30-11:50 CT / 11:30-12:50 ET	**Conference Welcome & Keynote Session I**	
10:15-11:15 PT / 12:15-13:15 CT / 13:15-14:15 ET	**Environmental Sensing and Signaling**	**Drug and Stress Resistance**
11:30-12:30 PT / 13:30-14:30 CT / 14:30-15:30 ET	**Applications and Resource Development I**	**Fungal Virulence**
12:45-13:14 PT / 14:45-15:45 CT / 15:45-16:45 ET	**Yeast as a Model**	**Insights into Metabolism**

THURSDAY, July 30		
8:30-9:50 PT / 10:30-11:50 CT / 11:30-12:50 ET	**Keynote Session II**	
10:15-11:15 PT / 12:15-13:15 CT / 13:15-14:15 ET	**Species Interactions**	**Fungal Diversity**
11:30-12:30 PT / 13:30-14:30 CT / 14:30-15:30 ET	**Applications and Resource Development II**	**Stress Response**
12:45-13:14 PT / 14:45-15:45 CT / 15:45-16:45 ET	**Molecular Biology**	**New Approaches and Techniques**

Keynote and Talk Sessions

Wednesday, July 29

Keynote - Room A

8:30 PT / 11:30 ET **Conference Welcome & Introductions**

8:40 PT / 11:40 ET Early fungi and the dawn of life on land. **Mary Berbee**

9:05 PT / 12:05 ET Nuclear dynamics in the arbuscular mycorrhizal fungus *Rhizophagus irregularis*. **Vasilis Kokkoris**

9:30 PT / 12:30 ET Exploring broad-spectrum and combination antifungal strategies to combat human fungal pathogens. **Nicole Revie**

Environmental Sensing and Signaling - Room A

Moderator: Patricia Larkin-Thomas

Standard Talks

10:15 PT / 13:15 ET The *Candida albicans* MAPKKK Ste11 has both regulatory and enzymatic functions in opaque cells under pheromone stimulation. **Anna Carolina Borges Pereira da Costa**

10:30 PT / 13:30 ET Role of the TOR (Target of Rapamycin) Pathway in circadian rhythmicity of *Neurospora crassa*. **Rosa Eskandari**

10:45 PT / 13:45 ET Mechanisms underlying the chemotropism of *Fusarium graminearum* on the wheat head. **Pooja Sridhar**

Flash Talks

11:00 PT / 14:00 ET Global phosphorylation analysis of the Protein Kinase A-regulated phosphoproteome of *C. neoformans*. **Brianna Ball**

11:05 PT / 14:05 ET Functional genomic analysis of protein kinases in the human fungal pathogen *Candida albicans*. **Yunjin (Rachel) Lee**

Drug and Stress Resistance - Room B

Moderator: Elizabeth Brauer

Standard Talks

10:15 PT / 13:15 ET Understanding *Candida auris*: Exploiting chemical matter to uncover cell biology and characterize resistance mechanisms in this emerging fungal pathogen. **Kali Iyer**

10:30 PT / 13:30 ET *Tra1* is required for antifungal drug resistance in yeast. **Matthew Berg**

10:45 PT / 13:45 ET A comparative study of boric acid and fluconazole drug responses in *C. albicans* planktonic and biofilm cells. **Ola Salama**

Flash Talks

11:00 PT / 14:00 ET Identification of novel bioactives to combat the emerging fungal pathogen *Candida auris*. **Emily Puumala**

11:05 PT / 14:05 ET Role of phosphatidylcholine on Cryptococcal capsule formation. **Christopher Lee**

Applications and Resource Development I - Room A

Moderator: Tara Rintoul

Standard Talks

11:30 PT / 14:30 ET Screening novel marine fungal strains for the production of polyunsaturated fatty acids, specifically eicosapentaenoic acid and arachidonic Acid. **Nicole Smith**

11:45 PT / 14:45 ET The impacts of gold mining on mycorrhiza in Northern Canada. **Sarah Mediouni**

12:00 PT / 15:00 ET The international dimension of Canadian mycology. **Keith Seifert**

Flash Talks

12:15 PT / 15:15 ET Trans-Kingdom Conjugation Enables Simple and Robust DNA Delivery to Fungi. **Maximillian Soltysiak**

12:20 PT / 15:20 ET *Saccharomyces*, at your *cerevisiae*: Does brewing equipment get kidney stones, and how can we make this the yeast of our problems? **Nykole Crevits**

Fungal Virulence - Room B

Moderator: Jennifer Geddes-McAlister

Standard Talks

11:30 PT / 14:30 ET Combatting fungal infections through the discovery and elucidation of novel anti-virulence strategies. **Jennifer Geddes-McAlister**

11:45 PT / 14:45 ET *Candida albicans* exhibits a cytoprotective response to anti-fungal drugs that facilitates the evolution of drug resistance. **Michael Hallett**

12:00 PT / 15:00 ET The zinc cluster transcription factor *Rha1* is a positive filamentation regulator in *Candida albicans*. **Raha Parvizi Omran**

Flash Talks

12:15 PT / 15:15 ET Integrating high-throughput screening of antifungal microbial interactions and system biology-based approaches to study complex gene-microbe interactions. **Gunjan Gupta**

12:20 PT / 15:20 ET Dissecting the mechanisms governing inter-kingdom interactions between *Candida albicans* and *Lactobacillus* species. **Jessie MacAlpine**

Yeast as a Model - Room A

Moderator: Aashiq Kachroo

Standard Talks

12:45 PT / 15:45 ET Yeast cytosolic J-chaperones perform distinct functions in cellular protein quality control. **Andrey Petropavlovskiy**

13:00 PT / 16:00 ET Is the main function of the Bowen-Conradi Syndrome protein Emg1 to displace snR35? **Courtney Harris**

13:15 PT / 16:15 ET Casein kinase 2 catalytic subunits a1/a2 of the small subunit processome's (SSU) UTP-C sub complex regulate growth likely through ribosome biosynthesis. **James Cluff**

Flash Talks

13:30 PT / 16:30 ET Lithium chloride sensitivity in translation mechanism of structured mRNAs in yeast. **Sasi Kumar Jagadeesan**

Insights in Metabolism - Room B

Moderator: Linda Harris

Standard Talks

12:45 PT / 15:45 ET Characterization of the roles of the monothiol glutaredoxin, Grx4, in the cell biology and virulence of *Ustilago maydis*. **Sean McCotter**

13:00 PT / 16:00 ET Enhanced stress tolerance in Norwegian kveik results in increased fermentation efficiencies at extreme temperatures. **Barret Foster**

13:15 PT / 16:15 ET Environmental factors and polyketide synthase gene expression in an usnic acid producing lichen-fungus. **W.G. Duleeka I. Gunawardana**

Flash Talks

13:30 PT / 16:30 ET How arbuscular mycorrhizal life history traits influence soil carbon cycling. **Caitlyn Horsch**

13:35 PT / 16:35 ET Anti-biofilm activity of unsaturated fatty acids with fluconazole. **Abdullahi Jamiu**

Keynote Session II - Room A

8:30 PT / 11:30 ET The Enterprise: A massive transposon carrying *Spok* meiotic drive genes. **Aaron Vogan**

8:55 PT / 11:55 ET Characterization of *TRI6*, a global regulator of secondary metabolism. **Kristina Shostak**

9:20 PT / 12:20 ET Genome mining *Aspergillus niger* for discovery of antimicrobial compounds. **Cameron Semper**

Species Interactions - Room A

Moderator: Alexandra Dallaire

Standard Talks

10:15 PT / 13:15 ET Analysis of incompatibility in het-6 locus in *Neurospora crassa*. **Ghazaleh Nourparvar**

10:30 PT / 13:30 ET Small non-coding RNAs and the development of Arbuscular Mycorrhizal fungi. **Alexandra Dallaire**

10:45 PT / 13:45 ET Global Analysis of circuitry governing *Candida albicans* morphogenesis within host immune cells. **Nicola Case**

Flash Talks

11:00 PT / 14:00 ET Investigating the effects of arbuscular mycorrhizae on *Crocanthemum canadense* (L.) Britt. (Cistaceae) propagated in tissue culture. **Kendra Sampson**

11:05 PT / 14:05 ET Effects of an *Epichloë* endophyte on foliar fungal communities in tall fescue grass (*Schedonorus arundinaceus*). **Jenna Dale**

Fungal Diversity - Room B

Moderator: Allison Walker

Standard Talks

10:15 PT / 13:15 ET Differences in genome content underlie standing genetic variation in an asexual eukaryote. **Emile Gluck-Thaler**

10:30 PT / 13:30 ET Sympatric divergence of ergot fungi populations in Canada and Midwestern USA. **Miao Liu**

Flash Talks

10:45 PT / 13:45 ET Survey of culturable fungal endosymbionts in Nova Scotia intertidal and subtidal macroalgae. **Caryn Leigh Cooper**

10:50 PT / 13:50 ET Vertical distribution of some crustose littoral zone lichens in the Bay of Fundy. **Cole Vail**

10:55 PT / 13:55 ET Four genomic clades of *Candida auris* identified in Canada, 2012-2019. **Domenica De Luca**

11:00 PT / 14:00 ET Pathogenic allodiploid hybrids of *Aspergillus* Fungi. **Jacob Steenwyk**

11:05 PT / 14:05 ET Microfungi diversity from the bark of *Acer saccharum*. **Jonathan Mack**

Applications and Resource Development II - Room A

Moderator: TBA

Standard Talks

11:30 PT / 14:30 ET The mycoflora of New Brunswick: First steps on a long road ahead. **Alfredo Justo**

11:45 PT / 14:45 ET The first report of a culturable microbiome from pollinated style tissue. **Michelle Thompson**

12:00 PT / 15:00 ET Biological control potential of ectomycorrhizal fungi against *F. circinatum* on Pinus patula seedlings. **Veronique Chartier-FitzGerald**

Flash Talks

12:15 PT / 15:15 ET Addressing Barriers of Ontario Cider Production. **Jordan Hofstra**

12:20 PT / 15:20 ET FungiDB: integrating genomic data for pathogens and model organisms and providing advanced search capabilities and large-scale data analysis. **Evelina Basenko**

Stress Response - Room B

Moderator: Aleeza Gerstein

Standard Talks

11:30 PT / 14:30 ET Regulation of phagosomal size and integrity during fungal infection. **Johannes Westman**

11:45 PT / 14:45 ET J domain co-chaperones contribute to host adaptation in *Cryptococcus neoformans* pathogenesis. **Linda Horianopoulos**

12:00 PT / 15:00 ET The proteasome governs fungal morphogenesis via functional connections with *Hsp90* and cAMP-PKA signaling. **Saif Hossain**

Flash Talks

12:15 PT / 15:15 ET Effect of melanin loss on *Exophiala dermatitidis* morphology and its ability to adapt to different stresses. **Kamaldeep Chhoker**

12:20 PT / 15:20 ET Using a CRISPR-Cas9-based gene drive to target stress response genes in *Candida albicans*. **Viola Halder**

Genes to Proteins - Room A

Moderator: Nicolas Corradi

Standard Talks

12:45 PT / 15:45 ET *VID28* and *VID30* Regulate Glucose Repression and Derepression in *Saccharomyces cerevisiae*. **Jordan Willis**

13:00 PT / 16:00 ET Modulation of the complex regulatory network for methionine biosynthesis in fungi. **Manjari Shrivastava**

13:15 PT / 16:15 ET Systematic perturbation of yeast essential genes using base editing. **Philippe Després**

Flash Talks

13:30 PT / 16:30 ET Transcriptomic profiling suggests a role for an ABA biosynthetic gene in *Fusarium graminearum* during its early infection of Wheat. **Tanya Sharma**

13:35 PT / 16:35 ET UhAVR1, an avirulence effector of *Ustilago hordei*, suppresses a conserved target(s) of plant immunity during the initial stages of fungal infection. **Ana Priscilla Montenegro Alonso**

New Approaches - Room B

Moderator: Yan Wang

Standard Talks

12:45 PT / 15:45 ET A haplotype-aware de novo assembly approach for the pathogenic dikaryotic fungus, *Puccinia triticina*. **Sean Formby**

13:00 PT / 16:00 ET Chromosome-scale genome assembly, dikaryon phasing, and centromere mapping using Hi-C. **Ivan Liachko**

Flash Talks

13:15 PT / 16:15 ET A deep learning approach to detect fungi in different morphologies from microscopy-based image analysis. **Van Bettauer**

13:20 PT / 16:20 ET The role of fungal adhesins in mediating morphogenesis and virulence in *Candida albicans*. **Sierra Rosiana**

13:25 PT / 16:25 ET Identification of novel NHEJ DNA repair genes using machine learning algorithms. **Minuka Hewapathirana**

13:30 PT / 16:30 ET Expanding the genetic toolbox for *Candida albicans*: the development of novel CRISPR interference and CRISPR base editing technologies. **Jehoshua Sharma**

Abstracts

UhAVR1, an avirulence effector of *Ustilago hordei*, suppresses a conserved target(s) of plant immunity during the initial stages of fungal infection

Ana Priscilla Montenegro Alonso*, Vasilis Kokkoris, Pierre-Luc Chagnon, Gökalp Yildirir, Stephanie Mathieu, Kelsey Clarke, Keith Hubbard, Franck Stefani , and Nicolas Corradi
*University of British Columbia

Ustilago hordei is a basidiomycete fungus that causes covered smut disease of barley and oats. Bioinformatic analysis of its genome has predicted approximately 333 candidate secreted effectors thought to aid the fungal infection process and support the pathogen's lifestyle. Out of those, UhAVR1 constitutes the only proven avirulence effector identified in smut pathogens. Infection of barley cultivars carrying the resistance gene Ruh1 with fungal strains harboring UhAvr1 leads to full immunity. UhAVR1 lacks homology to any protein or motifs with the exception of a predicted 19 amino-acid N-terminal signal peptide. Our results show that UhAVR1 is expressed during the early stages of fungal infection where it performs its virulence function or leads to HR triggering. Its secretion by the fungus occurs via the ER-Golgi and depends on the presence of its signal peptide. A fungal structure, hypothesized to be an appressorium, may aid Uh during the initial fungal establishment in barley. Delivery of UhAVR1 via foxtail mosaic virus in *Nicotiana benthamiana* and barley, or delivery using Pseudomonas species in barley indicates a role in the suppression of a component(s) of basal immunity which is conserved in both plant systems. Further studies are necessary to identify host components this effector targets and to establish its importance as a core effector of smut pathogens.

Global phosphorylation analysis of the Protein Kinase A-regulated phosphoproteome of *C. neoformans*

Brianna Ball*, Benjamin Muselius, and Jennifer Geddes-McAlister
*University of Guelph

Cryptococcus neoformans is a notorious opportunistic fungal pathogen that is an etiological agent of the dangerous disease cryptococcosis. *C. neoformans* targets immunocompromised individuals with a high infection rate within the HIV/AIDS demographic, inadequate antifungal intervention results in life-threatening manifestations of cryptococcal meningitis and meningoencephalitis. The cyclic AMP (cAMP)/protein kinase A (PKA) signal transduction pathway is a crucial regulator of virulence in many fungal species. *C. neoformans* employs PKA-induced phosphorylation to regulate its vital virulence factors, including capsule, melanin, and laccase production, however, direct targets of Pka1 phosphorylation to initiate such

regulatory events have not been completely defined. The aim of this research focuses on the regulation of Pka1 on the global phosphoproteome of *C. neoformans* to identify new components within this sophisticated signaling pathway. To assess Pka1-regulation, high-resolution mass spectrometry of Fe-NTA enriched phosphopeptides were quantified from wild-type *C. neoformans* and a pka1 gene deletion strain grown in culture. To complement the experimental data and reveal the complete landscape of phosphorylation dynamics in this fungal pathogen, in silico bioinformatic enrichment of the PKA motif was generated for phosphorylation site prediction from the *C. neoformans* proteome. This comprehensive profiling of phosphoproteins and phosphosites of C. neoformans deepens the understanding of the communication between Pka1 downstream phosphorylation events in coordination with diverse biological processes.

FungiDB: integrating genomic data for pathogens and model organisms and providing advanced search capabilities and large-scale data analysis

Evelina Basenko* presenting on behalf of the entire VEuPathDB team
*University of Liverpool

FungiDB (https://beta.fungidb.org) is a free, online data mining resource supporting fungi and oomycetes. FungiDB is a component of the Eukaryotic Pathogen, Vector and Host Informatics Resources (VEuPathDB.org), which integrates a diverse array of data for invertebrate vectors of human pathogens, pathogenic and non-pathogenic species and provides sophisticated data mining tools.VEuPathDB databases offer a one-stop-shop to enable: Browsing of genomes and gene record pages in an encyclopaedic manner to explore all available information and data; Searching integrated datasets via a unique search strategy system that utilises a web-based graphical interface to facilitate mining of genomes, functional data (e.g. transcriptomic, proteomic, phenomic and variation data), annotation, and the results of in-house analyses (protein domains, orthology predictions, metabolic pathways, etc.); Analysing your own data through a private VEuPathDB Galaxy workspace that offers preloaded genomes and sample workflows for RNA-Seq data and variant calling analysis. Users can transfer results to the private My Data Sets workspace, explore the data using the search strategy system and publicly available datasets, and view associated tracks in JBrowse, Annotating genomes through whole genome orthology mapping, the user comments system that captures expert knowledge about phenotypes, relevant PubMed records, etc. and editing of genome models via Apollo, a web-based genomic annotation editing platform; Learning via series of workshops, webinars, and tutorials; FungiDB is supported in part by NIH HHSN75N93019C00077 and the Wellcome Biomedical Resources #212929/Z/18/Z grants.

Tra1 is required for antifungal drug resistance in yeast

Matthew Berg*, Yuwei Jiang, Iqra Razzaq, Julie Genereaux, Christopher J. Brandl, Rebecca S. Shapiro, and Patrick Lajoie
*University of Western Ontario

The emergence of drug-resistant fungal strains is a threat to human health. Identifying targets that increase the susceptibility of fungal pathogens to current therapies will significantly improve outcomes. Tra1 is an essential component of the SAGA and NuA4 transcriptional co-activator complexes and is linked to multiple cellular processes associated with the yeast response to antifungal drugs. As a PIKK family member, Tra1 is characterized by a C-terminal phosphoinositide 3-kinase (PI3K) domain. Like TOR, another PIKK family member, Tra1 should be druggable. Interestingly, while the kinase domain structure is conserved, Tra1 lacks kinase activity. We recently identified specific residues within the putative ATP-binding cleft of *S. cerevisiae* Tra1 that compromises SAGA/NuA4 assembly and function. The mutant increases susceptibility to multiple stressors, including antifungal compounds such as caspofungin. Decreased resistance to antifungals is linked, in part, to a compromised fungal stress response through calcineurin signaling. Moreover, a similar mutant generated in the pathogenic yeast *C. albicans* displays similar phenotypes, suggesting that Tra1 broadly mediates antifungal response across yeast species. Transcriptional profiling in *C. albicans* identified 68 genes that were differentially expressed when the Tra1 mutant was treated with caspofungin, as compared to gene expression changes induced by either the Tra1 mutant or capsofungin alone. Included in this set were genes involved in cell wall maintenance, adhesion and filamentous growth. As Tra1 is a component of both SAGA and NuA4, it potentially has a unique regulatory role in the yeast antifungal response and is a promising new therapeutic target for fungal infections.

A deep learning approach to detect fungi in different morphologies from microscopy-based image analysis

Van Bettauer*, Kirbizakis E, Massahi S1, Simpson S, Harb M, Khurdia S, Law C, Costa ACBP, Omran RP, Whiteway M, and Hallett MT
*Concordia University

We developed a deep learning multi-object detection framework for microscopic images, and used it to automatically detect and label the full range of *C. albicans* morphology. Our method is based on a fully convolutional one-stage neural network object detection framework, which exploits the natural multi-scale structure of microscopic images. To train the model, we defined 11 classes based on known

C.albicans morphology and manually annotated hundreds of images with bounding boxes. We find that our system performs well across a diverse range of images, including both images with a single morphology and images consisting of cells of many different morphologies. By using a novel method for model interpretation, we are able to characterize features of *C.albicans* morphology which can be used to improve performance on other downstream tasks. In particular, we show that our system can count the number of instances of a morphological feature in a population of cells, and can broadly characterize features that indicate the likelihood of a morphological transition. Together our system is a robust classifier that can be used in an unsupervised fashion to discover de novo aspects of fungal morphology."

Global analysis of circuitry governing *Candida albicans* morphogenesis within host immune cells

Nicola Case*, Kwamaa Duah, Teresa O'Meara, Brett Larsen, Cassandra J. Wong, Sang Hu Kim, Robert T. Todd, Anna M. Selmecki, Anne-Claude Gingras, Luke Whitesell, Amanda O. Veri, and Leah E. Cowen
*University of Toronto

The evasion of killing by host immune cells is crucial for fungal survival in the host. For the human fungal pathogen *Candida albicans*, morphogenesis upon internalization by macrophages is a key intracellular survival strategy that occurs through mechanisms which remain largely enigmatic. To identify the *C. albicans* genes that orchestrate filamentation in the macrophage, we performed a functional genomic screen of conditional expression mutants covering ~40% of the genome and identified 298 genes important for filamentation upon phagocytosis. Notably, fifty-four of the genes were dispensable for filamentation in response to serum, demonstrating specificity in the program governing morphogenesis within macrophages. To discover circuitry through which one of these genes enables filamentation, we performed selection experiments to restore filamentation in a strain lacking MSN5, which encodes a predicted karyopherin for the nuclear import and export of proteins. Whole genome sequencing of evolved lineages uncovered potential filamentation-restoring genetic alterations. Further, we explored filamentation-inducing stimuli within the macrophage and determined that macrophage lysate is sufficient to induce morphogenesis. Bioactivity-guided fractionation coupled to mass spectrometry identified the immune modulator, prothymosin alpha (PTMA), as a potential macrophage-derived trigger of filamentation. Immunodepletion of PTMA from macrophage lysate abolished its ability to stimulate *C. albicans* filamentation, supporting PTMA as a filamentation-inducing component of the lysate. This work is the first to implicate a specific host protein as a trigger of filamentation and identifies key elements of the regulatory circuitry uniquely

governing *C. albicans* morphogenesis in response to phagocytosis by host immune cells.

Biological control potential of ectomycorrhizal fungi against *F. circinatum* on *Pinus patula* seedlings

Veronique Chartier-FitzGerald*, Dames, J.F., and Hawley, G.

Ectomycorrhizal fungal relationships are well known to act as biological control agents for their hosts along with providing increased access to nutrients and water. In the South African forestry industry, the fungal pathogen Fusarium circinatum is one of the largest limitations to commercial forestry both on plantations and within nurseries. In the present study the biological control and growth-promoting ability of local ECM fungi as an inoculum for Pinus patula seedlings exposed to F. circinatum strains is investigated. Four ECM fungal strains were isolated and molecularly identified as *Boletus edulis*, *Lactarius quieticolor*, *Suillus granulatus* and a *Suillus* strain. Their anti-fungal activity was shown to be highly varied in a dual assay with six *F. circinatum* strains: FC666, FC594, FC621, FC623, and FC701. The combined results of the anti-fungal assay and greenhouse trial showed that *B. edulis* and *S. granulatus* both produced indirect forms of pathogen inhibition while the Suillus strain and *L. quieticolor* both had much more direct forms of inhibition. Overall inoculation the Suillus strain and *L. quieticolor* resulted in the most significant growth of the *P. patula* seedlings. Thus, it is these isolates that require further research in the hopes of developing a commercial ECM inoculum for the South African forestry seedling industry to help combat *F. circinatum* and generally improve the health of *P. patula* seedlings in the nursery and field.

Effect of melanin loss on *Exophiala dermatitidis* morphology and its ability to adapt to different stresses

Kamaldeep Chhoker* and Dr. Steven Harris
*University of Manitoba

Black yeasts are fungi that have a black-brown appearance due to presence of melanins in the cells. Melanin helps the black yeasts survive various habitats that include extreme conditions, such as rock surfaces, and toxic niches that contain hydrocarbons and heavy metals. Melanin has been shown to provide various functions such as converting visible UV energy into heat, protecting fungi against lysis, and binding to various metals. *Exophiala dermatitidis* is one such black yeast that has melanin in the cell walls and shows meristematic growth. *E. dermatitidis* has been shown to grow in nutrient poor environments such as bathrooms, drains, and steam

baths, and is also an opportunistic pathogen of humans. This study aims to look at the effect of loss of melanin on cell count and colony morphology of *E. dermatitidis* mutants. This study also looks at the adaptability of these mutants under various stresses, including different temperatures, different media types and under different UV irradiation. The goal of this study is to use classical genetics, deep phenotyping and genome re-sequencing to identify candidate genes that might be responsible for these mutations and how certain mutations affect the adaptability of the *E. dermatitidis* mutants to different stresses.

Casein kinase 2 catalytic subunits a1/a2 of the small subunit processome's (SSU) UTP-C Sub Complex regulate growth likely through ribosome biosynthesis

James Cluff*, Elise Poole, and J. Michael Charette
*Brandon University

The nucleolus, site of ribosome assembly, is a diagnostic/prognostic marker of cancer, with cell growth being dependent on the rate of ribosome biosynthesis. Ribosome assembly defects (ribosomopathies) are associated with many cancers. The Small Subunit Processome (SSUP) is a large ribonucleoprotein complex responsible for the assembly of the SSU of the ribosome. It consists of five sub-complexes, one of which (UTP-C) likely contains the protein kinase CK2 complex, consisting of catalytic CKa1/CKa2 and regulatory CKb1/CKb2 proteins. CK2 is a ubiquitous and constitutively active serine/threonine kinase implicated in many cellular processes including growth, differentiation, and neoplasia.. CK2 regulates all three RNA polymerase and coordinates ribosomal protein production with ribosome assembly.
Demonstrate the presence of CK2 protein complex as a bona fide member of the SSU Processome and its regulatory role in ribosome assembly and cellular growth.
Using a yeast model system, we determined the role of CK2 in the regulation of ribosome assembly by genetically depleting cells of individual/pairs of CK2 subunits using a galactose inducible/glucose repressible promoter. As growth is directly correlated to ribosome assembly, growth curves are used as a surrogate for ribosome assembly. Membership of CK2 in the SSU processome will be confirmed by co-IP of each individual CK2 proteins with known SSU processome components. Genetic depletion of individual catalytic subunits CKa1 and CKa2 results in a reduction in growth while depletion of both catalytic subunits is lethal as seen in growth curves. Northern analysis of pre-rRNA processing will be used to identify defects in pre-rRNA processing in these strains. Co-IPs of CK2 with Kre33 confirm, CK2 membership in the SSU processome. We have shown for the first time that single and double depletion of the two catalytic CK2 proteins has an impact on cell growth, likely through a dysregulation of ribosome assembly.

Survey of culturable fungal endosymbionts in Nova Scotia intertidal and subtidal macroalgae

Caryn Leigh Cooper*, and Allison K. Walker
*Acadia University

Marine macroalgae are a polyphyletic group of photosynthetic eukaryotes which play critical roles in oxygen production, as primary producers, and in providing physical habitat structure. They harbor diverse microbial communities, including mutualistic, commensalistic, and parasitic fungi. These fungi may be obligately or facultatively marine and symbiotic. They are being investigated as a promising new resource for bioactive compounds, a wide variety of which likely remain undiscovered. In this investigation algicolous fungi were isolated from marine macroalgal samples collected from five intertidal ecosystems in Nova Scotia and identified using ITS rDNA Barcoding. From 11 species of marine macroalgae, 125 fungal isolates were identified to genus and 89 were identified species, representing 21 genera and 23 species. One potentially previously-undescribed fungus was also identified, and multilocus sequencing was undertaken to aid in its confirmation as a novel species.

The *Candida albicans* MAPKKK Ste11 has both regulatory and enzymatic functions in opaque cells under pheromone stimulation

Anna Carolina Borges Pereira da Costa*, Raha Parvizi Omran, Chris Law, Vanessa Dumeaux, and Malcolm Whiteway
*Concordia University

Saccharomyces cerevisiae cells and *C. albicans* opaque cells release pheromone to stimulate cells of opposite mating type. Although both organisms share the same orthologous proteins required for the activation of the pathway, the reaction cascade does not function the same way implying the regulation is different. In this study, we investigated the interactions among the pheromone players to identify the missing link amid the MAP kinase proteins. A set of mutant strains and GFP fusion strains were constructed and verified. The absence of STE2, CAG1, STE4, STE11, HST7 abrogated shmoo formation and mating. In the wild type strain, Cst5p and Cek1p tagged with GFP responded to pheromone stimulation by localizing in puncta at the tips of the shmoos and concentrating in the nucleus, while Cek2-GFP showed generalized localization with a nuclear concentration. The mutant strains showed no tagged-protein localization or mild response. Surprisingly, ste11Δ/Δ strain not only expressed Cst5-GFP at high levels under no stimulation but also exhibited comparable signal concentrated in puncta. ste11Δ/Δ also exhibited nuclear localization and expression of

Cek1 independent of pheromone stimulation but showed no influence on Cek2 localization or expression. However, the deletion of STE11 affected both Cek1 and Cek2 phosphorylation. The transcription profiles of ste11Δ/Δ cells compared to the pheromone-untreated and treated wild-type cells were marked by up-regulation RNA metabolism genes and down-regulation of pheromone responsive genes, respectively. The results suggest that CaSte11 is responsible for the phosphorylation and activation of Hst7 and also regulation and release of the MAPK Cek1 after activation.

Saccharomyces, at your cerevisiae: Does Brewing Equipment Get Kidney Stones, and How Can We Make This the Yeast of Our Problems?

Nykole Crevits* and iGEM Guelph
*University of Guelph

“The University of Guelph iGEM Team is a group of students tackling real-world problems with the use of synthetic biology. iGEM, the international Genetically Engineered Machine, is an annual global student-led research competition where students conceptualize, design and execute PhD-worthy scientific research projects as a team. Established in 2017, the Guelph iGEM team chose the issue of beerstone, a highly-insoluble and difficult to remove calcium-oxalate buildup that forms as a byproduct inside beer brewing equipment. Interestingly enough, beerstone is very similar in composition to kidney stones. Three metabolic enzymes from Oxalobacter formigenes, a human gut bacterium which solely metabolizes oxalate, were identified as a potential solution to prevent the buildup of beerstone. A proof-of-concept research project, iGEM Guelph investigated engineering of these genes into S. cerevisiae with the goal of modifying its metabolism to uptake and utilize calcium oxalate as an alternative energy source. They characterized cell viability, enzymatic activity and feasibility for use in an industrial brewery setting as a solution to a problem thousands of years old. This 2.5 year project provided invaluable experiential learning for the iGEM Guelph team members, including project management, technical synthetic biology skills, self-guided learning, teamwork, yeast metabolism and industrial applications, as well as networking with like-minded students and the next generation of innovative scientists from around the world. In 2019, iGEM Guelph brought home the gold medal for their work on agricultural antibiotic biosensors.

Effects of an *Epichloë* endophyte on foliar fungal communities in tall fescue grass (*Schedonorus arundinaceus*)

Jenna Dale* and Jonathan Newman
*University of Guelph

Tall fescue (*Schedonorus arundinaceus*) is a cool-season grass commonly used as a forage or turf grass. It has been studied extensively due to its agricultural and economic importance, and due to its symbiotic relationship with the fungal endophyte *Epichloë coenophiala*. This endophyte is strictly vertically transmitted (passed from a plant host to its offspring) and confers several benefits to its host including increased resistance to herbivores (mammalian and insect), drought, and some plant pathogens. Although the effects of *E. coenophiala* are well researched, less is known about the overall tall fescue microbiome and how it may be impacted by the presence of *E. coenophiala*. Research on other plant species indicates that plants contain diverse fungal and bacterial microbiomes, and recent studies suggest that this is also true of tall fescue and other grasses. We used next-generation sequencing of the ITS2 region to investigate the foliar fungal microbiome of 48 tall fescue plants grown at a field site since 2011. These plants comprised three endophyte treatments (two *E. coenophiala* strains and one Epichloë-free control) and samples were collected over two summers. Sequencing revealed that these plants contained diverse fungal communities which varied in composition between endophyte treatments. There was also a significant difference in community composition between the two years sampled, suggesting that these communities may fluctuate over time.

Small non-coding RNAs and the development of Arbuscular Mycorrhizal fungi

Alexandra Dallaire*, Bethan Manley, Navin Ramakrishna, Uta Paszkowski, and Eric Miska *Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, Cambridge, United Kingdom

80-90% of all land-plant species have the ability to develop symbioses with arbuscular mycorrhizal (AM) fungi. These mutualistic associations occur in the roots, where AM fungi form intracellular structures to trade goods: plants provide fixed carbon and in return, the fungus provides access to nutrients in the soil, particularly phosphates. The development of AM symbiosis is initiated by the exchange of signalling molecules between the two symbionts, followed by growth of fungal hyphae towards the root and entry into the root cortex. As endomycorrhizal species harbour striking expansions of small non-coding RNA pathway genes (Argonaute, RdRP), we hypothesize that RNA interference may play a central role in the physiology and biotic interactions of AM fungi. The aim of this project is to characterize and dissect small RNA pathway(s)

during AM development. In this talk I will introduce the in vitro cultivation system I use to mimic the presymbiotic communication stage of AM development. I will present my progress in using small RNA, RNA and protein sequencing to profile the RNAi response of *R. irregularis* to plant signals. I will then explain how this data can combine into a bioinformatics pipeline to annotate small RNAs in symbiotic fungi.

Four genomic clades of Candida auris identified in Canada, 2012-2019

Domenica De Luca*, David C. Alexander, Tanis C. Dingle, Philippe J. Dufresne, Linda M. Hoang, Julianne V. Kus, Ilan S. Schwartz, Michael R. Mulvey, and Amrita Bharat
*National Microbiology Laboratory

Candida auris is an emerging yeast that is associated with antifungal resistance and healthcare-associated outbreaks. From 2012-2019, there were twenty-four cases of *C. auris* colonization or infection in Canada. Isolates were from axilla/groin (n=6), ear (n=5), blood (n=4), toe (n=2), and a variety of other sites (n=7). Canadian isolates belonged to the four main genomic clades: Clade I (formerly called South Asian clade, n=12), Clade II (East Asian, n=3), Clade III (African, n=4), and Clade IV (South American, n=5). Isolates within each clade were clonal but WGS may be helpful in identifying potential clusters within healthcare facilities.

Systematic perturbation of yeast essential genes using base editing

Philippe Després*, Alexandre K. Dubé, Motoaki Seki, Nozomu Yachie and Christian R. Landry
*Université Laval

Base editors derived from CRISPR-Cas9 systems and DNA editing enzymes offer an unprecedented opportunity for the precise modification of genes, but have yet to be used at a genome-scale throughput. Here, we test the ability of the Target-AID base editor to systematically modify genes genome-wide by targeting yeast essential genes. We mutate around 17,000 individual sites in parallel across more than 1500 genes. We identify over 700 sites at which mutations have a significant impact on fitness. Using previously determined and preferred Target-AID mutational outcomes, we find that gRNAs with significant effects on fitness are enriched in variants predicted to be deleterious based on residue conservation and predicted protein destabilization. Our results show that base editing is a powerful tool to identify key amino acid residues at the scale of proteomes, and open new avenues for genome editing in fungal species."

Role of the TOR (Target of Rapamycin) pathway in circadian rhythmicity of *Neurospora crassa*

Rosa Eskandari*, Lalanthi Ratnayake, and Patricia L. Lakin-Thomas
York University

Almost all organisms have daily biological clocks (circadian rhythms). Many insights into the molecular basis of circadian rhythmicity have been obtained by studying the filamentous fungus *Neurospora crassa* as a model organism. The circadian clock in *Neurospora* regulates asexual spore formation (conidiation), and rhythmic spore formation can easily be monitored during growth. Research on the molecular machines that drive these rhythms in eukaryotes has been centered on rhythmic transcription/translation feedback loops (TTFL) of a number of "clock genes". Although much evidence supports TTFLs, certain rhythms in the filamentous fungus *Neurospora crassa* continue when clock genes (FRQ, WC-1/WC-2) are not rhythmically expressed. We previously identified the vta mutation that affects rhythmicity in the presence and absence of FRQ. We identified the vta gene product as a component of the nutrition-sensing pathway TOR (Target of Rapamycin) that is conserved in eukaryotes. Co-IP and mass spectrometry results demonstrated that VTA and another TOR pathway component GTR2 are binding partners. A gtr2ko strain was deficient in growth responses to different nutritional conditions. A FRQ-less gtr2 ko strain is defective in rhythmicity and is similar to vtako. Expression of GTR2 protein was found to be rhythmic and VTA was required for GTR2 rhythmicity. FRQ protein exhibited the expected rhythms in the presence of GTR2 but the rhythmicity and phosphorylation of FRQ dampened in the absence of GTR2. Our results indicate the role of VTA and GTR2 in maintaining functional circadian rhythms through the TOR pathway.

A haplotype-aware de novo assembly approach for the pathogenic dikaryotic fungus, *Puccinia triticina*

Sean Formby*, John P. Fellers, and Guus Bakkeren
*UBC

The wheat leaf rust, *Puccinia triticina* (Pt), is a major agricultural pathogen that significantly reduces wheat yields around the world every year. A high-quality reference genome assembly of the pathogen is required to identify a/virulence effectors and assess global gene diversity. This is essential to develop wheat cultivars with modified resistance. Currently, the Pt race 1 reference genome assembly is highly fragmented and represents only a single haplotype; rust fungi are dikaryotic, having two highly dissimilar haploid nuclei. Its relatively large genome of around 2x130 Mbp, high repeat content and heterozygosity make de novo genome assembly a difficult

task. Moreover, heterozygous genomic features involved in pathogenicity specific to a single haplotype are often collapsed and lost in conventional genome assembly pipelines, even with haplotype-aware parameters. Here, we present a haplotype-aware de novo genome assembly pipeline that utilizes the sequencing platforms of Pacbio, Nanopore and Illumina. The pipeline splits read according to their relative haplotype and subsequently assembles the separated reads resulting in a more contiguous and accurate representation of the dikaryotic genome useful for rust genomes in general.

Enhanced stress tolerance in Norwegian kveik results in increased fermentation efficiencies at extreme temperatures

Barret Foster*, Caroline Tyrawa, Julia Santos, Richard Preiss, and George van der Merwe
*University of Guelph

The application of yeast for beer production exposes them to several severe environmental stresses throughout growth, fermentation, and storage processes. Constant selective pressure has resulted in a variety of strains that exhibit strong phenotypic characteristics such as industry specific sugar utilization, enhanced stress tolerance and favourable flavour compound production. Recent research in our lab has identified a group of *Saccharomyces cerevisiae* ale yeast isolated from Western Norway, where unique farmhouse brewing techniques and centuries of isolation have resulted in a genetically distinct group of yeast, termed kveik, that differ from standard industrial brewing yeast. While kveik strains are known to be strong fermenters with increased thermotolerance, the fermentative capacity of these yeasts at a range of different temperatures are poorly characterized. A number of wort fermentations were performed at temperatures ranging from 12°C to 42°C to elucidate fermentation profiles, carbon metabolism, and flavour compound production of these yeast. These experiments show that Norwegian kveik are capable of fermenting more efficiently than industrial control strains at extreme temperatures and demonstrate a much wider temperature optima for fermentations. Kveik also demonstrate the capacity to efficiently metabolize wort sugars at this wide range of temperatures. Analysis of flavour metabolites produced during fermentation show that kveik yeast have a tendency to produce higher concentrations of esters which contribute to a fruitier finished product. These results indicate that Norwegian kveik yeast possess enhanced stress response mechanisms to survive and ferment under extreme conditions. Additional research is being done to further unravel the molecular mechanisms for this exceptional stress tolerance.

Combatting fungal infections through the discovery and elucidation of novel anti-virulence strategies

Jennifer Geddes-McAlister*, Brianna Ball, Elizabeth Woroszchuk, and Duncan Carruthers-Lay
*University of Guelph

Fungal pathogens are emerging as critically important threats to global health with over 300 million people affected by serious fungal diseases worldwide. Fungal pathogens are a highly diverse group of infectious agents, including yeast or yeast-like (e.g., *Candida* and *Cryptcoccus spp*.) and molds (e.g., *Aspergillus spp*.). These pathogens have evolved sophisticated strategies, including the secretion of virulence factors to interfere with host cell functions and to perturb immune responses. Our 'infectome' analysis identifies previously undescribed proteins involved in fungal virulence and host immune response, representing an opportunity to elucidate molecular mechanisms of host-pathogen interplay during disease. Using state-of-the-art mass spectrometry-based proteomics we profile the total proteome and secretome of *Cryptococcus neoformans* wild-type (H99) under *in vitro* growth conditions. We also define the infectome of *C. neoformans* and BALB/c macrophages in single runs using high resolution mass spectrometry on a Quadrupole Orbitrap instrument. The *in vitro* and infectome datasets were integrated using Perseus and candidate fungal proteins of interest were prioritized based on novelty, predicted secretory roles, and abundance profiles. Deletion strains were constructed by double-joint PCR followed by phenotypic screening and cell death assays. Virulence-associated candidates will be evaluated in a murine infection model and further characterized by immunofluorescence and interactome analyses. Our preliminary results demonstrate the deepest proteome to date of *C. neoformans* and highlight changes in protein abundance within the total proteome and secretome under in vitro growth conditions. In addition, profiling of the infectome (macrophages infected with *C. neoformans*) uncovers new fungal- and host-specific responses to infection. Moreover, integration of protein abundance profiles from in vitro growth and the infectome identify novel infection-associated proteins and comprehensively define the host response in a single experiment. An in-depth analysis from the host's perspective, defines proteins associated with phagocytosis, inflammatory response, and signaling. Conversely, from the pathogen's perspective, 43 proteins with significant increases in abundance were detected, 17 of which, were selected for further characterization, including three positive controls and 14 uncharacterized or hypothetical proteins. Currently, gene deletion strains of the first three candidates are being constructed and preliminary phenotypic screening analysis will evaluate a role for these proteins associated with capsule and melanin production, as well as growth at 37°C. Moreover, the candidates will be screened for impact on

macrophage cytotoxicity levels in vitro by lactate dehydrogenase release and potential differential roles during invasion and replication. Next, murine in vivo models of infection will be evaluated to determine roles for the proteins in virulence, as well as fungal proliferation and dissemination. We anticipate further characterization of the three proteins (and remaining candidates) by FLAG-tagging the proteins of interest, performing immunofluorescence to observe protein production and localization, along with immunoprecipitation to identify host binding partners. These interactions support elucidation of mechanisms of action for each of the fungal proteins and suggest opportunities for chemical or biological interference of these interactions to promote clearance of the pathogen and enhance host survival rates. Comprehensive profiling of infection from both host and pathogen perspectives unveils new anti-virulence strategies to combat fungal infection."

Differences in genome content underlie standing genetic variation in an asexual eukaryote

Emile Gluck-Thaler*, Timothy Ralston, Veena Devi Ganeshan, Cristhian Grabowski Ocampos, Anne E. Dorrance, Terry L. Niblack, Jason C. Slot, Corlett W. Wood, and Horacio Lopez-Nicola
*University of Pennsylvania

Macrophomina phaseolina is a globally distributed plant pathogen that can infect over 500 known hosts despite having a presumed asexual lifecycle. How this fungus overcomes the constraints imposed by clonal reproduction to generate the genetic variation necessary to adapt to multiple hosts and environments remains unclear. Here, we characterized the drivers of genetic diversity in *M. phaseolina* by testing specific hypotheses concerning the origins and distribution of variation in genome content, i.e., variation in the combinations of genes found in genomes. Using 12 newly sequenced isolates from North and South America, we found that while isolates differ by at most 1% at the nucleotide level, their genome contents can differ by up to 36%. Correspondingly, the *M. phaseolina* pangenome (the set of genes found across all isolates) is the second most diverse compared with 6 other fungal pangenomes and has likely not yet fully been described. We reconstructed all gains and losses in the pangenome, and found that genes experiencing high rates of gain and loss are compartmentalized into specific genomic regions that are occasionally shared among isolates, indicating a certain degree of evolutionary stability in these variation-generating hotspots. Finally, we explicitly connect mutational mechanisms operating at the level of individual genomes to processes generating variation in the pangenome and found that large insertions and deletions (>10,000bp) are significantly associated with gene gains and losses. Together, our results suggest that the evolutionary

potential of asexual eukaryotes is largely circumscribed by variation in genome content.

Environmental factors and polyketide synthase gene expression in an usnic acid producing lichen-fungus

W.G. Duleeka I. Gunawardana*, Natalia Sveshnikova, André Arsenault, and Michele D. Piercey-Normore
*Memorial University

Lichens are slow growing and exposed to prolonged environmental conditions, which resulted in the evolution of unique secondary metabolites for amelioration of extreme conditions. One of the most commonly produced secondary metabolites is usnic acid, which is thought to involve two genes methylphloracetophenone synthase (MPAS) and methylphloracetophenone oxidase (MPAO) as part of the polyketide synthase (PKS) gene cluster. The goal of this study was to examine the effect of two environmental factors (soil pH and moisture) on thallus growth and production of usnic acid by *Cladonia uncialis*. Lichen samples were collected within three locations in Newfoundland using a strip transect method (5 quadrats for each of 5 transects for each location). Soil pH and moisture were measured in each quadrat. Usnic acid concentration was measured using High Performance Liquid Chromatography and Quantitative PCR was performed using two genes (MPAO and MPAS) for each lichen thallus sample. There was a relationship between percent ground cover of *C. uncialis* and soil pH level but not soil moisture. Usnic acid concentration was also affected by soil pH level but not soil moisture. MPAS and MPAO gene expression levels were not significantly affected by soil pH level or soil moisture and neither did they correlate with the amount of usnic acid produced. These findings suggest that soil pH may influence thallus growth and the production of usnic acid by *C. uncialis*. However, several hypotheses are proposed to further explore the genes involved in usnic acid biosynthesis.

Integrating high-throughput screening of antifungal microbial interactions and system biology-based approaches to study complex gene-microbe interactions

Gunjan Gupta* and Marie Filteau
*Laval University

Fungi cause significant damage in the food sector, resulting in considerable economic losses. In the current context of increased regulatory restrictions to the use of antifungals and consumer demand for ‘clean label’ products, there is an urgent need for new antifungal strategies. The use of bioprotective microbial consortium is drawing

attention as an alternative to the use of chemical additives. Therefore, we decided to exploit microorganisms from a competitive natural extremophilic environment contaminated by yeasts and bacteria to study antimicrobial strategies against foodborne yeast. As we know, microorganisms are social beings that live in communities, communicate, defend their territories, and protect their resources. Therefore, we will use high-throughput methods to study the antifungal behaviours in pure or mixed culture conditions. Then, to elucidate the antifungal strategies, we will study the underlying mode of action using *Saccharomyces cerevisiae* as a model. To drive our systems-level understanding of the fungal genetic targets, we will use an approach we recently developed that combines co-culture and a functional genomic screen. In the end, we hope that interaction-mediated antifungal activity will be interesting from an applied perspective, in particular as it may lead to the development of novel antifungal applications in the food sector."

Using a CRISPR-Cas9-based gene drive to target stress response genes in *Candida albicans*

Viola Halder* and Rebecca Shapiro
*University of Guelph

Candida albicans is an opportunistic fungal pathogen found in the oral mucosa, the gut, the vaginal mucosa and the skin of humans. While *C. albicans* can cause mild and superficial infections, severe invasive infections can occur in immunocompromised patients. Understanding the survival and pathogenesis of *C. albicans* is critical for novel antifungal drug discovery and is the main objective of this project. Here, we exploit a CRISPR-Cas9-based genome editing platform to create stress response deletion libraries in *C. albicans*, in order to study their role in pathogen survival. Our strategy uses a CRISPR-Cas9-based gene drive array platform: a single plasmid with CAS9 and a pair of guide RNAs that direct Cas9 to create double-strand breaks flanking the open reading frame (ORF) of the gene of interest. Once the ORF is removed, the gene drive acts as a repair template to replace the gene of interest. The gene drive can further propagate to delete additional wild-type copies of the ORF, and thus, after the single-gene mutants are created, a diploid double-gene mutant library will be generated using a combinatorial mating strategy between mutant haploid strains. This library of single and double stress response mutants will be screened under diverse growth conditions to assess their relative fitness. Genetic interaction analysis will be exploited to map out genetic interactions between fungal genes involved in growth, survival and pathogenesis. We will identify combinations of fungal stress response genes with negative interaction or synthetic lethal interaction to uncover putative targets for combination antifungal therapy."

Candida albicans exhibits a cytoprotective response to anti-fungal drugs that facilitates the evolution of drug resistance

Michael T. Hallett*, Bettauer V, Massahi S, Khurdia S, Costa ACBP, Omran RP, Khosravi N, Simpson S, Harb M, Dumeaux V, and Whiteway M
*Concordia University

Candida albicans is both a human commensal and opportunistic pathogen. Nosocomial infections due to pathogenic *C. albicans* are the fourth most important in North America and are associated with significant socioeconomic burden. Systemic Candida infections of immune-compromised individuals are frequently lethal even when treated optimally. Drug resistance is sometimes due to the pre-existence of genetic polymorphisms that bypass the mode of action of the drug, thus conferring a long-term survival benefit. In other cases, resistance is acquired via the evolution of de novo genetic polymorphisms. There is evidence that *C. albicans* possess a drug tolerance response which “buys time” for individuals to evolve beneficial mutations. In fact, there is some evidence that this is facilitated by the (inherent or possibly induced) instability of the *C. albicans* genome. Our goal here is to characterize this poorly understood epigenetic cytoprotective program at the single cell molecular level. We tweaked a nano-litre droplet based single cell sequencing platform for the fungal setting. The system is capable of transcriptionally profiling several thousand individual cells in an efficient, cost effective manner. We exploit this platform to profile both untreated and anti-fungal drug exposed (incl. fluconazole, caspofungin and nystatin) populations at early time points post-treatment (tolerance) and late time points (resistance) in order to understand survival trajectories. We show that prototrophic Candida populations exhibit “bet hedging”, stochastically expressing cytoprotective epigenetic programs, and use live cell imaging to establish that these cells are more likely to survive drug exposure. Moreover, the drug tolerant individuals partition into distinct subpopulations, each with a unique survival strategy involving different transcriptional programs. Deep learning microscopy based approaches establish that “classic” morphologies of *C. albicans* are insufficient to capture the observed heterogeneity. Whole genome DNA-sequencing of these populations establishes a trend of increased instability during the tolerance phase until resistance, when the genome re-stabilizes. Our single cell approach highlights that survivor subpopulations pass through a tolerance phase that involves a multivariate epigenetic response including upregulation of efflux pumps, chaperones and transport mechanisms, and cell wall maintenance. Together this suggests that targeting the tolerance response concomitantly with standard therapies could represent an efficient approach to ablating clinical persistence.

Is the main function of the Bowen-Conradi Syndrome protein Emg1 to displace snR35?

Courtney Harris*, Taylor Goodon, Elise Poole, and J. Michael Charette
*Brandon University; Children's Hospital Research Institute of Manitoba

Ribosomes are complex cellular machines that translate genetic information into proteins. They are essential for development, cell growth, and basic cell functions. Disorders caused by ribosome mis-assembly and/or malfunction are called ribosomopathies and lead to severe health consequences. The most lethal ribosomopathy is Bowen-Conradi Syndrome (BCS), which is characterized by severe developmental delay, involving pre- and postnatal growth retardation and death in infancy. BCS is a rare genetic disorder as it exclusively affects the Hutterite population on the Canadian and U.S. Prairies at an incidence of 1/355 live births.
The cause of BCS is a mutation in the protein Emg1, an SSU processome component and rRNA methyltransferase. Emg1's additional, essential role in ribosome assembly is currently unknown. Therefore, it is imperative to investigate Emg1's function in order to gain a further understanding of ribosome assembly and the disease origins of BCS. We hypothesize that Emg1's main role is to remove a previous assembly factor, a small nucleolar RNA called snR35, to allow for subsequent ribosome assembly steps to occur. In BCS, we propose that mutated Emg1 can no longer displace snR35 and subsequent ribosome assembly steps are sterically blocked. To test this hypothesis, we are using a yeast model of BCS. Our preliminary results indicate an improvement in Emg1-BCS cell growth in the absence of snR35. By investigating the cellular mechanism of Emg1, we will further our understanding of ribosome assembly and of the pathogenesis of BCS. This work will extend to various other ribosomopathies and diseases, including cancer.

Identification of novel NHEJ DNA repair genes using machine learning algorithms

Minuka Hewapathirana*, Sasi Kumar Jagadeesan, and Ashkan Golshani
*Carleton University

In recent days, computational algorithms play a pivotal role in conducting biological simulation models that aid in the large-scale investigation of genomic research. To identify novel genes involved in Non-Homologous End Joining (NHEJ) double strand break repair, we established a PIPE Channelling system for conducting genome-wide array screening. Through this approach, protein-protein associations, genetic associations, co-expression databases are systematically assigned with a catalogue of DNA-repair related target mutants showing varying efficiency of NHEJ DNA repair. For

this purpose, we performed an interactive computational analysis using machine learning algorithm that resulted in numerous genetic variations.

Addressing barriers of Ontario cider production

Jordan Hofstra*, George van der Merwe, and Sydney Johnson
*University of Guelph

Cider, often referred to as 'hard cider' in North America to denote its alcohol content is a popular beverage made from fermenting apples (*Malus domestica Borkh*). Ontario is home to a burgeoning cider industry that generated $73.5 million in economic activity in 2017 and this number has since risen. Astoundingly, the industries growing success has been achieved while being supplied with dessert-apple cultivars which lack in organoleptic qualities possessed by European cider-apple cultivars and through the continued use of non-cider specific *Saccharomyces* yeast strains to complete fermentations. To address the latter, our lab has isolated 20 novel *Saccharomyces* strains from apples and the processed must and intend on analyzing multiple fermentation parameters to reveal novel yeast candidates ideal for cider fermentation. Fermentation capacity will be monitored in small-scale fermentation trials at 15°C using densitometry. Furthermore, metabolic preferences including effective metabolism of cider-saccharides and generation of ethanol, glycerol and acetic acid will be quantified by high performance liquid chromatography. Aromatic volatiles that have been identified as being influential on cider quality will be quantified for each strain using gas chromatography-mass spectrometry. Strains which demonstrate the ability to efficiently metabolize sugars and generate a desirable volatile flavour profile during a 15°C fermentation will have their full genomes sequenced to help elucidate the genetic mechanisms responsible for the elevated phenotype. I rationalize that increasing the yeast-related resources for Ontario cideries will aid in increasing product quality and diversity, furthering the growth of the industry.

J domain co-chaperones contribute to host adaptation in *Cryptococcus neoformans* pathogenesis

Linda Horianopoulos*, Guanggan Hu, Melissa Caza, Kerstin Schmitt, Oliver Valerius, Gerhard Braus, and James Kronstad
*University of British Columbia

As an opportunistic fungal pathogen, *Cryptococcus neoformans* must adapt to environmental stresses encountered upon entry into a mammalian host including immune responses and elevated temperature. Understanding the molecular mechanisms required for this adaptation can inform efforts to develop new treatments

for cryptococcosis. The molecular chaperones are a group of proteins which are important for mitigating proteotoxic stresses induced in these environmental stresses. There are several well conserved chaperones across kingdoms, however many of the co-chaperones which direct their functions and provide specificity towards clients vary drastically between species. We have focused on the 24 J domain protein co-chaperones (JDPs) which we have identified in *C. neoformans*. Mutants with single gene deletions of several genes encoding these JDPs were made. In particular, we focused on proteins lacking orthologs in Saccharomyces cerevisiae as candidates for JDPs that may be important for virulence. We found that several of these mutants were deficient in the elaboration of virulence factors including thermotolerance and capsule production in vitro. Furthermore, one of these mutants was avirulent and others had attenuated virulence in mouse models of cryptococcosis. In depth analysis of the roles of these virulence related JDPs by analyzing mutant phenotypes, characterizing localization, and using proteomics has confirmed the importance of known pathways such as mitochondrial and ER homeostasis to *C. neoformans* virulence. Since many of these JDPs are divergent from human JDPs they are strong candidates to target for antifungal drug development.

How arbuscular mycorrhizal life history traits influence soil carbon cycling

Caitlyn Horsch*, Dr. Cynthia Kallenbach, and Dr. Pedro M. Antunes
*McGill University and Algoma University

Soil is the largest terrestrial carbon (C) pool, containing nearly three times more C than the atmosphere. Thus, understanding the factors that encourage long-term soil C residence is important for climate mitigation. Fungal biomass is a major contributor to soil C. As such, differences in fungal traits that affect biomass production can impact soil C accumulation. Yet, we have not identified the key traits that favor soil C accumulation, especially in non-saprotrophic fungi like arbuscular mycorrhizal fungi (AMF). AMF Life-history strategies and functional traits tend to vary primarily at the family level. Species in the Gigasporaceae family are slower growing relative to those in the Glomeraceae family. Hyphal chemistry also differs between these families, influencing their decomposition into different soil C pools with varying temporal stability. We are investigating how different mycorrhizal traits influence soil C cycling and retention by growing Sudan grass with either Gigasporaceae species, Glomeraceae species, or a mixture of the two, and tracing all new fungal C inputs with 13C-CO2. After two months, we will measure AM biomass, hyphal chemistry, and 13C Fungal C in several soil C fractions. We predict that both AM fungal growth and hyphal chemistry will determine fungal C accumulation in the soil. Furthermore, we hypothesize that more total and stable C will accumulate with Glomeraceae species

than with Gigasporaceae species due to faster growth rates and nitrogen-rich hyphal chemistry.

The proteasome governs fungal morphogenesis via functional connections with Hsp90 and cAMP-PKA signaling

Saif Hossain*, Amanda O. Veri, and Leah E. Cowen
*University of Toronto

Protein homeostasis is critical for proliferation and viability of all organisms. For *Candida albicans*, protein homeostasis also modulates the ability to transition between yeast and filamentous forms, which is critical for virulence. A key regulator of morphogenesis is the molecular chaperone Hsp90, which mediates proteostasis under physiological and stress conditions. Hsp90 regulates filamentation by repressing cyclic AMP-protein kinase A (cAMP-PKA) signalling, such that inhibition of Hsp90 causes filamentation in the absence of an inducing cue. We explored the effect of perturbation of another facet of protein homeostasis and identified that morphogenesis is also regulated by the proteasome, a large 33 subunit protein complex consisting of a 20S catalytic core and two 19S regulatory particles, that controls degradation of intracellular proteins. We identified a conserved role of the proteasome in morphogenesis, as pharmacological inhibition of the proteasome induced morphogenesis of *C. albicans* and the related species *Candida dubliniensis*, *Candida tropicalis*, *Candida krusei*, and *Candida parapsilosis*. For *C. albicans*, genetic depletion of any of 27 subunits of the 19S or 20S particle induced filamentation. Filaments induced by inhibition of either the proteasome or Hsp90 have shared structural characteristics, such as aberrant nuclear content, and shared genetic requirements, such as intact cAMP-PKA signalling. Consistent with a functional connection between these facets of protein homeostasis that modulate morphogenesis, we observed that proteasome inhibition results in an accumulation of ubiquitinated proteins that overwhelm Hsp90 function, relieving Hsp90-mediated repression of morphogenesis. Together, this provides a mechanism whereby interconnected facets of proteostasis regulate *C. albicans* morphogenesis.

Understanding *Candida auris*: Exploiting chemical matter to uncover cell biology and characterize resistance mechanisms in this emerging fungal pathogen

Kali Iyer*, Kaddy Camara, Martin Daniel-Ivad, Richard M. Trilles, Sheila Elardo, José F. Muñoz, Thomas Henkel, Christina A. Cuomo, Justin R. Nodwell, John A. Porco, Lauren E. Brown, Luke Whitesell, Nicole Robbins, and Leah E. Cowen
*University of Toronto

Over the past decade, *Candida auris* has emerged as a critical threat to our most vulnerable populations, challenging current practices with its unprecedented drug resistance, extreme environmental persistence, and puzzling evolutionary history. To further our understanding of this emerging pathogen and develop novel therapeutic strategies, we screened a diversity-oriented synthesis collection to identify compounds that were bioactive alone and in combination with fluconazole, the most commonly administered systemic antifungal, which inhibits biosynthesis of the membrane sterol ergosterol. The rocaglates were the most prominent chemotype with cidal single agent activity, and inhibited translation in *C. auris* but not in its close pathogenic relative *Candida albicans*. This species-specific activity was contingent on a single amino acid change in the drug target, the translation initiation factor Tif1. Strikingly, rocaglate-mediated translation inhibition induced a non-canonical form of programmed cell death in *C. auris*, but not in a sensitized *C. albicans* strain, suggesting evolutionary divergence in programmed cell death pathways in response to translation inhibition. From the azole-potentiation screen, we discovered that the most potent compound against *C. auris* enhanced fluconazole efficacy through increasing azole intracellular accumulation. This activity was dependent on expression of the multidrug transporter gene CDR1, suggesting that this compound targets efflux mechanisms. This has allowed us to explore the role of efflux in diverse *C. auris* isolates as well as drug-resistant *C. albicans*. Overall, this work identifies compounds with novel bioactivity against the drug-resistant pathogen *C. auris*, revealing important biology and paving the way for development of much-needed therapeutic strategies.

Lithium chloride sensitivity in translation mechanism of structured mRNAs in yeast

Sasi Kumar Jagadeesan*, Sasi Kumar Jagadeesan, and Ashkan Golshani
*Carleton University

“For decades, lithium chloride (LiCl) has been used as a treatment option for those living with bipolar disorder (BD). As a result, many studies have been conducted to examine its mode of action, toxicity, and downstream cellular responses. We know that LiCl is able to affect cell signaling and signaling transduction pathways through protein

kinase C and glycogen synthase kinase-3 which are considered to be important in regulating gene expression at the translational level. However, additional downstream effects require further investigation, especially in translation pathway. In yeast, LiCl treatment affects the expression, and thus the activity, of PGM2, a phosphoglucomutase involved in sugar metabolism. Inhibition of PGM2 leads to the accumulation of intermediate metabolites of galactose metabolism causing cell toxicity. However, it is not fully understood how LiCl affects gene expression in this matter. In this study, we identified three genes, SAS1, SAS2 and SAS3 which increase LiCl sensitivity when deleted. We further demonstrate that SAS1, SAS2 and SAS3 influence translation and exert their activity through the 5'-UTR of PGM2 mRNA."

Anti-biofilm activity of unsaturated fatty acids with fluconazole

Abdullahi Jamiu*, Oluwasegun Kuloyo, Nthabiseng Mokoena, Jacobus Albertyn, and Carolina H. Pohl
*University of the Free State, South Africa

Biofilms are responsible for 80% of all microbial infections, they are usually recalcitrant to therapy, seed recurrent infections, and consequently lead to treatment failure. A fungal yeast, *Candida krusei* with innate and rapid acquired resistance to antifungal drugs also forms recalcitrant biofilm. To this end, the dire need for anti-biofilm agents is undoubtable and the use polyunsaturated fatty acids (PUFA) such as arachidonic acid (AA), which increases the susceptibility of some yeasts to antifungals might be a probable option. In this study, the anti-biofilm and synergistic activity of fluconazole (FLC) and AA against biofilm of *C. krusei in vitro* and *in vivo* in a *Caenorhabditis elegans* infection model was investigated. For the *in vitro* assay, *C. krusei* biofilm was grown in the presence of either FLC, AA or combination of AA and FLC at 37 °C for 48 h and the biofilm mitochondrial metabolism was evaluated using XTT assay. A *C. elegans* in vivo infection model was also established using *C. albicans* and *C. krusei* strains, and infected nematodes were treated with FLC combined with either exogenous or dietary AA. The results showed that the combination of AA and FLC had anti-biofilm and synergistic effects on *C. krusei* biofilm *in* vitro, probably due to membrane organisation disruption and/or increased oxidative stress as a result of AA incorporation. However, neither exogenous nor dietary AA combination with FLC could prolong the overall survival of infected nematodes in vivo possibly due to low concentration of FLC used.

The Mycoflora of New Brunswick: First steps on a long road ahead

Alfredo Justo*
*New Brunswick Museum

“We evaluated the current knowledge about macrofungi (Ascomycota, Basidiomycota) in New Brunswick, in the context of the North American Mycoflora Project. We will present an overview of the data currently at hand, and what is needed to produce a complete catalog of all macrofungi (a Mycoflora or Funga) occurring in the province, backed up with voucher specimens and molecular data. Multiple challenges exist to accomplish this goal, including: modern interpretation of old names in the North American mycological literature; wide misapplication of European names to yet-undescribed North American taxa; and the dwindling numbers of taxonomic expertise across North America. We will also explore the opportunities for collection-based biodiversity research, including citizen-science driven projects.”

C-DRIVE - CRISPR/Cas9 induced gene drive to perform combinatorial genome editing in yeast

Aashiq Kachroo*, Brittany M. Greco, Mudabir Abdullah, Jon M. Laurent, Riddhiman K. Garge, and Edward M. Marcotte
*Concordia University

For decades, Baker’s yeast (*Saccharomyces cerevisiae*) has served as a tremendous model for biomedical research. Genome-scale engineering in yeast is feasible primarily due to prodigious homology-directed DNA repair (HDR), a plethora of genetic tools/ selection markers, and simple conversion between haploid and diploid forms. However, with the emergence of yeast as a model for systems and synthetic biology research, there is a need for highly efficient and scalable genome engineering strategies. Previously, using CRISPR-Cas9, we developed a method for one-step, marker-free editing of the yeast genome using HDR and an appropriate repair template (Kachroo et al., 2017; Akhmetov et al., 2018). In this work, by combining CRISPR-Cas9, DNA repair via HDR, and yeast-mating, we demonstrate a highly efficient gene drive. First, by targeting the functional copy of the ADE2 gene via CRISPR-Cas9 in a heterozygous diploid strain (ADE2 / Δade2::KanMX), we show the conversion of the ADE2 to Δade2::KanMX locus at near 100% efficiency. The KanMX copy of the homologous chromosome, thus serves as a highly effective repair template for HDR. Next, we show that this method is equally efficient at singly replacing yeast genes with their corresponding human orthologs in a similar fashion. Finally, we demonstrate this method's scalability by converting two or more heterozygous human-yeast loci to

become homozygous for human genes. Therefore, this strategy lays the foundation for large-scale combinatorial engineering of any biological process in budding yeast.

Nuclear dynamics in the arbuscular mycorhizal fungus *Rhizophagus irregularis*
Vasilis Kokkoris*, Pierre-Luc Chagnon, Gökalp Yildirir, Stephanie Mathieu, Kelsey Clarke, Keith Hubbard, Franck Stefani, and Nicolas Corradi
*University of Ottawa and Agriculture and Agri-Food Canada

Arbuscular mycorrhizal fungi (AMF) are plant symbionts with a distinct nuclear organization, -thousands of nuclei flow simultaneously within their coenocytic hyphae and spores. Recently was revealed that these nuclei are either genetically homogeneous (homokaryons) or heterogeneous (dikaryons), whereby thousands of nuclei of two distinct mating types co-exist at all times. Dikaryotic strains carry higher genetic diversity compared to homokaryotic relatives, yet many questions about this distinct genetic makeup remain unanswered. For example, what is the frequency of AMF dikaryosis, and is there evidence of spatial heterogeneity or inter-nuclear dominance across individual spores of dikaryotic strains? Moreover, how do dikaryons compare in terms of nuclear counts to homokaryotic relatives? We address these questions by combining molecular approaches with advanced microscopy and mathematical modeling. We found that AMF dikaryosis is a rare genetic condition and that dikaryotic strains have higher nuclear counts compared to homokaryotic. We also found that some strains harbor both nucleotypes in exact equal proportions while others appear to have a stable but unequal ratio of their nucleotypes, possibly indicating function dominance or cooperation among nucleotypes. Variation in nuclear counts and nuclear ratios can have important implication in AMF ecology, protein expression and host response.

Role of phosphatidylcholine on Cryptococcal capsule formation
Christopher Lee*, Francois Mayer, and James Kronstad
*Kronstad Lab, University of British Columbia

The basidiomycete fungus *Cryptococcus neoformans* serves as a useful model for investigating mechanisms of fungal pathogenesis. This pathogen is the causative agent of cryptococcal meningitis in immunocompromised patients, contributing to over a million cases a year worldwide. The high mortality of cryptococcosis can be attributed to its ability to avoid recognition by the immune system and its high neurotropism leading to preferential colonization of brain tissue. Several virulence factors contribute to the proliferative growth of *C. neoformans* in immunocompromised patients including production of a capsule, extracellular phospholipases, and melanin. Between these, the capsule remains the most distinct and impactful virulence factor due to its ability to

act as a shield from pathogen recognition receptors and phagocytosis within the host. It is known that phospholipids from macrophages and amoebae trigger capsule formation, and this highlights the potential for phospholipids to act as a signal for capsule enlargement. A mutant defective in phosphatidylcholine (PC) biosynthesis because of loss of Opi3 has a reduced capsule size in capsule-inducing medium (CIM). Moreover, this mutant exhibits a unique accumulation of lipid droplets observable though staining with Nile Red. We show that Opi3's unique phenotype can be rescued though supplementation with exogenous choline that feeds into the phosphatidylcholine biosynthetic pathway. Overall, the results to date suggest an important connection between phosphatidylcholine biosynthesis and capsule formation.

Functional genomic analysis of protein kinases in the human fungal pathogen *Candida albicans*

Yunjin (Rachel) Lee*, Dongyeob Lee, Nicole Robbins, and Leah Cowen
*University of Toronto

Fungal diseases kill approximately 1.5 million people globally each year. Candida albicans is a leading cause of mycotic infection with unacceptably high mortality rates of ~40% despite therapeutic intervention. Poor patient outcomes are a result of the limited number of antifungal drug classes, as well as the unprecedented increase in drug resistance. Protein kinases are key regulators of eukaryotic signaling pathways and select studies suggest targeting these enzymes is effective at combatting *C. albicans* infections. However, a systematic exploration into the roles of kinases in *C. albicans* pathobiology is lacking. The *C. albicans* gene replacement and conditional expression (GRACE) library is a powerful resource that can be employed to systematically probe gene function. For these mutants, one allele of a given gene is deleted and the remaining allele is controlled by a tetracycline-repressible promoter. Initially, the GRACE library consisted of ~2,400 strains, encompassing ~40% of the genes in the *C. albicans* genome, with 45% of the 139 *C. albicans* kinases represented in this collection. This work expanded the coverage of *C. albicans* kinases to 82%. Using this expanded collection, phenotypic screens were conducted to identify kinases essential for growth, antifungal drug tolerance, and morphogenesis, a key virulence trait. Collectively, these functional genomics screens led to the discovery of kinases with previously undescribed roles in these processes. Future work involves identifying kinases required for in vivo pathogenicity and commensalism. This project advances our understanding of kinases important for *C. albicans* pathobiology and expands our repertoire of fungal targets for therapeutic intervention.

Chromosome-scale genome assembly, dikaryon phasing, and centromere mapping using Hi-C

Ivan Liachko*
*Phase Genomics

The process of purifying DNA for next-generation sequencing results in the loss of long-range sequence contiguity. This obstacle prevents the assembly of end-to-end chromosomes and impedes the phasing of haplotypes. In cases of dikaryons, standard sequencing methods fail to deconvolve which chromosomes occupy the same nuclei, making it difficult to understand dikaryon evolution and mechanisms of somatic hybridization. We have adapted the chromosome conformation method Hi-C to the assembly of fungal genomes. This technology captures physical interactions between DNA sequences that are proximal to each other in vivo and, using simple short-read sequencing, can measure the 3D organization of virtually any genome. Because Hi-C is performed on intact cells with intact chromosomes, it captures the ultra-long-range genomic information lost during the act of DNA purification for other methods. And due to a power-law relationship between physical proximity and genomic distance, the information recovered from this assay can be used to assemble a genome into full chromosomes, no matter their size, as well as separate haplotypes on a chromosome scale and fix errors in long-read assemblies. This method also recovers unique epigenetic information which allows the de novo annotation of centromeres and rDNA arrays and identification of patterns of higher-order genome organization.
We have recently published a number of papers of fungal genomes assembled with Hi-C data, spanning from complex dikaryotic rust pathogens to simple yeasts and mixed metagenomic communities. In this talk we will discuss published and unpublished work enabling fungal genomic discovery efforts.

Sympatric divergence of ergot fungi populations in Canada and Midwestern USA

Miao Liu*, Parivash Shoukouhi, Kassandra R. Bisson, Stephen A. Wyka, Kirk Broders, and Jim G. Menzies
*Agriculture & Agri-Food Canada

The ergot diseases of cereal crops and forage grasses are caused by the infection of *Claviceps spp*. (Hypocreales, Ascomycetes) on florets, producing dark spur-like sclerotia on spikes that are toxic to human and animals, leading to detrimental impacts on agriculture and economy due to the downgrading of cereal grains, import-export barriers, reduced yield and ecological concerns. At least seven phylogenetic lineages (phylogenetic species) were identified within pre-molecular concept of *C. purpurea* s.l. (sensu lato) in agricultural areas and vicinities in Canada. However, *Claviceps purpurea*

s.str. remained as the most inclusive species with a wide host range, including cereal crops, comprising 90% of samples collected. Genetic diversity in natural population in Canada is largely unknown. Multi-locus genotyping data combined with population network analyses and population demographic parameters was explored to shed light on genetic differentiation and evolution of the natural populations Canada and the Midwestern USA. Results showed that three distinct genetically subdivided populations exist, and the subdivision is not correlated with geographic or host differentiations. Some intrinsic mechanisms might play roles in leading to the cessation of gene flows among the subpopulations.

Dissecting the mechanisms governing inter-kingdom interactions between *Candida albicans* and *Lactobacillus* species

Jessie MacAlpine*, Martin Daniel-Ivad, Robert Todd, Junko Yano, Amanda Veri, Luke Whitesell, Paul L. Fidel Jr., Mairi C. Noverr, Anna Selmecki, Justin Nodwell, and Leah E. Cowen *University of Toronto

Interactions between bacteria and fungi are ubiquitous in nature, yet little is known about the mechanisms governing these interactions. In humans, the opportunistic fungal pathogen *Candida albicans* is a common member of the mucosal microbiota that is capable of causing both superficial infections and life threatening systemic disease. Vaginal candidiasis occurs in approximately 75% of healthy women at least once in their lifetime, with fungal overgrowth often developing after a decline in bacterial abundance due to antibiotic use. *Lactobacillus* species are prominent constituents of the vaginal microbial community and the most common industrial probiotic, with current research focusing on exploiting probiotic bacterial species to promote a healthy vaginal microbiome. With the goal of identifying the mechanism(s) by which specific probiotic organisms affect *C. albicans* virulence, we examined the effects of Lactobacillus strains on the ability of *C. albicans* to switch from yeast to filamentous morphologies, a cellular transition important for virulence. We observed that several species of Lactobacillus secrete a factor that is able to repress *C. albicans* morphogenesis. Bioassay-guided fractionation and subsequent structural elucidation work linked this activity to a defined molecular entity. Functional genomics and selection-based strategies were then leveraged to identify *C. albicans* genes important for this interaction. My future work aims to further elucidate the mechanisms underlying this inter-kingdom interaction and facilitate the development of optimized probiotics and novel therapeutic strategies.

Microfungi diversity from the bark of *Acer saccharum*

Jonathan Mack* and David Overy
*Carleton University

Acer saccharum, also known as Sugar Maple is a common staple tree in most of Eastern Canada. Unfortunately its fungal diversity is quite poorly known, especially the diversity of hyphomycetes and other microfungi from its bark. I will present the preliminary results from an ongoing project which aim at isolating and characterizing the hyphomycetes diversity from the bark of *A. saccharum* using various isolation technique and comparing their efficiency in isolating hyphomycetes. Interesting relevant findings will also be briefly discussed.

Characterization of the roles of the monothiol glutaredoxin, Grx4, in the cell biology and virulence of *Ustilago maydis*

Sean McCotter* and Kronstad, JW
*UBC Vancouver

The corn smut fungus, *Ustilago maydis*, is the premier basidiomycete model for studying plant-pathogen interactions. Monothiol glutaredoxins are central regulators of cellular functions such as iron homeostasis, cell wall integrity and the redox status of fungal cells. In this study we characterized the novel role of the monothiol glutaredoxin Grx4 in the pathogenesis of *U. maydis* on its host, *Zea mays*. Mutants expressing a conditional allele of grx4 under the control of the arabinose-induced/glucose-repressed promoter Pcrg exhibited decreased virulence on maize. When grown in glucose, Pcrg:grx4 strains showed increased sensitivity to reactive oxygen stress and cell-wall damaging agents. Unlike the related basidiomycete *Cryptococcus neoformans*, grx4 mutants do not show increased sensitivity to some iron-related stressors, suggesting a novel role for Grx4 in the cell biology of *U. maydis*. Together, these data suggest that glutaredoxins could play in important role in the virulence of plant pathogenic fungi, in addition to their established roles as key regulators of fundamental cellular processes.

The impacts of gold mining on mycorrhiza in Northern Canada

Sarah Mediouni* and Kevin Stevens
*Wilfrid Laurier University

Gold mining continues to be an important part of economic development in Northern Canada. A large portion of the gold in the Northern territories is contained within arsenopyrite ores, therefore, arsenic byproduct is of special concern. Little research has been conducted on the impacts of arsenic on arbuscular mycorrhizae in Northern Ecosystems, and it is unknown whether they are a naturally occurring in the north.

Previous research on temperate regions has shown that mycorrhizal symbiosis can accelerate the remediation process in gold mines by supporting plant growth in poor soil conditions. However, it has also been shown that at high arsenic concentrations can reduce mycorrhizal colonization. The mine studied in this project is Tundra Mine, an inactive gold mine 250 km northeast of Yellowknife. Eight sites were chosen at varying distances from the tailings area to include a range of arsenic contamination levels. Colonization was compared in one species at a time across several sites or several species were compared across one site. Mycorrhizal colonization at the highest arsenic site (Hambone Lake = 2677 704.6 mg/kg) ranged from 59.83 ± 7.27 % in *Calamagrostis canadensis* to 33.20 ± 10.61% in *Epilobium angustifolium*. At the site with the lowest arsenic concentration (Reference 1 = 64.82 ± 19.32 mg/kg), colonization ranged from 66.47 ± 9.32 % in *Agrostis scabra* to 37.05 ± 9.31% in *Calamagrostis deschampsioides*. No trends of reduced colonization at higher arsenic sites were observed in this study, indicating a tolerance to arsenic. There were also no clear trends of colonization between plant species. This study has shown that arbuscular mycorrhizae are part of the Northern ecosystem and can be used as a tool in revegetation strategies.

Analysis of incompatibility in het-6 locus in *Neurospora crassa*

Ghazaleh Nourparvar*, Denis Lafontaine, and Myron L. Smith
*Carleton University

“Fungi can be pathogenic to plants and animals and finding novel antifungal agents is an ongoing endeavor. Non-self-recognition as an innate immune system in multinucleated fungi has the potential to work as a switch to hinder colony growth and promote cell death. In these organisms, presence of nuclei that bear variation at certain loci (called het loci) in the same cytoplasm results in a non-self-recognition response. In *Neurospora crassa*, het-6 (one of the 11 het loci) has two genes (het-6 and un-24) and two haplotypes (OR and PA). Non-allelic interaction between un-24PA and het-6OR exhibit high rates of programmed cell death and a slow aberrant growth phenotype. Remarkably, after about 4.5 days of growth, rapidly growing wild-type sectors emerge from these un-24PA-het-6OR colonies through a process called ‘escape’. We hypothesize that escape is a result of a genetic instability and is regulated by checkpoints active during replication. Sequencing showed that escapes are due to point mutations that are targeted to the het-6 gene (94%), and rarely to vib-1 (3%), a suppressor of incompatibility, or other unknown factors (3%). 80 gene knockouts were separately introgressed into the un-24PA-het-6OR strain and tested whether their absence suppress escape. Results show that deletions of the DNA damage checkpoint factors and vib-1 suppress and/or delay escape. VIB-1 is of interest since it functions

as a control hub, including modulating het-6 transcription and programmed cell death. RT-qPCR data shows that transcription levels of vib-1 and other incompatibility genes is modulated when exposed to DNA damaging agents.

The zinc cluster transcription factor Rha1 is a positive filamentation regulator in *Candida albicans*

Raha Parvizi Omran*, Chris Law, Vanessa Dumeaux, Bernardo Ramírez-Zavala, Joachim Morschhäuser, and Malcolm Whiteway
*Department of Biology, Concordia University, Montreal, Canada

Zinc cluster transcription factors are essential fungal regulators of gene expression. In the pathogen *Candida albicans*, they control processes ranging from metabolism to mating, virulence, and antifungal resistance. Here, we have identified gene ORF19.1604 as encoding a zinc cluster transcription factor acting as a regulator of filament development. Hyperactivation of ORF19.1604, which we have named RHA1 for Regulator of Hyphal Activity, leads to a wrinkled colony morphology under non-hyphal growth conditions, to filament formation, to invasiveness, and enhanced biofilm formation. RNA-sequencing of the activated Rha1 strain reveals the up-regulation of genes for filamentation and cell-wall-adhesion-related proteins such as Als1, Als3, Ece1, and Hwp1. Upregulation is also seen for the hyphal-inducing transcription factors Brg1 and Ume6, in addition to the downregulation of hyphal repressor Nrg1. The deletion of BRG1 blocks the filamentation caused by activated Rha1, while null mutants of UME6 result in a partial block. Deletion of RHA1 cause filamentation reduction under a variety of filament-inducing conditions. In contrast to the partial effect of either single mutant, the double rha1 ume6 deletion strain is defective in both serum- and Spider-medium-stimulated hyphal development. While the loss of Brg1 function blocks serum-stimulated hyphal development, this block can be significantly bypassed by Rha1 hyperactivity, and the combination of Rha1 hyperactivity and serum addition can generate significant polarization in even brg1 ume6 double mutants. Thus, in response to an external signal, Rha1 as new morphogenesis regulators mediate filamentation by the functional relationship with Brg1 and Ume6.

Yeast cytosolic J-chaperones perform distinct functions in cellular protein quality control

Andrey Petropavlovskiy*, Amandeep Sandhu, Rahim Ahmed, and Martin L. Duennwald
*The University of Western Ontario

To maintain the correct conformation of proteins and counteract detrimental effects of protein misfolding, cells have evolved multiple conserved mechanisms of protein

quality control. Several structural and functional classes of molecular chaperones are central to these mechanisms. Translation-associated and stress-associated chaperones are two prominent functional chaperone classes. Translation-associated chaperones mainly fold nascent polypeptides, whereas stress-associated chaperones re-fold misfolded proteins after exposure to stress. J-chaperones, also known as Hsp40s, is the most diverse class of molecular chaperones in the eukaryotic cell. All J-chaperones possess a conserved J-domain, which facilitates their interaction with Hsp70s. Even though the function of J-chaperones in aiding Hsp70s is well-characterized, their specific role in protein quality control remains largely ambiguous. Specifically, it remains unclear whether specific J-chaperones function as translation- or stress-associated chaperones. To explore the specific functions of cytosolic J-chaperones, we examined their role in maintaining Saccharomyces cerevisiae cell viability under a set of different stress and growth conditions. Here, we demonstrate that some cytosolic J-chaperones are strongly specialized as either translation- or stress-associated. By contrast, there is a considerable overlap between these functions for other J-chaperones. Specifically, we have found that some J-chaperones that are essential in translationally active cells, are mostly dispensable in slowly dividing cells. Furthermore, we demonstrate that specific J-chaperones are required for coping with different types of cell stress and possess a distinct transcriptional profile. Together our findings begin to decipher the specific functions of J-chaperones in maintaining protein quality control under different cellular stress conditions.

Identification of novel bioactives to combat the emerging fungal pathogen *Candida auris*

Emily Puumala*, Nicole Robbins, and Leah E. Cowen
*University of Toronto

Candida species are amongst the most prevalent causes of systemic fungal infections, which account for over one million annual fatalities and pose a growing threat to public health. *Candida albicans* represents the most common etiological agent however, the rate of infections caused by non-albicans Candida species continues to rise. Among these emerging drug-resistant pathogens is Candida auris, which since its discovery in 2009, has been identified worldwide and exhibits resistance to all three antifungal classes: the azoles, polyenes and echinocandins. My research endeavours to identify novel efficacious compounds, as well as those capable of potentiating established antifungals, against *C. auris*. Leveraging compound libraries through screening for antifungal activity against *C. auris*, I prioritized seven novel bioactives and echinocandin potentiators from the Medicines for Malaria Venture's Pathogen Box library. Chemogenomic profiling of six compounds identified several candidate genes

and pathways that suggest diverse modes-of-action. For example, heterozygous deletion of the fatty acid synthetase gene, FAS1, as well as sphingolipid biosynthesis genes, AUR1 and LCB2, resulted in hypersensitivity to the molecule MMV688766, suggesting it modulates fungal lipid homeostasis. In support of this model, supplementation of growth medium with fatty acids rescued the toxic effects associated with MMV688766 treatment. Future work will focus on further defining the mechanism of action of MMV688766, as well as using the developed framework for continued analysis of other compounds' modes of action. Collectively, this work highlights exciting new molecules with efficacy against an emerging fungal pathogen, which may pave the way for future antifungal drug development."

Exploring broad-spectrum and combination antifungal strategies to combat human fungal pathogens

Nicole Revie*, Kali Iyer, Sheena Li, Yoko Yashiroda, Jennifer Lou, Mohammad Mazhab-Jafari, Hiroyuki Hirano, Minoru Yoshida, Hiroyuki Osada, Charles Boone, and Leah E. Cowen
*University of Toronto

Invasive fungal infections have transitioned from a rare curiosity to a major cause of human mortality. The dependence on azole antifungals for the treatment of systemic mycotic disease has led to the development of widespread resistance. A promising strategy to expand the antifungal target space and overcome resistance is combination therapy, as combining drugs has the potential to confer enhanced efficacy and fungal selectivity, and to slow the evolution of resistance. Here, we aim to identify and characterize novel molecules that exhibit broad spectrum antifungal activity as well as augment the efficacy of current antifungals. To this end, we performed a high-throughput screen with ~20,000 compounds from the RIKEN Natural Product Depository in the absence and presence of the azole fluconazole against four prominent fungal pathogens: *Candida albicans*, *Candida glabrata*, *Candida auris*, and *Cryptococcus neoformans*. Mode-of-action studies were pursued for NPD6433, a compound with broad-spectrum activity that inhibits the fatty acid synthase Fas1 by targeting a fungal-selective pocket within the enoyl reductase domain. Further, we identified an azole potentiator that acts synergistically with fluconazole against azole-sensitive and -resistant *C. albicans* strains via disruption of lipid homeostasis. Selection experiments have implicated the AAA-ATPase Vps4 and ESCRT-III complex members involved in the multi-vesicular body (MVB) trafficking pathway. Future work aims to investigate the proximal target of this molecule and its interaction with MVB components. Collectively, this work has identified promising antifungal scaffolds with broad-spectrum and azole potentiating activity, which will aid in the development of

novel therapeutics to combat life-threatening fungal infections.

The role of fungal adhesins in mediating morphogenesis and virulence in *Candida albicans*

Sierra Rosiana* and Rebecca Shapiro
*University of Guelph

Candida albicans is a microbial fungus that exists as both a commensal member of the human microbiome, as well as an opportunistic pathogen. *C. albicans* is considered to be a polymorphic yeast and can switch between both yeast and filamentous morphologies. The genetic mechanisms that govern this yeast to filamentous cellular morphogenesis are numerous and involve a complex genetic interaction network that remains to be fully explored. One family of fungal proteins that likely play a role in morphogenesis are cell surface adhesin proteins. While preliminary research suggests adhesins are involved in morphogenesis, a complete analysis of the role and relationship between these adhesins has not been explored. Previous research from Dr. Shapiro's lab established a CRISPR platform for efficient generation of single- and double-gene deletions in *C. albicans*, which was applied to construct a library of 144 mutants, comprising 12 unique adhesin genes deleted singly, or in every possible combination of double deletions. My research aims to explore the role of adhesin proteins, singly and in combination, in *C. albicans* virulence. I performed a comprehensive screen of this adhesin mutant library, using the model nematode worm *Caenorhabditis elegans* as a simplified model host system. This screen identified single and double gene mutants that were critical for virulence, and further identified genetic interactions where deletion of two adhesins rendered strains significantly more avirulent than deletion of either gene on its own. This screen was followed up with in vitro biofilm and morphogenesis assays, which uncovered that some strains that were critical for virulence were also attenuated in biofilm formation and morphogenesis. The results of this project yield important new insight regarding the role of adhesins in mediating virulence of this critical fungal pathogen and identifying these important virulence regulators may ultimately lead to new genetic targets for antifungal therapeutics.

A comparative study of boric acid and fluconazole drug responses in *C. albicans* planktonic and biofilm cells

Ola Salama* and Aleeza Gerstein
*University of Manitoba

Candida albicans is the most prevalent fungal pathogen that causes vulvovaginal candidiasis (VVC) and recurrent VVC (RVVC, defined as four or more episodes in a year), accounting for 85-90% of VVC/RVVC cases. Incidences of non-albicans species, which have a reduced antifungal susceptibility baseline, in VVC/RVVC have increased recently. Biofilm formation is an important virulence factor in Candida spp. pathogenicity and could be responsible for treatment failure in VVC/RVVC; however, the involvement of biofilms in VVC, and particularly RVVC is understudied. Boric acid (BA) is used to treat VVC; however, it is considered as a second-line treatment because of uncertainty surrounding the mechanism of action and the potential long-term side effects. The aim of this research is to investigate BA efficacy against planktonic cells, biofilm formation, and effectiveness against preformed biofilms in diverse clinical strains and species and compare it to fluconazole (FLC). Using diskimageR, we determined the population-level distribution of BA and FLC resistance and tolerance across 77 clinical isolates of *Candida spp*. and showed that drug response parameters differ intra- and inter-species. We found that planktonic *C. albicans* yeast cells cannot form biofilms in the presence of BA. BA was shown to reduce the biomass of biofilms, implying eradication effect, while FLC did not inhibit biofilm growth. A significant reduction in metabolic activity was also identified in biofilms treated with BA, while metabolic activity did not change in biofilms treated with FLC. Our findings suggest that BA could be a potentially effective drug against fungal biofilms.

Investigating the effects of arbuscular mycorrhizae on *Crocanthemum canadense* (L.) Britt. (Cistaceae) propagated in tissue culture

Kendra Sampson*, Robin Browne, Allison Walker, and Rodger Evans
*Acadia University

"*Crocanthemum canadense* (L.) Britt. (Cistaceae), Rockrose, is a small perennial herb found in Eastern North America sand barrens. It is classified as critically imperiled in Nova Scotia under the Nova Scotia Endangered Species Act. Nova Scotia Rockrose populations continue to decline; recent counts estimate only 5000-5500 plants remain. To better understand Rockrose biology, we analyzed symbiotic mycorrhizal associations among native Nova Scotia populations. Recent research from our group has documented the presence of arbuscular mycorrhizal fungi (AMF) within Rockrose roots of plants in their native habitat; the present study is the first to focus primarily on

the benefits of this symbiotic relationship between Rockrose and AMF, and to identify the fungal partner. AMF improves water and nutrient uptake (phosphorus and nitrogen) by the plant. In return, they rely on the plant as a host and carbon source. In a greenhouse trial with varying percentages of AMF inocula, we determined how AMF would affect the growth of Rockrose plants propagated from tissue culture. The 1:25 inoculum had the most significant effect on the height of the shoots (cm) while the 1:50 inoculated plants had the largest root mass (g). Previous studies indicate that the lack of phosphorus in the experimental trial could have had a negative effect on the growth and symbiosis of *C. canadense* and AMF. The identity of AMF in the roots and the inoculum was determined using rDNA barcoding. A potentially novel species of AMF sister to *Funneliformis mosseae* was identified. This research will aid in the conservation and restoration of this critically imperiled species by further understanding beneficial soil fungi, an understudied component of the declining sand barrens habitat.

The international dimension of Canadian mycology

Keith Seifert* and Allison Walker
*Carelton University / ex Agriculture & Agri-food Canada

Canada has long had a strong international reputation in mycological research. Canadian mycologists participate in a variety of Canadian, American and international societies. There has usually been Canadian representation on the International Mycological Association, but in the absence of a Canadian mycological society, our interests there are passed through the Mycological Society of America. The activities of Canadian labs that don't participate in the MSA thus tend not to be recognized at this international level. Formalizing our nascent Canadian mycological initiative into an official organization would be the first step to addressing this problem.

Genome mining *Aspergillus niger* for discovery of antimicrobial compounds

Cameron Semper*, Isabelle Benoit-Gelber, Adrian Tsang, and Alexei Savchenko
*University of Calgary

Organisms produce an array of organic compounds that are not required for their growth under normal laboratory conditions. These compounds, termed secondary metabolites (SM), are rich in chemical diversity and have been developed into several industrially and medically relevant natural products. The genes responsible for the biosynthesis of specific SM are typically co-localized within microbial genomes. This clustering phenomenon is observed extensively in the filamentous fungus Aspergillus niger, with recent genome sequencing projects revealing more than 75 secondary metabolite gene clusters (SMGC). The majority of these SMGC are not expressed (are

"silent") under standard laboratory conditions but many (~60) co-localize with a putative transcriptional regulator. As a strategy to awaken these silent SMGC in A. niger, we inserted each of these transcriptional regulators downstream of the inducible glucoamylase promoter. The resulting library consisting of 60 overexpression (OE) strains has been profiled extensively at the level of gene expression and metabolically. In an effort to further characterize the strains in the OE library, antibacterial activity screening was performed on crude extracts obtained from each of the 60 strains grown under multiple growth conditions. Results of these experiments identified multiple strains that appear to produce compounds capable of inhibiting bacterial growth.

Expanding the genetic toolbox for *Candida albicans*: the development of novel CRISPR interference and CRISPR base editing technologies

Jehoshua Sharma*, Deeva Uthayakumar, Lauren Wensing, Yannic Proteau, Madison Best, Philippe Després, Christian Landry, Alejandro Chavez, and Rebecca Shapiro
*University of Guelph

Candida albicans is a pathogenic fungus that can cause severe invasive infections and is emerging as a significant global threat, with rising incidences of morbidity and mortality. Treatment of *C. albicans* and other fungal infections is notoriously challenging, with a limited selection of clinically-effective antifungal drugs and increasing rates of antifungal drug resistance. Studying the virulence and drug response of these life-threatening fungal pathogens is of imminent importance, yet progress is hindered by challenges associated with manipulating these fungal species genetically. Since their development, CRISPR-Cas-based genetic technologies have revolutionized genome editing, and democratized these powerful tools across diverse organisms. Previously, our lab developed a novel CRISPR-Cas9-based 'gene drive' platform for efficient creation of single and double homozygous gene knockouts in *C. albicans*. Currently, we are expanding upon this research and developing novel CRISPR-based genetic manipulation systems for *C. albicans*. Here, I discuss the development and optimization of two of these novel CRISPR systems: CRISPR interference (CRISPRi)-dCas9 which can modulate gene expression, and CRISPR base editor (CRISPRbe)-nCas9, which can be used for precise genetic base editing at a codon level of resolution. I will discuss our optimization of these platforms, using ADE1 and ADE2 genes are markers, and will further highlight how these emerging technologies can be applied more broadly to the study of *C. albicans* biology. Together, these two new platforms allow for diverse mechanisms of interrogation to help study *C. albicans*' genetics, and can ultimately be used to probe the genetic mechanisms of drug resistance.

Transcriptomic profiling suggests a role for an ABA biosynthetic gene in *Fusarium graminearum* during its early infection of Wheat

Tanya Sharma*, Leann M. Buhrow, Youlian Pan, and Michele C. Loewen
*University of Ottawa

Fusarium head Blight is a major cereal crop disease caused by the pathogenic fungus *Fusarium graminearum*. Recent studies have explored how various pathogenic fungi use abscisic acid (ABA) as a virulence factor to mediate host-microbe interactions. While fungal species are not known to encode ABA receptors, they are known to biosynthesize ABA. Presently, no ABA biosynthesis gene has been reported in *F. graminearum*. The sequences of known members of the fungal ABA biosynthetic pathway from *Botrytis cinerea* were used to BLAST against *F. graminearum* sequences in NCBI and ENSEMBL databases. A single hit was obtained with 75.8 % identity to BcCPR1, a known ABA biosynthetic cytochrome p450 oxidoreductase from *B. cinera*. RNA-seq analysis of *F. graminearum* 24 hours after challenge of *Triticum aestivum* cultivar 'Fielder' under different conditions (with co-application of ABA, gibberellic acid (GA) and in presence of ABA signaling inhibitor AS6) showed that this gene is expressed under all conditions containing *F. graminearum*, suggesting that expression of the fungal ABA biosynthetic pathway is relevant at the 24 h time point after infection. Ongoing analyses are looking at other fungal phytohormone biosynthesis pathways, as well as the corresponding wheat transcriptomes.

Characterization of TRI6, a global regulator of secondary metabolism

Kristina Shostak*, A. SPROULE, I. THAPA, D. OVERY, B. BLACKWELL, and G. SUBRAMANIAM
*Agriculture & Agri-Food Canada

Fusarium graminearum, causative agent of Fusarium head blight results in major economic losses worldwide. To develop effective disease management strategies, a comprehensive understanding of the organism's pathogenicity is required. Currently, our understanding of transcription factor TRI6 in pathogenicity is limited to its role in production of a mycotoxin deoxynivalenol (DON). However, several lines of evidence indicate that TRI6 is a global regulator affecting different pathways involved in virulence. Our goal is to establish its role in the production of novel secondary metabolites (SM) with a role in infection cycle in wheat (*Triticum aestivum*). *F. graminearum* genome is predicted to possess 67 secondary metabolic gene clusters encoding many secondary metabolites. We constitutively expressed TRI6 to cryptically activate SM clusters in vitro. RNA sequencing analysis identified a total of 109 genes in 38 secondary metabolic gene clusters whose expression was affected by constitutive

expression of TRI6. To validate RNA-Seq data, we performed metabolite profiling and identified several compounds whose production levels were affected by TRI6. These included trichothecenes, gramillins A and B, fusaoctaxin A, and seven novel secondary metabolites produced by the trichothecene and fusaoctaxin SMCs.

Modulation of the complex regulatory network for methionine biosynthesis in fungi

Manjari Shrivastava*, Jinrong Feng, Mark Coles, Benjamin Clark, Amjad Islam, and Malcolm Whiteway
*Concordia University

The assimilation of inorganic sulphate and the synthesis of the sulphur-containing amino acids methionine and cysteine is mediated by a multi-branched biosynthetic pathway. In *S. cerevisiae* this pathway is regulated by a collection of five transcription factors (Met4, Cbf1, Met28 and Met31/Met32), while in the filamentous fungi the pathway is controlled by a single Met4-like factor. We have investigated this circuitry in the fungal pathogen *Candida albicans*, which is phylogenetically intermediate between the filamentous fungi and *S. cerevisiae*. We found that in the pathogen the Met4 ortholog is also a core regulator of methionine biosynthesis, but it functions in consort with Cbf1. Unlike *S. cerevisiae*, *C. albicans* Met4 and Cbf1 do not show a protein-protein interaction, which suggests that they may independently co-regulate the pathway. While *C. albicans* encodes a Met4 protein, a Met4 paralog designated Met28 (orf19.7046), and a Met31 protein, deletion and activation constructs suggest that of these proteins only Met4 is actually involved in regulation of methionine biosynthesis. Both Met28 and Met31 are linked to other functions; Met28 appears essential, while Met32 appears implicated in aspects of growth control. While *S. cerevisiae* and *C. albicans* share Cbf1 and Met4 as central elements of the methionine biosynthesis control, the other proteins that make up the circuit in *S. cerevisiae* are not members of the *C. albicans* control network, and thus the *S. cerevisiae* circuit likely represents a recently evolved arrangement.

Screening novel marine fungal strains for the production of polyunsaturated fatty acids, specifically Eicosapentaenoic acid and Arachidonic Acid

Nicole Smith* and Jeremy Benjamin
*Acadia University

The polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and arachidonic acid (ARA) are important for human health. Alternate sources of these valuable PUFAs for human consumption are being sought to replace the current, unsustainable,

sources: fish oil and animal byproducts respectively. Marine fungi show promise as a novel and potentially lucrative source of PUFAs, which could provide a renewable source of EPA and ARA. In collaboration with Mara Renewables Corporation in Dartmouth Nova Scotia, this study established growth and screening methods for marine fungi, isolated novel strains from the Atlantic Ocean, identified them by ITS rDNA barcoding and determined their fatty acid profiles. Marine fungal strains were grown in liquid cultures and screened for EPA and ARA production using fatty acid methyl esters (FAMEs) analyzed by gas-liquid chromatography. Of the 74 isolated marine microorganisms belonging to 25 genera; four Mortierella strains produced ARA and one Pythium strain (oomycete) produced both EPA and ARA, at commercially competitive quantities. Future research will target the isolation and screening of additional strains from these genera and scale up promising strains in bioreactors for optimization of growth and fatty acid production.

Trans-kingdom conjugation enables simple and robust DNA delivery to fungi

Maximillian Soltysiak*, Ryan R. Cochrane, Stephanie Brumwell, Rebecca S. Meaney, Samir Hamadache, Mariana Severo, Arina Shrestha, and Bogumil J. Karas
*University of Western Ontario

Prokaryotic organisms use conjugation, a mechanism for direct DNA transfer, in the spread of antibiotic resistance and virulence genes. While donors must be bacterial or archaeal, conjugation can be used to deliver DNA to a broad range of recipient species, including fungi. Donor species, such as *Escherichia coli* and *Agrobacterium tumafaciens*, have been used to transform yeast and filamentous fungi with simplified protocols, with little required optimization, that have been previously described as “mixing two cultures and waiting an hour.” Unlike conventional DNA delivery techniques, like chemical/lithium acetate transformation and electroporation, conjugation does not require DNA isolation, eliminating the risk for DNA damage during extraction and handling. Here, we developed a novel method for conjugation, using donor E. coli to deliver large episomal plasmids, over 130 kbp in length, to the *Saccharomyces cerevisiae*. Our protocol expands the conditions in which conjugation can occur, no longer requiring DNA delivery in liquid or on top of agar, but rather within the solid media itself, which may prove useful for recipient species that require such media for growth. Furthermore, we highlight our designer conjugative plasmids tailored for increased conjugation rates to *S. cerevisiae*. Moving forward, conjugation represents an attractive, simple method for DNA delivery of plasmids—up to the size of chromosomes and complete biosynthetic pathways—to yeast and other fungi.

Mechanisms underlying the chemotropism of *Fusarium graminearum* on the wheat head

Pooja Sridhar*, Zerihun Demissie, Simon Foote, John Allingham, and Michele Loewen
*Queen's University

Fusarium Head Blight (FHB) of wheat is caused by the filamentous fungus *Fusarium graminearum* and leads to food shortages and economic losses worldwide. While there is much knowledge about *F. graminearum* biology, less is known about the factors that enable it to sense and mediate directed growth (chemotropism) towards the host cells it colonizes. Our work showed that the pheromone sensing G protein-coupled receptor Ste2 of *F. graminearum* (FgSte2) is responsible for sensing and mediating chemotropism towards the catalytic product of a wheat-secreted peroxidase. Ste2-mediated chemotropic growth towards this peroxidase-derived factor was found to be transduced through the cell wall integrity (CWI) MAPK signaling cascade. Our ongoing work is exploring both the wheat and fungus for potential wheat peroxidase substrates that form the chemoattractant product. We are using in vitro and in vivo reporter-based assays to screen potential compounds for their ability to activate FgSte2 and induce signal transduction through the CWI pathway. In addition, a transcriptomic approach is being applied to elucidate the differential gene expression in *F. graminearum* upon induction by the peroxidase-derived product. Our preliminary work and new findings from these methods will be presented. Characterization of the chemoattractant, as well as the cellular changes it induces, will deepen our understanding of the mechanisms of host-sensing by this important pathogen."

Pathogenic allodiploid hybrids of Aspergillus fungi

Jacob Steenwyk*, A.L. Lind, L.N.A. Ries, T.F. dos Reis, L.P. Silva, F. Almeida, R.W. Bastos, T.F. de Campos Fraga da Silva, V.L.D. Bonato, A.M. Pessoni, F. Rodrigues, H.A. Raja, S.L. Knowles, N.H. Oberlies, K. Lagrou, G.H. Goldman, and A. Rokas
*Vanderbilt University

Interspecific hybridization substantially alters genotypes and phenotypes and can give rise to new lineages. Hybrid isolates that differ from their parental species in infection-relevant traits have been observed in several human-pathogenic yeasts and plant-pathogenic filamentous fungi but have yet to be found in human-pathogenic filamentous fungi. We discovered 6 clinical isolates from patients with Aspergillosis originally identified as *Aspergillus nidulans* (section Nidulantes) that are actually allodiploid hybrids formed by the fusion of *Aspergillus spinulosporus* with an unknown close relative of *Aspergillus quadrilineatus*, both in section Nidulantes. Evolutionary genomic analyses revealed that these isolates belong to *Aspergillus latus*, an allodiploid

hybrid species. Characterization of diverse infection-relevant traits further showed that *A. latus* hybrid isolates are genomically and phenotypically heterogeneous but also differ from *A. nidulans*, *A. spinulosporus*, and *A. quadrilineatus*. These results suggest that allodiploid hybridization contributes to the genomic and phenotypic diversity of filamentous fungal pathogens of humans.

The first report of a culturable microbiome from pollinated style tissue

Michelle Thompson*, Anuja Shrestha, Jeffrey Rinne, Charles Shearer, Victor Limay-Rios, Lana Reid, and Manish N. Raizada
*University of Guelph

The plant style tissue microbiome is a new frontier to investigate, and an area to seek probiotic microbes to protect against *Fusarium graminearum*. This pathogenic fungus causes Gibberella ear rot (GER) in maize, producing dangerous mycotoxins before and after harvest which are harmful to human and livestock health. *Fusarium graminearum* (and other mycotoxigenic fungi) frequently invade maize through exposed silks, which are unusually long style tissue. We hypothesized that maize genotypes that are partially resistant silk-invading pathogens house microbes in the silk to defend the entryway to the developing grain. Fourteen genotypes of maize were grown in 2017, treated with and without *F. graminearum*, and the cobs were harvested at maturity. The portion of the silks protected by the husk were split into tip and base samples. Over 1000 microbes were isolated from these silks. Taxonomic identification of these strains revealed that silks do indeed host a diversity of culturable bacteria and fungi! So far, anti-Fusarium assays have discovered over 30 unique candidate bacteria which suppress *F. graminearum* growth in vitro. These microbes may have coevolved with maize to protect the grain from mycotoxigenic fungi. They could potentially be used as a treatment to prevent toxins such as vomitoxin (Deoxynivalenol), zearalenone, or aflatoxins from contaminating food. Better understanding of plant microbiome-pathogen relationships can provide new tools to combat dangerous diseases. This thesis may help farmers worldwide adapt to the spread of serious fungal diseases.

Vertical Distribution of Some Crustose Littoral Zone Lichens in the Bay of Fundy

Cole Vail* and Allison Walker
*Acadia University

Despite wave action, salinity, UV exposure, and other abiotic and biotic pressures; lichens account for much of the substrate cover in the rocky shore littoral zone, providing primary production, habitat and a food source for other organisms. Some species with cyanobacterium symbionts can also provide a source of nitrogen fixation. One lichen family, the Verrucariaceae, represents one of the few families of lichens

present between the supralittoral and sublittoral zones. This research project documented the vertical zonation and general ecology of several members of this understudied family of lichens in the littoral zone. Specimens from seven different sites along the Nova Scotia coast were identified using DNA barcoding and morphology to provide biodiversity data for the Bay of Fundy.

The Enterprise: A massive transposon carrying Spok meiotic drive genes

Aaron Vogan*, S. Lorena Ament-Velásquez, Eric Bastiaans, Ola Wallerman, Sven J. Saupe, Alexander Suh, and Hanna Johannesson
*Uppsala University

The genomes of eukaryotes are full of parasitic sequences known as transposable elements (TEs). Most TEs studied to date are relatively small (50 -- 12000 bp), but can contribute to very large proportions of genomes. Here we report the discovery of a giant tyrosine-recombinase-mobilized DNA transposon, Enterprise, from the model fungus *Podospora anserina*. Previously, we described a large genomic feature called the Spok block which is notable due to the presence of meiotic drive genes of the Spok gene family). The Spok block ranges from 110 kb to 247 kb and can be present in at least four different genomic locations within *P. anserina*, despite what is an otherwise highly conserved genome structure. We have determined that the reason for its varying positions is that the Spok block is not only capable of meiotic drive, but is also capable of transposition. More precisely, the Spok block represents a unique case where the Enterprise has captured the Spoks, thereby parasitizing a resident genomic parasite to become a genomic hyperparasite. Furthermore, we demonstrate that Enterprise (without the Spoks) is found in other fungal lineages, where it can be as large as 70 kb. Lastly, we provide experimental evidence that the Spok block is deleterious, with detrimental effects on spore production in strains which carry it. In contrast to the selfish role of the Enterprise in *P. anserina*, we speculate that the mobility of the Enterprise may also play an adaptive role in many other fungi, through the horizontal transfer of metabolic genes. This union of meiotic drivers and a transposon has created a selfish element of impressive size in *Podospora*, challenging our perception of how TEs influence genome evolution and broadening the horizons in terms of what the upper limit of transposition may be.

Regulation of phagosomal size and integrity during fungal infection

Johannes Westman*, Glenn F.W. Walpole, Lydia Kasper, Bessie Y. Xue, Osama Elshafee, Bernhard Hube, and Sergio Grinstein
*The Hospital for Sick Children

Phagosomes must maintain their membrane integrity to exert their microbicidal function. Some microorganisms, however, survive and can grow within phagosomes. In such instances, phagosomes need to expand to avoid rupture and microbial escape. We used the polymorphic fungus *Candida albicans* to study whether macrophage phagosomes regulate their size to preserve their integrity during infection. We demonstrate that as *C. albicans* hyphae elongate within the phagosome, luminal calcium is released, which induces recruitment and insertion of lysosomes, thereby increasing the phagosomal surface area. As the hyphae grow, the expanding phagosome consumes the majority of free (unfused) lysosomes. Simultaneously, lysosomal biogenesis is stimulated by activation of the transcription factor TFEB. Preventing lysosomal insertion leads to phagosomal rupture, inflammasome activation, IL-1beta secretion, and increased hyphal growth leading to pathogen escape and eventual host cell death. Moreover, whole-genome transcriptomic analysis demonstrated that the stress responses elicited in *C. albicans* upon engulfment are reversed if phagosome expansion is prevented. Our findings reveal a mechanism whereby phagosomes maintain their integrity while expanding, thereby ensuring that growing pathogens remain entrapped within this microbicidal compartment."

VID28 and VID30 regulate glucose repression and derepression in *Saccharomyces cerevisiae*

Jordan Willis*, Jesse Rentz, and George van der Merwe
*University of Guelph

The yeast *Saccharomyces cerevisiae* has evolved to utilize a wide range of carbon sources but typically preferentially ferments glucose, resulting in the inhibition of gluconeogenic processes through carbon catabolite repression. Fermentative metabolism requires a concert of synergistic mechanisms involved in carbon sensing, transcriptional inhibition and protein degradation, thereby enabling the cell to quickly react to changes in environmental and intracellular sugar availability. The Vid30 Complex (Vid30c), also known as the Gid Complex, is an E3 ubiquitin ligase required for the degradation of gluconeogenic enzymes, including fructose-1,6-bisphosphatase (FBPase), to inhibit gluconeogenic processes. The transcriptional repressor Mig1 prevents gluconeogenic gene expression during glycolysis by binding to the promoter regions of gluconeogenic genes to prevent their transcription. When glucose becomes

limited, the Snf1 kinase phosphorylates Mig1, resulting in the upregulation (derepression) of glucose-repressed genes. Here, we use SDS-PAGE Western blotting techniques to show that the carbon source-sensitive posttranslational modification of Mig1 requires VID28 and VID30. Additionally, our qRT-PCR analyses show that these Vid30c subunits are required for the efficient regulation of transcriptional repressors (including Mig1) and activators of glucose-repressed genes, as well as the expression of their targets. In combination, these results describe a new role for the Vid30c in the transcriptional regulation of the glucose repression mechanism."