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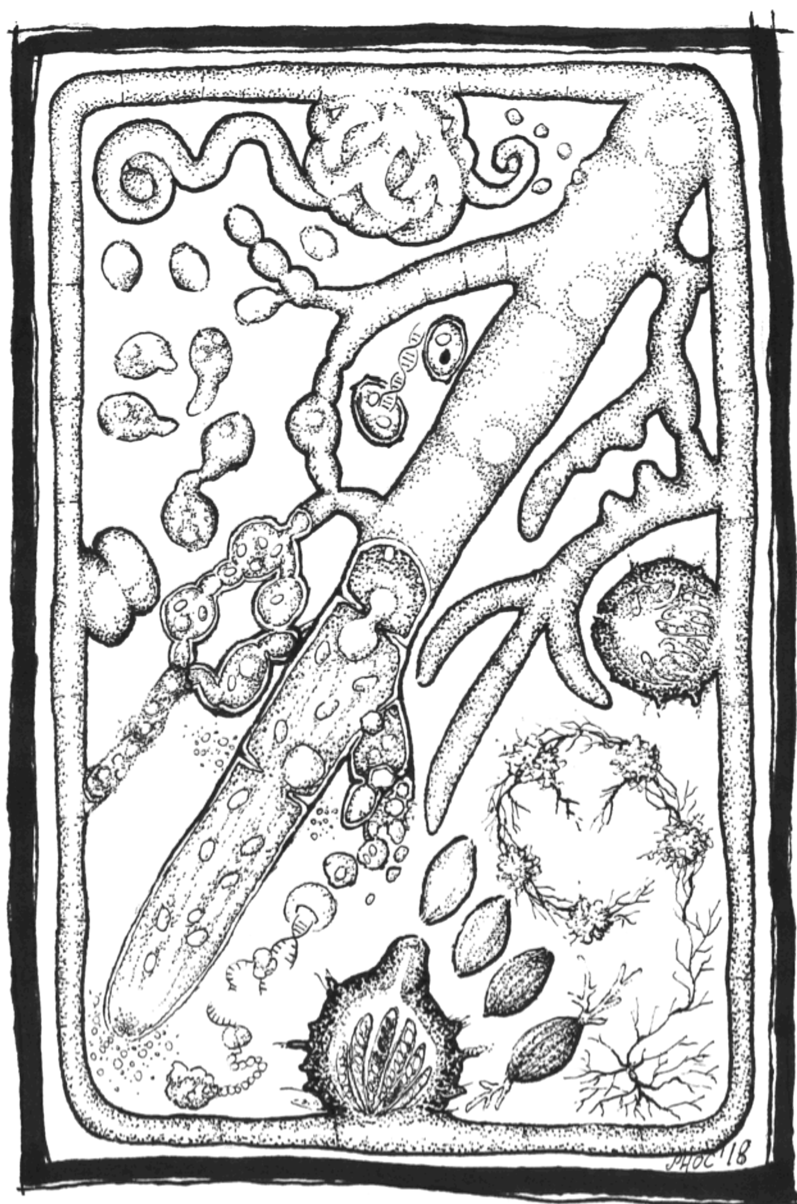
Program and Abstracts



NEUROSPORA 2018

October 18-21

Asilomar Conference Center

Pacific Grove

California

Cover design by Stephanie Herzog, Technische Universität Braunschweig

Neurospora 2018

October 18-21
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Pacific Grove
California

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Brief Schedule

	Morning	Afternoon	Evening
Thursday October 18		Arrival Registration	Dinner Mixer (Heather)
Friday October 19	Breakfast Plenary Session I Cell Biology and Morphogenesis	Lunch Plenary Session II Metabolism, Signaling and Development	Dinner Poster Session
Saturday October 20	Breakfast Plenary Session III Gene Expression and Epigenetics	Lunch Plenary Session IV Genomics, Evolution, and Tools	Banquet Speaker Poster Session
Sunday October 21	Breakfast Plenary Session V Circadian Clocks and Environmental Sensing	Lunch Departure	

All Plenary Sessions will be held in Heather. Posters will be displayed in Heather and Toyon throughout the meeting. They should be set up Friday and displayed until the end of the poster session/reception on Saturday evening.

Schedule of Activities

Thursday, October 18

15:00 - 18:00 p.m. Registration: Hearst Social Hall

18:00 - 19:00 p.m. Dinner: Crocker

20:00 - 22:30 p.m. Mixer: Heather

Friday, October 19

7:30-8:30 Breakfast, Crocker Dining Hall

Morning (Heather)	Cell Biology & Morphogenesis (Session 1)	
Chair	Barry Bowman	
08:30-08:40	Welcome & Announcements	
08:40-09:00	Meritxell Riquelme	Imaging vesicular cargoes and cargo adaptors through the secretory pathway in *Neurospora crassa*
09:00-09:20	Rosa Mourino	The actin motor MYO-5 effect in the intracellular organization of *Neurospora crassa*
09:20-09:40	Olga Alicia Callejas-Negrete	The phospholipid flippase DNF-4 and its role in polarized growth in *Neurospora crassa*
09:40-10:00	Oded Yarden	*gul-1*, a suppressor of *cot-1*, encodes an RNA-binding protein which is involved in cell wall remodeling
10:00-10:30	Coffee Break	
10:30-10:50	Stephen Free	Formation of cell wall melanin in *Neurospora crassa*
10:50-11:10	Jens Heller	Innate immunity proteins mediate allorecognition and cell death in *Neurospora crassa*
11:10-11:30	Brad Bartholomai	Spatiotemporal regulation of a core clock gene products in *Neurospora crassa*
11:30-11:50	David Roos	FungiDB: An integrated functional genomics database resource

12:00-13:00 Lunch, Crocker Dining Hall.

Afternoon (Heather)	Metabolism, Signaling, & Development (Session 2)	
Chair	Andre Fleißner	
14:30-14:50	Marcus Roper	Cooperative dynamics underlie Neurospora spore germination
14:50-15:10	Elizabeth Hutchison	Transcript structure, expression, and localization of the *Neurospora crassa* sexual development regulator *fsd-1*
15:10-15:30	Shinji Honda	*Ascospore sleepless-1* and *-2* are required for ascospore dormancy in *Neurospora crassa*
15:30-15.50	Liran Aharoni-Kats	MOB2A Tyr117 and Tyr119 are required for interactions with the NDR kinase COT1 and for proper sexual development
15:50-16:20	Coffee Break	
16:20-16:40	Ernestina Castro-Longoria	First report of a functional agmatinase in *Neurospora crassa* and its essentiality for cell survival
16:40-17:00	Shin Hatakeyama	Association of fungal MSH1 protein to mitochondrial DNA is critical for lifespan in *Neurospora*
17:00-17:20	Raphael Gabriel	Reverse engineering of enzyme hypersecretion in filamentous fungi
17:20-17:40	Michael T. Judge	Real-time in-vivo metabolomics of *Neurospora crassa*

18:00-19:00 Dinner, Crocker Dining Hall

Evening (Heather)

19:30 - 20:30 In memorium: Charles Yanofsky (Matt Sachs and others)
Beadle and Tatum lecture (Zack Lewis)

20:30 - 22.00 Poster session

Saturday, October 20

07:30-08:30 Breakfast, Crocker Dining Hall

Morning (Heather)	Gene Expression and Epigenetics (Session 3)	
Chair	Tom Hammond	
08:40-09:00	Hua Xiao	Cap-binding proteins mediate meiotic silencing by unpaired DNA
09:00-09:20	Eugene Gladyshev	Recombination-independent recognition of DNA homology for Repeat-Induced Point mutation (RIP) and meiotic silencing by unpaired DNA (MSUD)
09:20-09:40	Ashish Giri	The *Neurospora crassa* Oak Ridge (OR) background exhibits an atypically efficient meiotic silencing by unpaired DNA
09:40-10:00	Matt Sachs	The impact of modulating translation initiation, elongation, and termination on gene expression
10:00-10:30	Coffee Break	
10:30-10:50	Zhipeng Zhou	Codon usage determines gene expression level by regulating transcription
10:50-11:10	Allyson Erlendson	Control of gene regulation by Polycomb Repressive Complex 2 and its partners
11:10-11:30	Andrew Klocko	Nucleosome positioning by DIM-1 prevents aberrant DNA methylation in Neurospora
11:30-11:50	Vince Bicocca	ASH-1-catalyzed H3K36 methylation drives repression and marks H3K27me2/3-competent chromatin

12:00-13:00 Lunch and Business Meeting. Please sit at tables within the **Woodwinds** Room of Crocker Dining Hall.

Afternoon (Heather)	Evolution, Genomics, and New Tools (Session 4)	
Chair	Jason Stajich	
14:30-14:50	Nicholas A. Rhoades	Identification of a genetic element required for spore killing in Neurospora
14:50-15:10	Michael Freitag	Comparative studies on chromosome structure and gene silencing in fungi
15:10-15:30	Lori Huberman	Developing a high-throughput functional genomics platform for filamentous fungi
15:30-15:50	Katherine Borkovich	Lessons from high-throughput functional genomics analyses in *Neurospora crassa*
15:50-16:20	Coffee Break	
16:20-16:40	Aaron Robinson	Genetics of thermotolerance in *Neurospora discreta*
16:40-17:00	Christopher Hann-Soden	Geography and demography of clonal and sexual populations of Neurospora
17:00-17:20	Jason Stajich	Fungal growth suppression by bacteria emitted volatiles
17:20-17:40	Scott Baker	Team science and a cutting edge suite of analytical tools accelerate molecular analyses of Neurospora and other filamentous fungi

18:00-19:00 Banquet, **Woodwinds** Room of Crocker Dining Hall,
19:30-20:20 Banquet Speaker (CICESE group, B.O. Dodge Award)

20:20-22:00 **Heather -** Post-banquet Social / Poster Session

Sunday, October 21st

07:30 -08:30 Breakfast, Crocker Dining Hall

Morning (Heather)	Circadian Clocks & Environmental Sensing (Session 5)	
Chair	Meritxell Riquelme	*FEBS Letters Poster Award Announcement*
08:40-09:00	Luis Corrochano	Regulation of conidiation and carotenoid biosynthesis by the velvet complex in *Neurospora crassa*
09:00-09:20	André Fleißner	Intra- and interspecies communication in filamentous fungi
09:20-9:40	Michael Brunner	Regulation of the circadian clock of Neurospora by *frq* antisense transcription
09:40-10:00	Deborah Bell-Pedersen	Circadian clock regulation of mRNA Translation in Neurospora
10:00-10:30	Coffee Break	
10:30-10:50	Jay Dunlap	The Phospho-Code determining circadian feedback loop closure and output in Neurospora
10:50-11:10	Christina M. Kelliher	Regulators of the Neurospora circadian period length in a low glucose environment
11:10-11:30	Meaghan Jankowski	Circadian regulation of rhythmic proteins leads to coordination of metabolic pathways within *Neurospora crassa*
11:30-11:50	Hannah De los Santos	The ECHO app: An application utilizing extended harmonic oscillators to identify non-harmonic circadian oscillations in large datasets

12:00-13:00 Lunch, Crocker Dining Hall / Box Lunches

Departure

Plenary Session Abstracts

Session 1

1.1 Imaging vesicular cargoes and cargo adaptors through the secretory pathway in *Neurospora crassa*

Meritxell Riquelme[1], Adriana M. Rico-Ramírez[1], Luis Enrique Sastré-Velásquez[1] and Robert W. Roberson[2]

[1]Department of Microbiology. Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, BC 22860, Mexico. [2]School of Life Sciences, Arizona State University, Tempe, AZ, USA

Hyphal polarized growth is supported by the directional transport of secretory vesicles to the apex, where they accumulate at the Spitzenkörper (SPK). In the last stages of the secretory pathway, vesicles fuse with the plasma membrane (PM) providing all the materials and enzymes necessary for cell wall expansion. Noticeably, chitosomes containing chitin synthases (CHSs) concentrate at the core of the SPK, while macrovesicles containing β-1,3-glucan synthases occupy the outer layer of the SPK. The selective packaging of proteins into distinct vesicles initiates at the endoplasmic reticulum (ER) in an essential step that involves the action of coat protein complexes, sorting signals and cargo receptors. We have explored the mechanisms of synthesis, traffic and sorting of chitosomes containing CHS-4, a protein with multiple transmembrane spanning domains. While it had been formerly suggested that CHSs are delivered to the PM via a non-classical secretory pathway, hyphal exposure to Brefeldin A, an inhibitor of the ER-to-Golgi COPII dependent-secretion, interrupted the arrival of CHS-4 to the SPK. We identified CSE-7 as the putative ER cargo receptor for CHS-4 and showed that CSE-7 is necessary for the localization of CHS-4 at hyphal tips and septa. CSE-7 localized primarily at delimited regions of the ER and at presumably tubular vacuoles. By transmission electron microscopy a very complex endomembranous system was revealed in subapical hyphal areas, with abundant rough ER sheets and smooth flattened cisternae that collectively we coined as network of elongated cisternae (NEC). Remarkably, CSE-7 was also found at septa and at the core of the SPK, indicating that in addition to having a role at ER sites for the correct exit of CHS-4, CSE-7 has a potential role at apical sites for localized delivery or activity of CHS-4.

Recently, we have identified in the genome of *N. crassa cni*, encoding a putative ER cargo receptor belonging to the Cornichon/Erv14 family. CNI showed partial co-localization with CSE-7 at the NEC. Additionally, CNI co-localized partially with the Golgi cisternae-associated Rab GTPase YPT-1, suggesting that CNI exits the ER in COPII coated vesicles until reaching the Golgi cisternae, where it will be packed and returned to the ER.
Our current efforts are oriented towards further understanding the many possible secretory pathway/s followed by the cell wall building and remodeling nanomachinery.

1.2 The actin motor MYO-5 effect in the Intracellular organization of *Neurospora crassa*

Arianne Ramírez-del Villar[1], Robert W. Roberson[2], Rosa R. Mouriño-Pérez[1]

[1]Departamento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada. Ensenada, Baja California, Mexico. [2]School of Life Sciences, Arizona State University, Tempe, Arizona, USA

In filamentous fungi, polarized growth is the result of vesicle secretion at the hyphal apex. Motor proteins mediate vesicle transport to target destinations on the plasma membrane via actin and microtubule cytoskeletons. Myosins are motor proteins associated with actin filaments. Specifically, class V myosins are responsible for cargo transport in eukaryotes. We studied the dynamics and localization of myosin V in wild type hyphae of *Neurospora crassa* and in hyphae that lacked MYO-5. In wild type hyphae, MYO-5-GFP was localized concentrated in the hyphal apex and colocalized with Spitzenkörper. Photobleaching studies showed that MYO-5-GFP was transported to the apex from subapical hyphal regions. The deletion of the class V myosin resulted in reduced rate of hyphal growth, apical hyperbranching, and intermittent loss of hyphal polarity. MYO-5 did not participate in breaking the symmetrical growth during germination but contributed in the apical organization upon establishment of polarized growth. In the Δmyo-5 mutant, actin was organized into thick cables in the apical and subapical hyphal regions, and the number of endocytic patches was reduced. The microvesicles observed with CHS-1-GFP were distributed as a cloud occupying the apical dome and not in the Spitzenkörper as the WT strain. The mitochondrial movement was not associated with MYO-5, but tubular vacuole position is MYO-5-dependent. These results suggest that MYO-5 plays a role in maintaining apical organization and a robust Spitzenkörper and is required for hyphal growth, polarity, septation, conidiation, and proper conidial germination

1.3 The phospholipid flippase DNF-4 and its role in polarized growth in *Neurospora crassa*

Olga Alicia Callejas-Negrete, Alejandra Irene Hernández-Saiz, and Rosa Mouriño-Pérez

Departamento de Microbiología. Centro de Investigación Científica y de Educación Superior de Ensenada. Carretera Ensenada-Tijuana No. 3918, Zona Playitas, C.P. 22860, Ensenada, B.C. México

Phospholipid flippases are transmembrane proteins that translocate specific population of phospholipids from the luminal to the cytosolic leaflet of the plasma membrane and have been associated with apoptosis signaling, protein sorting, vesicular traffic, and secretion. The *dnf-4* gene of *Neurospora crassa* has high identity with the *neo-1* gene of *Saccharomyces cerevisiae* that is essential and belongs to a highly conserved subfamily of P-type ATPase. To understand the cellular dynamics and functions of DNF-4 in *N. crass*a, we used live-cell imaging methods to record and analyze growing hyphae after labeling these proteins with sGFP and also examined the deletion mutant Δ*dnf-4*. DNF-4-GFP was present as bright spots, apparently surrounding the endoplasmic reticulum and the Golgi. The fluorescent spots moved in anterograde and retrograde fashion; some of them with the cytoplasmic bulk flow, but some others moved at a higher seed, probably driven by motors associated to microtubules, as was shown with the anti-microtubule drug (benomyl) experiments. Additionally, we observed vacuole-like structures, containing smaller membranous compartments moving towards the hyphal tip. The Δ*dnf-4* mutant had a decrease of 41% in the elongation rate, 30% in biomass production and 76% in conidia production ($p<0.05$). Branching rate was three-fold higher in the Δ*dnf-4* mutant ($p<0.05$). Mature hyphae showed a small and unstable Spitzenkörper. Hyphal morphology was affected, presenting a meandering profile. The mutation *dnf*-4 gene in *N. crassa* is not essential but strongly affects the growth rate, increase the frequency of branching and reduce significantly conidial production.

1.4 *gul-1*, a suppressor of *cot-1*, encodes an RNA-binding protein which is involved in cell wall remodeling

Inbal Herold[1], David Kowbel[2], Diego L. Delgado-Alvarez[3], Marisela Garduno-Rosales[3], Rosa R. Mouriño-Pérez[3] and Oded Yarden[1]

[1]Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jeruslaem, Rehovot 7610000, Israel, [2]Department of Plant and Microbial Biology, UC Berkeley, USA and [3]Departamento de Microbiología, CICESE (Centro de Investigación Científica y Educación Superior de Ensenada), Mexico

COT1 is the founding member of the highly conserved nuclear Dbf2-related (NDR) Ser/Thr kinase family and plays a role in the regulation of polar growth and development in *Neurospora crassa* and other fungi. COT1 interacts with several proteins, including the co-activators MOB2A/B, subunits of the heterotrimeric phosphatase PP2A and the downstream effector GUL1 (the homologue of the yeast Ssd1p). Under standard growth conditions GUL1 is mostly dispersed within the cytoplasm yet also produces aggregates that exhibit high mobility within the cell, which is dependent on a functional cytoskeleton. Some of these aggregates are associated with nuclei and in the proximity of the cell wall. Under stress conditions, a significant increase in GUL1 aggregate association with nuclei was observed. Inactivation of *gul-1* partially suppresses the *cot-1* phenotype along with curbing the increased (20-250%) expression of chitin synthase (*chs*) 1-7, glucan synthase 1 (*fks-1*) and the chitinase *gh18-5*. Overall, the expression of over 500 genes related to the COT1 pathway is altered in a *gul-1* background, including those involved in cell wall integrity, amino acid and nitrogen metabolism as well as over 250 genes who encode hypothetical proteins. Results of RNA antisense purification (RAP) and RNA immunoprecipitation (RIP) experiments show that GUL1 is a bona fide RNA-binding protein and can physically associate with several RNA species.

1.5 Formation of cell wall melanin in *Neurospora crassa*

Jie Ao, Sumit Bandyopadhyay, and Stephen J. Free

Department of Biological Sciences, SUNY University at Buffalo, Buffalo, NY, USA

Neurospora crassa contains all four enzymes for the synthesis of DHN (dihydroxynaphthalene) the substrate for melanin formation. We show that the DHN melanin pathway functions during *N. crassa* female development to generate melanized cell walls in peridum and ascospore cells. The *N. crassa* contains one polyketide synthase gene (*per-1*/NCU03584), two polyketide hydrolase genes (*pkh-1*/NCU01903 and *pkh-2*/NCU05821), two THN (tetrahydroxynaphthalene) reductase genes (*pkr-1*/NCU09390 and *pkr-2*/NCU06905) and one scytalone dehydratase (*scy-1*/NCU07823). We show that the *per-1*, *pkh-1*, *pkr-1*, and *scy-1* are required for melanization of the developing ascospores. We also identified the laccase that functions in the conversion of DHN into melanin via a free radical oxidative polymerization reaction, and have named the gene *lacm-1* (laccase for melanin formation-1/NCU02201). In maturing perithecia, we show that LACM-1 is localized to the peridium cell wall space while the DHN pathway enzymes are localized to intracellular vesicles. We present a model for melanin formation in which melanin is formed within the cell wall space and the melanized cell wall structure is similar to "reinforced concrete" in that the cell wall glucan, chitin, and glycoproteins are encased within the forming melanin polymer. This arrangement provides for a very strong and resilient cell wall and protects the glucan/chitin/glycoprotein matrix from digestion from enzymes and damage from free radicals.

1.6 Innate immunity proteins mediate allorecognition and cell death in *Neurospora crassa*

Jens Heller, Asen Daskalov, and N. Louise Glass

Koshland Hall 341, University of California at Berkeley

In plants and metazoans, intracellular receptors that belong to the NOD-like receptor (NLR) family recognize pathogen associated molecular patterns and are major contributors to innate immunity. In response to infection or danger signals some animal NLRs trigger signaling cascades eventually activating gasdermins, which induce cell death by generating membrane pores (pyroptosis).

Filamentous fungal genomes contain large repertoires of genes encoding for proteins with similar architecture to plant and animal NLRs with mostly unknown function. Here, we identify and molecularly characterize PLP-1, an NLR-like protein containing an N-terminal patatin-like phospholipase domain, a nucleotide-binding domain (NBD), and a C-terminal tetratricopeptide repeat (TPR) domain. PLP-1 guards the essential SNARE protein SEC-9; genetic differences at *plp-1* and *sec-9* function to trigger allorecognition and cell death in *Neurospora crassa*. Analyses of *Neurospora* population samples revealed that *plp-1* and *sec-9* alleles are highly polymorphic, segregate into discrete haplotypes, and show trans-species polymorphism, all of which are signatures of balancing selection. We also observed these signatures for a fungal gasdermin-like protein, RCD-1. Upon fusion between cells bearing incompatible *sec-9* and *plp-1* alleles or incompatible RCD-1 alleles, allorecognition and cell death are induced.

Our data indicate that fungal proteins involved in allorecognition function similar to immune receptors/executers in plants and animals, showing that these proteins are major contributors to innate immunity in plants and animals and for allorecognition in fungi.

1.7 Spatiotemporal regulation of a core clock gene products in *Neurospora crassa*

B.M. Bartholomai[1], A.S. Gladfelter[2], J.J. Loros[3], J.C. Dunlap[1]

[1]Department of Molecular and Systems Biology, Geisel School of Medicine at Dartmouth, Hanover, NH, [2]Department of Biology, University of North Carolina, Chapel Hill, NC, [3]Department of Biochemistry and Cell Biology, Geisel School of Medicine at Dartmouth, Hanover, NH

Over the past few decades, the molecular clock of *Neurospora crassa* has been well defined at the population level. However, little is known about the spatiotemporal dynamics of core clock genes and their products in individual hyphae. Advances in imaging techniques and instruments finally allows us to investigate how, when, and where core clock components are trafficked and regulated at the cellular level. Recently, it has been reported that the clock is spatially constrained within a mycelium. Is it possible that spatial regulation of the clock begins immediately after transcription? In other fungi, we know that mRNA can be sequestered and targeted by forming phase separated compartments with intrinsically disordered proteins. To explore whether or not such a phenomenon might be involved in clock regulation, we set out to investigate whether or not *frequency (frq)* mRNA is non-randomly positioned in the cytoplasm, using single molecule RNA FISH coupled with quantitative image processing. The image analysis suggests that there is clustering of *frq* that is not due to chance. We are currently investigating what factors are involved in facilitating this clustering to better understand the spatiotemporal dynamics of *frq* throughout the circadian cycle.

1.8 FungiDB: An integrated functional genomics database resource

Evelina Basenko[1] and David Roos[2] … on behalf of the EuPathDB project

[1] Centre for Genomic Research, Functional & Comparative Genomics, Univ Liverpool, UK
[2] Department of Biology, Univ Pennsylvania, Philadelphia PA, US

FungiDB.org is a free online resource designed to facilitate the mining and functional analysis of genomic-scale fungal and oomycete datasets. The parent EuPathDB project supports and integrates diverse information, including:

- genome sequences and population-level diversity data on microbial eukaryotes, including >200 fungal taxa (both pathogenic and non-pathogenic species)
- diverse functional genomics information, generated on multiple platforms: chromatin marks, transcriptomes, proteomes, metabolomes, genome-wide phenotypic information, *etc*
- information on subcellular localization, structure, molecular interactions, metabolic pathways and signaling networks, immunogenicity, reagents, publications, *etc*
- automated analyses (InterPro scan, orthology predictions, *etc*), along with annotations and experimental metadata contributed by community experts and/or professional curators

'User Workspaces' permit researchers to analyze their own data using Galaxy workflows, in the context of publicly-accessible datasets, *e.g.* RNAseq mapping & differential expression analysis, SNP calling from user-provided isolate sequences, *etc*. Analytical tools and sophisticated 'Search Strategies' support *in silico* queries against a wealth of integrated data and ancillary information, encouraging scientists to ask their own questions, rather than merely browsing pre-computed results. These resources are visited by >75K unique users per month, from >100 countries, and have been cited >13K times in the scientific literature to date. FungiDB is relatively new, but the fastest growing component of EuPathDB: usage has more than doubled over the past year, to ~7K unique users/mo & 150 citations/yr.

FungiDB supports exploration of various *Neurospora* genomes (*N. crassa*, *N. discreta*, *N. tetrasperma*), including functional genomic and phenotypic information from the *Neurospora* genome project and knockout collection. >16K images, corresponding to 1234 *N. crassa* genes, have recently been integrated, *cf:* http://fungidb.org/fungidb/app/record/gene/NCU00007#phenomics. FungiDB also accommodates community-driven submission of images, phenotype descriptions, supporting files, PubMed records, *etc* as 'User Comments', which become immediately visible and searchable; such comments have been attached to 379 *N. crassa* genes to date.

Interested in joining FungiDB advisory calls or nominating a dataset for integration? Visit https://fungidbcommunity.wordpress.com/ Additional questions? email help@FungiDB.org FungiDB is supported in part by NIH #HHSN272201400030C and Wellcome Trust #WT108443MA

Session 2

2.1 Cooperative dynamics underlie Neurospora spore germination

Marcus Roper, Claire Chang, Qiang Fei, Sierra Foshe / UCLA
Ariel Fitzmorris / Santa Monica Community College
George Stepaniants / U. Washington

The advantages that cooperation confers upon microbes, and how cooperation is maintained when selection favors selfish behaviors, is a central puzzle in the emergence of multicellular life. Although there are now many theories for the conditions under which cooperation can evolve, few of them have been quantitatively tested against experiments. We revisit the question of how Neurospora conidial germination rates depend on interactions between spores, using microfluidics and automated image analysis. We focus on quantifying two forms of spore cooperation: 1. Rates of fusion and subsequent growth among spores that are somatically incompatible, but auxotrophically interdependent. 2. The effect of spore concentration and genotype upon the rates at which growing germlings attain exponential growth. Our data, taken together, reveal that conidial germination is shaped by a suite of sophisticated cooperative effects, including the ability to identify and count nearby genetically similar spores.

2.2 Transcript structure, expression, and localization of the *Neurospora crassa* sexual development regulator *fsd-1*

Elizabeth Hutchison, Thomas Hurysz, Natalie Craig, Kathryn Emmens, Bryce Gebhardt, Mary Pyatt, and Christine Toufexis

SUNY Geneseo Biology Department, 1 College Circle, Geneseo, NY 14454

FSD-1 is a *Neurospora crassa* transcription factor that controls both early and late stages of sexual development. Mutants in *fsd-1* are sterile and previous work showed that *fsd-1* regulates formation of female sexual tissues and spore maturation. Intron and exon boundaries of *fsd-1* were mapped using reverse transcription (RT)-PCR and sequencing, and we determined that *fsd-1* is transcribed into three transcripts which differ in length and intron/exon structure. RT-qPCR during a time course of sexual development showed that expression of *fsd-1* transcripts is not detectable until *N. crassa* initiates sexual development. In addition, one transcript variant is predominantly expressed during sexual development, and expression of this transcript peaks approximately five days after crossing. We also monitored FSD-1 protein expression using immunoprecipitation and western blotting and observed one protein band, consistent with our data that only one transcript is primarily expressed. To investigate FSD-1 localization, we constructed a GFP-tagged version of FSD-1 using double-joint PCR and targeted this construct to the native locus. FSD-1-GFP localization was monitored via fluorescence microscopy throughout a time course of development. We found that FSD-1-GFP localized to the nuclei of female reproductive tissues, such as paraphyses, but not to tissues where meiosis occurs. In addition, we confirmed that the FSD-1-GFP strain complements the sexual development-related phenotypes that occur in an *fsd-1* knockout strain, such as sterility and lack of ascospore melanization. Finally, we constructed strains where *fsd-1* has an overexpression promoter, and we will determine whether overexpression of *fsd-1* has a dominant negative effect on wild type strains during mating.

2.3 Ascospore *sleepless-1* and *-2* are required for ascospore dormancy in *Neurospora crassa*

Miki Uesaka, Ayumi Yokoyama and Shinji Honda

Faculty of Medical Sciences, University of Fukui, Fukui, Japan

The heat-resistance ascospores of the filamentous fungi Neurospora break their semi-permanent dormancy by heat shock or smoke water containing furfural converted from the plant compound xylose by burning vegetation. The mechanisms of dormant maintenance, heat/smoke activation and their relationship, however, remain enigmatic. Taking advantage of the Neurospora knockout collection, we isolated mutants spontaneously germinating without heat shock, named ascospore sleepless-1 and -2 (*as-1* and *as-2*). We showed that AS-1 controls an ascospore-specific MAPK pathway and mutants lacking the pathway lose ascospore dormancy. Furthermore we demonstrated that the pathway keeps activated during semi-permanent dormancy and is aggressively inactivated by heat stimuli, supporting the importance of dormant maintenance. We also identified a smoke-activated G protein signaling by isolating mutants insensitive to the less-toxic furfural derivative furfuryl alcohol and revealed that AS-2 negatively regulated the signaling. We confirmed that furfuryl alcohol stimuli via the G protein signaling regulates the tight threshold temperature of dormant break in a dose-dependent manner. We conclude that ascospore dormancy break by heat and smoke is finely controlled by at least two major pathways in Neurospora.

2.4 MOB2A Tyr117 and Tyr119 are required for interactions with the NDR kinase COT1 and for proper sexual development

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MOB (MPS-1 binding) proteins act as activating subunits which are required for NDR kinase function. In *N. crassa*, MOB2A and MOB2B have been shown to have overlapping functions. Both MOB2 proteins physically and genetically interact with COT1, a Ser/Thr kinase that is involved in the regulation of hyphal polarity and branching. Phosphorylation has been suggested to play a role in the regulation of MOB function. Using mass spectrometry analysis we showed that two Tyr residues (Tyr117 and Tyr119) of MOB2A can potentially undergo phosphorylation. These residues were altered by site directed mutagenesis to produce mutants harboring two Phe or Glu residues (mimicking the putative unphosphorylated or constantly phosphorylated MOB2A forms, respectively). *mob-2a(Y117E,Y119E)* in a Δ*mob-2b* background is a temperature-sensitive mutant that exhibited slow growth with extreme hyperbranching at 34°C (optimal for the wild type). We have also determined that MOB2 proteins negatively regulate conidial germination and that even though MOB2A and MOB2B have some overlapping functions, MOB2B cannot compensate for MOB2A's role in conidiation and germination. In addition to their role in a-sexual development, MOB2A/B are also involved in different stages during the sexual reproductive cycle. Mutating MOB2A/B reduced protoperithecia formation. Impaired, developmental was also evident 6 days after fertilization. In 11 day old perithecia of the mutants, asci were disorganized within the perithecial cavity. Furthermore, while shot ascospores were viable, their length was abnormal (ranging about 5-9µm versus 6-7µm in the wild type). Altering Tyr117 and Tyr119 residues also affected the physical interactions between MOB2A and COT1, as determined by yeast two hybrid analyses.

2.5 First report of a functional agmatinase in *Neurospora crassa* and its essentiality for cell survival

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Agmatinase is a metalloenzyme which hydrolyzes agmatine to produce putrescine and urea; being the alternative pathway to produce polyamines in bacteria, plants, and mammals. In fungi, only the constitutive pathway via ornithine decarboxylase (ODC) has been reported. In this study, we report for the first time the presence of agmatinase (AGM-1) (NCU 01348) in the filamentous fungus *Neurospora crassa*. AGM-1 seems to have an essential role besides being an alternative pathway of polyamine production since the null mutant Δ*agm-1* strain was unable to grow and the deficiency of AGM-1 in the heterokaryon strain provoked an affected phenotype. Interestingly, the intracellular localization of the enzyme led us to hypothesize that AGM-1 could be involved in the regulation and stability of the actin cytoskeleton, an essential component for cell survival. The enzymatic character of AGM-1 was confirmed by an enzymatic assay with the purified enzyme from *N. crassa*, which displayed enzymatic activity. Therefore, the results presented here provide robust evidence about the existence and importance of AGM-1 in the filamentous fungus *N. crassa*.

2.6 Association of fungal MSH1 protein to mitochondrial DNA is critical for lifespan in Neurospora

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Among the various eukaryotic MSH (MutS homolog) proteins, fungal MSH1 possesses unique characteristics regarding mitochondrial genome maintenance. A knockout of the *msh1* gene in *Neurospora crassa* ($msh1^{KO}$) caused very early cessation of hyphal growth accompanied by accumulation of aberrant mitochondrial DNA (mtDNA). In the $msh1^{KO}$ strain, large-scale mtDNA deletion immediately occurred at the beginning of the inoculation passage and branched moieties of mtDNA appeared as well. According to the polypeptide sequence, MSH1 belongs to the MutS-II family, members of which are believed to suppress genetic recombination by binding to DNA. In order to verify that the MSH1 protein did indeed bind to the DNA, we tried to express the entire recombinant MSH1 protein in *E. coli* and *Pichia* but failed. However, we were able to determine, by Electrophoresis Mobility Shift Assay, that a partial version of recombinant MSH1 domain I, not domain V, bound to branched DNA such as Holliday-junction mimic and fork-shaped DNA substrates. We also observed that truncating the mitochondrial localization signal in the N-terminus of MSH1 produced phenotypes similar to the $msh1^{KO}$ strain, *i.e.* short life span and mtDNA aberration, and saw that the protein failed to localize to mitochondria. We conclude that MSH1 protein may stabilize mtDNA by participating in suppression of its recombination, and that a defect in this function may cause mitochondrial dysfunction resulting in a shortened life span.

2.7 Reverse engineering of enzyme hypersecretion in filamentous fungi

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Fungal carbohydrate active enzymes (CAZys) are vital for various industrial applications such as the biorefinery of renewable plant biomass. With the advent of high throughput strain engineering, automated screening and advanced genetic engineering through CRISPR/Cas9, current limitations lie in the identification of promising target genes and genetic mechanisms for improving enzyme secretion. Historically, most hypersecreting fungal strains were created through random mutagenesis and extensive screening. Despite genome resequencing of several mutant strains, the responsible mutations could not be pinpointed in many cases. The *Neurospora crassa* UV mutant *exo-1* is a hypersecreter of amylases, invertase and pectinases. Here, we report the successful identification of the causative mutation through genome resequencing and genetic tests: a premature stop in the F-box protein-encoding gene *frp-1*. As known for *exo-1*, high invertase, amylase and pectinase activity was found in the corresponding deletion strain Δ*frp-1* upon sugar depletion. Moreover, the *exo-1*/Δ*frp-1* phenotype could be successfully reverse-engineered into the industrially employed fungus *Myceliophthora thermophila* through CRISPR/Cas9.

The Δ*frp-1* phenotype was further characterized with enzyme assays and RNA-Seq in *N. crassa*. Measured amylase and invertase activities in the absence of any inducer exceeded activities in the carbon catabolite derepressed strain Δ*cre-1*, while glucose-repression was still functional in Δ*frp-1*. Transcriptomics analysis detected high induction of 18 CAZy genes and 8 sugar transporters in the mutant strain in no carbohydrate and Avicel medium, while, surprisingly, a large number of other CAZys failed to be expressed at wild type levels. Moreover, high up-regulation of amino acid, organic acid and sugar metabolism was found in Δ*frp-1* compared to the wild type.

Aiming to elucidate the underlying mechanism of hyper-derepression, we found the high expression of amylases and invertase, but not pectinases, in the Δ*frp-1* mutant to be completely dependent on the transcriptional regulator COL-26. Current efforts are focusing on uncovering additional factors involved using a proteomics approach and further genetic studies.

2.8 Real-time *in-vivo* metabolomics of *Neurospora crassa*

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Metabolomics relies on analytical methods to provide holistic information about metabolites, their distributions across samples, and the connections underlying the dynamic properties of metabolism. While a temporal dimension of metabolomics data has been difficult to obtain, recent methods have allowed for collection of densely sampled time series on populations of single cells. We extend High-Resolution-Magic Angle Spinning (HR-MAS) NMR as a flexible and untargeted way of conducting *in-vivo* measurements of metabolism in real time, and collected time-resolved metabolomes for *Neurospora crassa* under aerobic and oxygen-limited conditions. We find that this method requires virtually no sample preparation and allows for continuous collection of data over more than 11h at ~4-min temporal resolution with little noise. Additionally, our study reveals time-dependency in known connections between central carbon metabolism, amino acid metabolism, energy storage molecules, and structural molecules in *N. crassa*. Real-time *in-vivo* metabolomics is rapid and simple, and is easily extensible to different organisms, giving it the potential to drive improved kinetic models for diverse biological systems.

Session 3

3.1 Cap-binding proteins mediate meiotic silencing by unpaired DNA

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In *Neurospora crassa*, cross walls between individual cells are normally incomplete, making the entire fungal network vulnerable to attack by viruses and selfish DNAs. Accordingly, several genome surveillance mechanisms are maintained to help the fungus combat these repetitive elements. One of these defense mechanisms is known as meiotic silencing by unpaired DNA (MSUD), which is an RNA silencing system that identifies and silences unpaired genes during meiosis. The silencing process begins in the nucleus, where single-stranded aberrant RNAs (aRNAs) are made from any gene lacking a pairing partner. These aRNAs are then exported to the perinuclear region, where the meiotic silencing complex (MSC) converts them into small interfering RNAs (siRNAs) that can target homologous messenger RNAs (mRNAs). In this study, we have identified another silencing component known as the cap-binding complex (CBC). Made up of cap-binding proteins CBP20 and CBP80, CBC associates with the 5' cap of mRNA transcripts in eukaryotes. CBC interacts with MSC, directly linking the two cellular factors.

3.2 Recombination-independent recognition of DNA homology for Repeat-Induced Point mutation and meiotic silencing by unpaired DNA

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Pairing interactions between apparently intact homologous chromosomal segments are ubiquitous in nature. The general mechanism(s) by which DNA sequence homology can be recognized in such situations, i.e., in the absence of DNA breakage and recombination, represent(s) a fundamental unanswered question in chromosomal biology. Using the process of Repeat-Induced Point mutation (RIP) in *Neurospora crassa* as a model system, we previously showed that DNA homology can be sensed by a new mechanism that (i) does not depend on the canonical recombination-mediated pathway, and (ii) likely involves direct interactions between homologous double-stranded DNA molecules. In our ongoing work, we now find that RIP shares its homology-recognition principles with Meiotic Silencing by Unpaired DNA (MSUD). Taken together, these findings raise a possibility that recombination-independent pairing interactions between intact DNA double helices represent a general, perhaps fundamental, mode of DNA homology recognition.

3.3 The *Neurospora crassa* Oak Ridge (OR) background exhibits an atypically efficient meiotic silencing by unpaired DNA

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The meiotic silencing by unpaired DNA (MSUD) discovered in the OR genetic background of *Neurospora crassa* efficiently silences any unpaired gene during meiosis. Interestingly, we found that most strains of non-OR background exhibit a modulated MSUD response where the silencing is not all-or-none like in OR. We created new MSUD testers in the novel B/S1 genetic background derived from Bichpuri-1 *a* and Spurger-3 *A* wild-isolated *N. crassa* strains and found that the tester-heterozygous crosses are inefficient for MSUD (produce only ~25% of round ascospores) as compared to the same crosses in OR (>97%). MSUD was also inefficient in *N. tetrasperma* (7%). Possibly, the MSUD response is adjusted to multiple other cues in the non-OR backgrounds. Loci on chromosome 1, 2 and 5 appear to harbour genes determining the OR vs B/S1 difference, identification of these genes will enable us to understand the molecular basis of such modulation. Inefficient MSUD relieves the barren phenotype in *Dp*-heterozygous crosses, presumably making *Dp*-mediated RIP suppression significant in non-OR backgrounds. We also found the occasional presence of >8 nuclei in the ascus during ascospore packaging.

3.4 The impact of modulating translation initiation, elongation, and termination on gene expression

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The central dogma of molecular biology, generally stated, is that DNA makes RNA (transcription), and RNA makes protein (translation). Studies of model fungi have been instrumental in establishing the strategies and mechanisms that eukaryotes use to control transcription and translation. While the importance of transcriptional control is universally acknowledged, there is now a deepening and widening appreciation of the diverse roles of translation in controlling gene expression. For example, it is now apparent that upstream open reading frames (uORFs), once thought of as rarities, are present in more than 25% of eukaryotic mRNAs, and that the translation of these uORFs can be critical for controlling gene expression levels. It is now apparent that initiation of translation of these uORFs - as well as initiation of translation of the major predicted gene products of mRNAs – can occur at codons other than AUG codons, and these non-AUG initiation events have functional significance. The rate of translation elongation can also be modulated at the level of specific mRNAs by codon-choice or by encoded nascent peptides that stall ribosomes by affecting the function of the ribosome's peptidyltransferase center. Initiation and elongation can both be modulated by controlling the activity of the translation machinery. Events associated with translation termination can have regulatory functions critical for controlling gene expression and also can have a major impact on mRNA stability through the mRNA quality control pathway known as nonsense-mediated mRNA decay (NMD). Here we discuss these processes of translational control and focus on the impact of translational control in *Neurospora crassa*'s regulatory response to inositol availability.

3.5 Codon usage biases co-evolve with transcription termination machinery to suppress premature cleavage and polyadenylation

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Codon usage biases are found in all genomes and influence protein expression levels. The codon usage effect on protein expression was thought to be mainly due to its impact on translation. Here, we show that transcription termination is an important driving force for codon usage bias in eukaryotes. Using *Neurospora crassa* as a model organism, we demonstrated that introduction of rare codons results in premature transcription termination (PTT) within open reading frames and abolishment of full-length mRNA. PTT is a wide-spread phenomenon in Neurospora, and there is a strong negative correlation between codon usage bias and PTT events. Rare codons lead to the formation of putative poly(A) signals and PTT. A similar role for codon usage bias was also observed in mouse cells. Together, these results suggest that codon usage biases co-evolve with the transcription termination machinery to suppress premature termination of transcription and thus allow for optimal gene expression.

3.6 Control of gene regulation by Polycomb Repressive Complex 2 and its partners

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Polycomb Group (PcG) proteins generate facultative heterochromatin by trimethylating histone H3 lysine 27 (H3K27me3). Members of the conserved Polycomb Repressive Complex 2 (PRC2) include the H3K27 methyltransferase, KMT6 (*Neurospora crassa* SET-7), and its binding partners SUZ12, EED, and –at least sometimes– MSL1 (*Nc*NPF). In humans, mutation of PRC2 components result in developmental defects, inherited diseases and sporadic cancers. Deletion of core PRC2 genes in both *N. crassa* and *Fusarium graminearum* leads to complete loss of H3K27me3, and is accompanied by pleiotropic developmental defects in *Fusarium*, but deletion of $msl1^{npf}$ has no such drastic effects. Here we report on *in vivo* and *in vitro* effects of PRC2 subunit mutations. Minor changes in the primary sequence of KMT6 resulted in complete or intermediate loss of function. Cytology and ChIP-seq showed partial mislocalization of KMT6-GFP in some of these strains. Several mutations affected the allosteric regulation of KMT6 by the EED or SUZ12 subunits. To uncover suppressors of H3K27me3 silencing, and to identify functional equivalents of PRC1 (an animal complex that binds H3K27me3 yet does not exist in fungi), we developed a forward genetics approach, relying on de-repression of a *neo* reporter gene in a reliably silenced region. Dozens of primary mutants, called *defective in silencing* (*dis*) or *drug response attenuated* (*dat*), were classified by distinct growth phenotypes and global gene expression patterns evidenced by transcriptome sequencing. Mutations in *dis* and *dat* genes were identified by bulk segregant analyses followed by sequencing. Here we report on studies on the *Neurospora* and *Fusarium* DIS2 protein, a novel component of the PcG silencing system.

3.7 Nucleosome positioning by DIM-1 prevents aberrant DNA methylation in Neurospora

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In the filamentous fungus *Neurospora crassa*, constitutive heterochromatin is marked by tri-methylation of histone H3 lysine 9 (H3K9me3) and DNA methylation. We identified mutations in the Neurospora *d*efective *i*n *m*ethylation-1 (*dim-1*) gene that cause defects in cytosine methylation and implicate a putative AAA-ATPase chromatin remodeler. Although it was well established that chromatin remodelers can impact transcription by influencing DNA accessibility with nucleosomes, little was known about the role of remodelers on chromatin that is normally not transcribed, including regions of constitutive heterochromatin. We found that *dim-1* mutants display both reduced DNA methylation in heterochromatic regions and increased DNA methylation, and H3K9me3, in some intergenic regions associated with highly expressed genes. Deletion of *dim-1* leads to atypically-spaced nucleosomes throughout the genome and numerous changes in gene expression. DIM-1 localizes to both heterochromatin and intergenic regions that become hypermethylated in *dim-1* strains. We show that cytosine methylation occurs predominantly on linker DNA and suggest that nucleosome disorder limiting available linker DNA in *dim-1* strains may account for the hypomethylation of constitutive heterochromatic sequences. Our findings indicate that DIM-1 normally positions nucleosomes in both heterochromatin and euchromatin and that the standard arrangement and density of nucleosomes is required for proper heterochromatin machinery function.

3.8 ASH-1-catalyzed H3K36 methylation drives repression and marks H3K27me2/3-competent chromatin

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Methylation of histone H3 at lysine 36 (H3K36me), a widely-distributed chromatin mark, largely results from association of the lysine methyltransferase (KMT) SET-2 with RNA polymerase II (RNAPII), but most eukaryotes have one or more additional H3K36me KMTs that act independently of RNAPII, including the orthologs of ASH-1, which are conserved in animals, plants, and fungi but whose function and control are poorly understood. We found that *Neurospora crassa* has just two
H3K36 KMTs, ASH-1 and SET-2, and were able to explore the function and distribution of each enzyme independently. While H3K36me deposited by
SET-2 marks active genes, inactive genes are modified by ASH-1 and its activity is critical for their repression. ASH-1-marked chromatin can be further modified with overlapping domains of H3K27me, and ASH-1 catalytic activity modulates the accumulation of H3K27me2/3 both positively and negatively. These findings provide new insight into ASH-1 function, H3K27me2/3 establishment, and repression in facultative heterochromatin.

Session 4

4.1 Identification of a genetic element required for spore killing in Neurospora

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Standard Mendelian genetics dictates that each allele in a sexual cross has an equal probability of being inherited by the proceeding generation. Meiotic drive elements are "selfish" genetic elements that are able to bias inheritance frequency in their favor. An example of a meiotic drive element is Neurospora *Spore killer-2* (*Sk-2*). Crosses of *Sk-2* × Sk^S (*Spore killer*-sensitive) produce asci with four black, viable ascospores and four white, inviable, "killed" ascospores. The four surviving ascospores almost always inherit the *Sk-2* element, resulting in a nearly 100% biased transmission of *Sk-2* to the surviving population. Previous work has identified one gene, *rsk* (*resistant to Spore killer),* and one locus, *rfk-1* (*required for killing*), that are involved in the spore killing mechanism. Here we identify a 1481 bp region within the previously identified 45 kb *rfk-1* locus that is associated with spore killing. Deletion of this 1481 bp region from *Sk-2* results in the loss of spore killing. In a *Sk-2* strain that has lost the ability to kill (ISU-3211), this 1481 bp region contains seven point mutations, one of which (G28326A) we found to be sufficient for the loss of spore killing. A potential start codon for the RFK-1 protein was identified 62 bp upstream of the G28326A mutation. Spore killing occurs normally when a non-native promoter is fused to this start codon, suggesting that *rfk-1* encodes a protein of at least 39 amino acids. We also show that the *rfk-1* gene has evolved from Neurospora gene *ncu07086*. The significance of this finding with respect to the killing mechanism will be discussed.

4.2 Comparative studies on chromosome structure and gene silencing in fungi

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Chromatin, i.e. the collection of DNA, RNA and proteins that forms the material for chromosomes, is not static over the cell cycle or even during gene expression events that measure on the second scale. How changes in chromatin structure are regulated to generate stable chromosomes yet allow rapid access to the DNA for transcription is still largely unknown. We focus on the interplay between protein complexes that are involved in gene activation (Trithorax Group proteins; TrxG) and gene silencing (Polycomb Group proteins; PcG), and for this purpose have studied these complexes and the histone modification marks they generate (H3 lysine 4 and lysine 27 methylation, respectively) in several taxa. While we found underlying similarities, we show that major aspects of chromatin biology are not conserved, even in relatively closely related fungi. Here we will report on findings with *Neurospora*, *Fusarium*, and *Zymoseptoria* species and discuss effects of H3K27me3 and H3K9me3 distribution on genome stability.

4.3 Developing a high-throughput functional genomics platform for filamentous fungi

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Advances in sequencing technology that allow for rapid and inexpensive whole-genome sequencing highlight how few genes have been functionally characterized. This problem is particularly acute in fungal biology, where even in the best studied organisms upwards of half of genes are annotated as hypothetical. High-throughput tools to identify gene function exist for planktonic organisms, such as yeast and bacteria. However, filamentous fungi present a number of challenges to high-throughput gene characterization, including multinucleate cells and asexual fusion. We are currently working to overcome these challenges and develop high-throughput functional genomics tools for filamentous fungi using the model fungus *Neurospora crassa*. To assign roles to novel genes, we will transform fungal cells with barcoded cassettes, which are stably inserted into genomic DNA. Using high-throughput sequencing, we will associate barcodes with insertion locations. We can then expose these barcoded fungal libraries to experimental conditions. Quantifying relative barcode abundance will allow us to determine the relative importance of disrupted genes in experimental conditions and assign functions to hypothetical genes. Generating barcoded insertional mutagenesis libraries in a number of fungal systems will enable the rapid characterization of gene function and allow us to expand our understanding of fungal biology.

4.4 Lessons from high-throughput functional genomics analyses in *Neurospora crassa*

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Filamentous fungi are important for carbon recycling in the biosphere, as devastating plant pathogens and largely opportunistic animal pathogens, and as model systems for aspects of mammalian biology. *Neurospora crassa* is a major model organism for filamentous fungi. Available tools include a sequenced and annotated genome, 9,758 protein-coding genes and a nearly complete gene knockout strain collection. Phenotypic data for 10 different growth or developmental attributes have been obtained for nearly 1300 mutants in *N. crassa*. Much of the data has been collected by undergraduates during summer programs or while enrolled in research-based courses. Large groupings of mutants that have been analyzed include transcription factors, serine-threonine protein kinases, protein phosphatases and G protein coupled receptors. In many cases, chemical sensitivity or nutritional phenotypes are also available. We have employed statistical clustering approaches to assign mutants to different groupings based on their growth and developmental phenotypes. Publically available data from RNAseq analysis and patterns of Mitogen-Activated Protein Kinase and cAMP-dependent Protein Kinase-mediated phosphorylation of protein targets in *N. crassa* and other closely related filamentous fungi are being incorporated to confirm clustering relationships. The overall goal is to identify genes that are new members of identified or unknown pathways.

4.5 Genetics of thermotolerance in *Neurospora discreta*

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Differences in maximal growth temperature among *Neurospora discreta* isolates from the western United States correlate with differences in mean annual environmental temperature. Isolates from New Mexico and Alaska exhibit comparable growth rates below 35°C, while isolates from New Mexico grow much better near and above 40°C. Individual progeny from crosses between isolates from New Mexico and Alaska either possess one of the two parental temperature phenotypes or have an intermediate phenotype. The range of progeny phenotypes suggests the involvement of multiple gene regions. With support from the DOE Joint Genome Institute (JGI) Community Science Program, we obtained complete genome sequences for 82 progeny that strongly resembled either the New Mexico parental temperature phenotype or the Alaska parental phenotype (39 NM-like and 43 AK-like progeny). High-quality genome assemblies of the parental strains were obtained utilizing sequence data from both Illumina (JGI) and Oxford Nanopore MinION platforms. Comparative analyses of genomes from these two progeny pools demonstrate that a region on chromosome III plays a major role in determining whether strains grow well above 40°C, while suggesting a secondary role for a region on chromosome I.

4.6 Geography and demography of clonal and sexual populations of Neurospora

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The study of wild populations of *Neurospora* has yielded insights into how microbial populations evolve and structure themselves. Yet the majority of *Neurospora* research has been done on a few, relatively similar species. We have employed the soil sampling methodology of Glass, Metzenberg, and Raju (1990) to collect populations of diverse *Neurospora* species from regions where collection had previously been dominated by *N. discreta*. Additionally, we identified strains of *Neurospora* from collections of endolichenic fungi and found them to be members of the same populations collected from soil. The addition of over a hundred *Neurospora* strains from species that previously existed as dark matter sheds light upon the mysterious ecology of this model. Moreover, from our population genomic analysis we find profound differences in population structure, demography, and evolutionary paradigm between these species. We find that a self-fertile breeding system necessitates clonal reproduction, but that the production of mitotic spores, while being associated with greater population structure, does not lead to dominantly clonal reproduction. However, the consequences of self-fertility may be mitigated by a system of alternating generations, as we find the well-mixed yet self-fertile *N. tetraspora* to be lichen associated in it's self-infertile haploid stage. These findings leverage new advances in sequencing to understand how distinct ecologies can affect evolutionary paradigms, and pave the way for applying the wealth of knowledge on model species of *Neurospora* to wider swaths of life.

4.7 Fungal growth suppression by bacteria emitted volatiles

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Fungi sense biotic and abiotic inputs from their environment. These signals are processed and elicit a reaction inducing defense, immunity, or growth genes. What molecules do fungi recognize and use to sense another organism? When fungi interact with other microbes they may change growth patterns, secrete antibiotics, or effectively ignore them altogether. Our experiments testing fungi and bacteria co-cultures noted growth suppression of some fungi including Neurospora. By physically separating the organisms but allowing them to share the same headspace of a Petri dish we demonstrated the inhibition is through volatilized molecules. Through published and our own Gas Chromatography–Mass Spectrometry profiling of the volatiles we identified several candidate compounds at high abundance in growth suppressing strains including one with approved usage in insect repellant. Tests of single compounds alone found several sufficient to induce similar levels of growth inhibition. Mutant strains of *N. crassa* that that failed to sense or be repressed by the compound were generated by mutagenizing conidia and selecting for escapers. Mapping of the mutant alleles after a backcross is underway. Gene expression of hyphae exposed to one of the most potent compounds was compared to unexposed colony to identify candidate genetic and enzymatic pathways induced by the chemical.

4.8 Team science and a cutting edge suite of analytical tools accelerate molecular analyses of Neurospora and other filamentous fungi

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Rapid advances in mass spectrometry, microscopy, cell sorting and DNA sequencing have the potential to accelerate fungal biology research. Two DOE National User Facilities, the Environmental Molecular Sciences Laboratory (EMSL) and the DOE Joint Genome Institute (JGI) offer researchers access via a peer reviewed proposal process to cutting edge analytical capabilities. A variety of molecular analysis capabilities such as mass spectrometry, NMR, light and electron microscopy, microfluidics and high performance computing are available from EMSL. These capabilities have been utilized to advance understanding of a variety of fungal bioprocesses including secondary metabolite production and the secretome. Next generation sequencing platforms available at the JGI were utilized to re-sequence over 550 *Neurospora crassa* Fungal Genetics Stock Center strains with mutant phenotypes that had not been associated with genomic mutations. Other studies ongoing at the JGI include "whole genus sequencing" of multiple ascomycete genera.

Session 5

5.1 Regulation of conidiation and carotenoid biosynthesis by the velvet complex in *Neurospora crassa*

Luis M. Corrochano, M. del Mar Gil-Sánchez, Sara Cea-Sánchez, Alejandro Miralles-Durán, Cristina De Andrés-Gil, Eva M. Luque, Guilherme TP Brancini, Gabriel Gutiérrez, and David Cánovas

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The *velvet* regulators are members of a family of proteins with a conserved domain that help to coordinate growth, differentiation and secondary metabolism in fungi. In *Neurospora* crassa the *ve-1* mutant has defects in aerial hyphal growth, conidiation and reduced carotenoid accumulation. We have detected the presence of VE-1, VE-2 and the methyltransferase LAE-1 in vegetative mycelia where they form a protein complex, and during conidiation. In addition, we noted that VE-1 was absent in aerial hyphae grown in the dark despite the presence of *ve-1* mRNA. The absence of VE-1 in aerial hyphae in the dark is due to protein degradation through the proteasome with a key role for the adaptor protein FWD-1. We propose that the light-dependent regulation of VE-1 stability modifies the components of the *velvet* complex resulting in changes in the transcriptome during conidiation.

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5.2 Intra- and interspecies communication in filamentous fungi

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In many filamentous fungi, germinating spores undergo mutual attraction and fusion, thereby forming a supracellular network, which further develops into the mycelial colony. Germling fusion in *Neurospora crassa* employs an unusual signaling mechanism, in which the two fusion partners take turns in signal sending and receiving. The highly coordinated cellular behavior is mediated by an intricate signaling network, which includes two MAP kinase cascades and the fungal specific SO protein. Function and activity of these proteins are highly dependent on their subcellular localization, indicated by the alternating membrane recruitment of the MAK-2 MAP kinase and SO during the tropic cellular interaction.

Recent studies identified SIP-1, a fungal specific protein of unknown function, as an interaction partner of SO. While both proteins co-localize during the cell-cell interaction, their dynamics differ in isolated non-interacting germlings. While SO is fully cytoplasmic under these conditions, SIP-1 localizes to the plasma membrane of the growing germ tube in an alternating manner comparable to the dynamics during cell fusion. These observations indicate that germlings rapidly alternate between two physiological stages, which are probably associated with the initiation of cell-cell interactions. In addition, we recently found that the cell-cell communication mechanism first described in *N. crassa* is highly conserved in the phytopathogenic grey mold *Botrytis cinerea*. During germling fusion, the MAK-2 and SO homologs of this fungus show dynamics identical to the ones observed in the red bread mold. Germinating spores of *N. crassa* and *B. cinerea* readily undergo interspecies interactions, suggesting that also the so far unknown signals and receptors are conserved. Interestingly, germling fusion in *B. cinerea* is fully absent during growth on plant surfaces, which induce pathogenic development, suggesting that cell fusion and host infection are two alternative, mutually exclusive developmental routes.

5.3 Regulation of the circadian clock of Neurospora by *frq* antisense transcription

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Expression of the core clock gene *frequency* (*frq*) of *Neurospora* is tightly regulated to maintain its temporal expression. A noncoding antisense transcript, *qrf*, completely overlaps the *frq* mRNA and has been shown to be an integral part of *frq* regulation. Here we show that the expression of *qrf* is regulated by two major environmental stimuli, light and glucose. The light-dependent expression of the antisense transcript is dependent on the clock activator, WCC. The glucose-dependent expression is driven by an unknown transcriptional factor and is regulated by the circadian repressor CSP1. The CSP1-dependent regulation generates a dusk-phased expression profile of *qrf* that is in antiphase to the WCC-dependent rhythm of *frq*. This antiphasic *qrf* rhythm is independent of transcription of the sense RNA and does not require regulation of *qrf* by WCC. *qrf* deficient strains are rhythmic, indicating that the antisense transcription is not required for the core clock function. However, in absence of *qrf* the phase of the circadian clock is delayed in a glucose and light-dependent manner. The mechanism allows fine-tuning *frq* expression in response to light and glucose levels. Mechanistically *frq* and *qrf* transcription mutually affect each other by promoter exclusion rather than by direct interference of sense and antisense transcription. Together, these results provide a better understanding of the regulation of the circadian clock by the antisense *frq* transcript.

5.4 Circadian clock regulation of mRNA translation in Neurospora

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Our long-term goal is to understand the fundamental mechanisms by which the circadian clock, important in human health and drug metabolism, regulates rhythmic gene expression and thus cell function and metabolism. Most of the focus on understanding clock control of gene expression has been at the level of transcription. However, in many systems, there are examples of specific proteins that show a circadian rhythm in levels, while levels of the associated mRNA are relatively constant throughout the day. This suggests that the clock may regulate mRNA translation, but which proteins cycle in abundance in cells over the day, and the mechanisms of clock control of translation, are not known. Using *Neurospora crassa* as a model organism, we discovered that the circadian clock, through regulation of specific kinases and phosphatases, regulates the phosphorylation state and activity of eukaryotic elongation factor 2 (eEF2) and cap-dependent eukaryotic initiation factor 2α subunit (eIF2α). Both eEF2 and eIF2α peak in activity during the night, coincident with reduced stress and high energy levels. Circadian ribosome profiling, coupled with transcriptome analyses from WT and eEF2 and eIF2α kinase mutants revealed that clock control of translation elongation and cap-dependent initiation leads to rhythmic translation of specific mRNAs, rather than acting globally. Work is currently in progress to identify and validate elements that confer specificity.

5.5 The Phospho-Code determining circadian feedback loop closure and output in Neurospora

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In the negative feedback loop driving fungal and animal circadian oscillators, negative elements (FRQ, PERs, CRYs) are believed to regulate their own expression by promoting the phosphorylation, and therefore inhibition, of their heterodimeric transcriptional activators (e.g. WC-1/WC-2 (WCC), BMAL1/CLOCK). However, contradictions exist and mechanistic details are lacking; in *Neurospora*, correlations between WCC activity and phosphorylation are lost in rhythmic strains lacking phosphatase CSP-6, and strains lacking all phosphosites on either WC-1 or WC-2 remain rhythmic. We mapped >95 phosphosites on the WCC and describe the time-of-day-specific code, requiring phosphoevents on both WC-1 and WC-2, governing repression and feedback loop closure. Combinatorial control via phosphorylation also governs rhythmic WCC binding to *clock-controlled genes* indicating that this time-of-day-specific phosphorylation mediates the essential first step in circadian output. These data provide a basic mechanistic understanding for fundamental events underlying circadian negative feedback and output, key aspects of circadian biology.

5.6 Regulators of the *Neurospora* circadian period length in a low glucose environment

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Many organisms across the tree of life possess a circadian clock to anticipate and align cellular metabolic demands with daily fluctuations in the environment. Circadian clocks are simultaneously buffered to generate a period length of approximately 24 hours at a range of physiologically relevant external conditions, such as temperature or nutrient levels, in a phenomenon called compensation. In *Neurospora crassa*, temperature compensation (TC) is achieved, in part, by Casein Kinase 2 phosphorylation and regulation of the stability of the core negative element of the clock, Frequency (FRQ). Less is known about the mechanisms underlying nutritional compensation (NC) in *Neurospora*. Three transcriptional regulators—CSP-1, RCO-1, and PRD-1—are known to play a role in NC because absence of each of these genes leads to alterations in the circadian period length at various glucose concentrations. It is completely unknown if TC and NC mechanisms are distinct or if changes in the external environment lead to the same regulation on the core clock. We have designed a genetic screen to identify defects in nutritional compensation using a luciferase reporter system for circadian period length. To ask if compensation regulators are universal, we first screened ~100 viable kinase knockouts on medium containing 0% glucose. Our preliminary results suggest that regulatory pathways for TC and NC are separate in *Neurospora*, as TC mutants do not also display NC defects. Despite their homeothermic properties, mammalian cells are also temperature compensated for circadian period length. Thus, results from this work could apply across circadian systems.

5.7 Circadian regulation of rhythmic proteins leads to coordination of metabolic pathways within *Neurospora crassa*

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Molecular clocks based around a transcription-translation negative feedback loop are ubiquitous among higher eukaryotes, allowing organisms to anticipate daily changes in their environment. Circadian studies have typically focused on clock regulation at the transcriptional level, with daily rhythmic genes termed *ccgs* (clock controlled genes). Daily oscillations in *ccg* mRNA levels were thought to directly result in matching oscillations at the protein level, but such correlations have varied widely in recent studies. Therefore, to more accurately know what the clock regulates at the protein level, we used Tandem-Mass-Tag Mass Spectrometry (TMT-MS) to allow relative quantification of protein levels over a 48 hr timecourse (2 hr resolution, in triplicate) yielding the most complete circadian proteome to date. Approximately one quarter of our detected proteins were considered significantly rhythmic, but approximately 40% of those did not arise from significantly rhythmic transcripts, when compared to previous RNA-seq data. The mechanism of post-transcriptional regulation that led to such a high discrepancy in clock regulated transcripts versus proteins is therefore of interest. Rather than degradation via E3 Ubiquitin ligases, our data suggest that the main mechanism of circadian regulation of the proteome is found at the translational level, through the clock's control of translation elongation. From previous studies, we know that the clock regulates many metabolic genes, and metabolism was still highly regulated at the proteomic level; rhythmic proteins within pathways showed coordinated peak phases, resulting in overall pathways that were mostly in-phase, such as Glycolysis and the TCA cycle, while alternative pathways like the Pentose Phosphate Pathway were in anti-phase. The clock's coordination of other metabolic pathways was only apparent at the protein level rather than the transcript level, such as the anti-phase relationship between lipid/fatty acid biosynthesis and degradation pathways. Overall, our study highlights the importance of directly studying the circadian proteome to further understand how the molecular clock regulates metabolism and other components of the cell environment.

5.8 The ECHO app: an application utilizing extended harmonic oscillators to identify non-harmonic circadian oscillations in large datasets

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Circadian rhythms are endogenous cycles of approximately 24 hours reinforced by external cues such as light. These cycles are typically modeled as harmonic oscillators with fixed amplitude peaks. Using experimental data measuring global gene transcription in *Neurospora crassa* over 48 hours in the dark (i.e. with external queues removed), we demonstrate that many circadian genes frequently exhibit either damped harmonic oscillations, in which the peak amplitudes decrease each day, or forced harmonic oscillations, in which the peak amplitudes increase each day. By fitting Extended Harmonic Circadian Oscillator (ECHO) models which include an amplitude change (AC) coefficient, we detected additional circadian genes that were not identified by the current standard tools that use fixed amplitude waves as reference, e.g. JTK_CYCLE (JTK). In order to confirm our method in a controlled setting, we build a synthetic data set of circadian and noncircadian genes, varying amounts of noise and time point resolution, as well as forcing coefficient values. When run in comparison to JTK and MetaCycle, ECHO maintains high accuracy and phase recall despite increases in noise and resolution for all AC coefficient categories. JTK and MetaCycle, however, are unable to recall circadian genes and their phase with precision as one increases noise and resolution in all AC coefficient categories. Thus, extended harmonic oscillators provide a powerful new tool for circadian systems biology. We build the ECHO functionality into a freely available, easy-to-use interface for circadian biologists. This interface contains 2 main sections: 1) finding rhythms in uploaded data and 2) visualizing these results. In the finding rhythms section, users can choose from a variety of preprocessing steps before running ECHO and, if desired, JTK. Once results are obtained, users can explore results and generate automatic visualizations including Venn Diagrams for comparisons between methods, gene expression plots, heat maps, and parameter density graphs. Current methods that have developed interfaces are not easily used, nor do they provide the extensive visualization component that allows users to explore

results instantaneously. By building this functionality into an extensive application, we have made a powerful, high-throughput method more accessible to circadian biologists.

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1.9 Prevacuolar Compartments and Tubular Vacuoles: A Microscopic Analysis

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Neurospora crassa has an unusual cellular structure, seen only near the hyphal tip, that appears to function as a prevacuolar compartment (PVC). Analysis by confocal microscopy shows that integral membrane vacuolar proteins are located there, but soluble proteins inside mature vacuoles are not. In fact, defining an "inside space" for the PVC has been challenging. Examination with new "super-resolution" microscopes suggests that the PVC is composed of multiple tiny vesicles that form an irregular, incomplete ring. These vesicles may be organized by the dynein/dynactin complex which can be observed within the ring of some, but not all, PVC-forming vesicles. PVCs are typically observed between the hyphal tip and the cluster of tubular vacuoles that are present in the first hyphal compartment. When viewed with a "super-resolution" microscope the tubular vacuoles appear not to be actual tubes but are strings of small vesicles. Unlike the PVCs, the tubular vacuoles contain both the integral membrane and the soluble proteins seen in mature vacuoles. These observations suggest that PVCs and tubular vacuoles may function to form larger mature vacuoles but this process has not yet been directly observed.

1.10 The penta-EF-hand Protein PEF-1 is part of the membrane damage response in *Neurospora crassa* and *Botrytis cinerea*

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During its life cycle *Neurospora crassa* is exposed to the risk of plasma membrane damage. Germinating vegetative spores of *N. crassa* fuse with each other to form a network during colony establishment. This process involves the breakdown of the cell wall and plasma membrane merger and bears the risk of membrane rupture. Earlier studies of the plasma membrane fusion mutant ΔPrm1 revealed the presence of Ca2+-mediated membrane repair mechanisms. The Ca2+-binding penta-EF-hand protein PEF-1 was identified as a potential part of this proposed repair mechanism. In *N. crassa* PEF-1 is recruited to the plasma membrane at the fusion point of lysing germlings and the lack of *pef-1* results in increased lysis rates in the Δ*Prm1* mutant. Interestingly, PEF-1 also translocates to the plasma membrane in response to membrane damage induced by Nystatin, which is an important membrane destabilizing antifungal drug. Cell membranes are the primary target for common antifungal drugs as well as plant defense compounds against pathogenic fungi. Understanding membrane repair mechanisms can provide important insight into host pathogen interactions and possible plant protection strategies. We have therefore analyzed the role of BcPEF-1 in the common plant pathogen *Botrytis cinerea*. Its host spectrum covers more than 200 organisms worldwide, including many food crops such as tomato. An important defense compound of the tomato plant is α-tomatine, a plasma membrane destabilizing saponin. Subcellular localization and live-cell imaging revealed that BcPEF-1-GFP is recruited to the plasma membrane of *B. cinerea* in response to treatment with α-tomatine. The tolerance of the BcPEF-1 knockout mutants toward α-tomatine slightly decreased compared to the wild type. This points to a role of BcPEF-1 in the defense against α-tomatine that complements its enzymatic degradation. Further studies aim to fully characterize this repair mechanism and its potential role in pathogenicity.

1.11 The role of ANX14 in the calcium-dependent response to cell lysis in *Neurospora crassa*

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Vegetative spores of *Neurospora crassa* germinate and fuse with each other to establish a mycelial colony. The fusion process, during which the cell wall is degraded and the cell membranes merge, is prone to failure resulting in membrane rupture, cell lysis and cell death. Earlier analysis of the membrane fusion deficient *ΔPrm1* mutant suggested that a Calcium-dependent membrane repair mechanism exists in *N. crassa*, which involves the Ca^{2+}-binding penta-EF-hand protein PEF-1. In lysing germling pairs PEF-1 is recruited to the fusion point, and it accumulates at the membrane in response to the treatment with the membrane destabilizing compound α-tomatine. Another protein family that is considered to play a role in membrane repair are annexins. They are Ca^{2+}-sensing proteins that bind phospholipids and potentially bring membranes into proximity before merging. The fungal annexin ANX14 was therefore considered to take part in the membrane repair mechanism of *N. crassa*.

Live cell imaging revealed that ANX14 accumulates at the fusion point upon lysis during fusion and is recruited to the membrane in response to the treatment with α-tomatine in a Ca^{2+}-dependent manner. The deletion of *anx14* results in increased lysis rates of germling pairs during fusion. Lysis rates further increase by 2- and 4- fold in the double mutants *Δanx14 Δpef-1 and ΔPrm1 Δanx14* respectively, suggesting that PEF-1 and ANX14 function independently.
These observations suggest that ANX14 is part of a general membrane repair mechanism, that is highly dependent on Ca^{2+}. Further studies aim at fully understanding the membrane repair mechanism of *N. crassa.*

1.12 Characterization of conidiophore architecture in *Neurospora crassa*

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While genetic, temporal, and environmental regulation of conidiophore development has been well characterized in *N. crassa*, little is known about the architecture of these structures. We are particularly interested in natural variation of conidiophore development and how genetic polymorphisms contribute to it. To study this, we are using the Louisiana wild population collection. We are employing Digital Imaging of Root Traits (DIRT), a program developed to phenotype root architecture, to quantify over 70 structural traits relating to conidiophore development over time. We hope to integrate these structural phenotypes with genetic polymorphisms to better understand conidiophore architecture and how it is genetically regulated.

1.13 Exploring Nuclear-independent Microtubule Organizing Centers in *Neurospora crassa*

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Microtubule Organizing Centers (MTOCs) control where and when microtubules are newly formed and organized in eukaryotic cells. Centrosomes and the Spindle Pole Bodies (SPBs), in metazoan and fungal cells respectively, are the main known MTOCs. In addition to the SPB, the cytoplasm and the equatorial plate contribute to microtubule nucleation in the fission yeast *Schizosaccharomyces pombe*. In *Aspergillus nidulans,* the SPB and MTOCs associated with septa polymerize microtubules. To elucidate whether there are MTOCs independent of the SPBs in *N. crassa,* we performed experiments analyzing the polymerization of microtubules after treating mature hypha expressing β -tubulin-GFP and dRFP-H1 with 7.5 ug ml^{-1}-benomyl or after fluorescence recovery after photobleaching (FRAP) experiments. Using benomyl, we observed that after microtubule depolymerization, fluorescent β-tubulin spots were present spread in the cytoplasm, once the drug was removed, the fluorescent spots migrated to the hyphal tip, and new microtubules were repolymerized from the very tip. FRAP experiments showed similar results, corroborating microtubule polymerization from the hyphal apex. We also observed microtubule nucleation from septa. In the apex and septa, microtubule repolymerization was independent of the SPBs. These results indicate the presence of MTOC independent of SPBs in *N. crassa*. Currently we are analyzing the biochemical composition of these MTOCs.

1.14 The evolutionary significance of fungal endocytosis

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The highly successful life style of fungi relies on rapid conquest of the environment by fast growth rates and abundant secretion of polymer-digesting enzymes. Both these features originate in the hyphal apices and are attained by the exocytosis of vesicles carrying the components for cell wall construction and an array of enzymes to be secreted. By confocal microscopy, we measured the rate of exocytosis and endocytosis in a strain of *Neurospora crassa* carrying a fluorescent tag on fimbrin, a protein specifically involved in endocytosis (FIM-1-GFP). We thus discovered that a substantial amount of plasma membrane deposited by the exocytic process (about 12%) becomes internalized by endocytosis. In hyphae, the exocytic and endocytic zones are highly localized and in relatively close proximity, the former at the apex, the latter several micrometers away in the subapex suggesting the possibility of a coordinated tandem operation. The internalization of plasma membrane by endocytosis must require considerable energy to overcome the high turgor of the fungal cytosol. We propose that such expensive process evolved primarily to remove the excess membrane generated by the high rates of exocytosis that allow fungal hyphae to exert their superior qualities, namely rapid colonization of the environment by fast growth rate and effective substrate utilization by abundant secretion of substrate degrading enzymes. In addition to membrane recycling, the endocytic process is being actively investigated elsewhere as a mechanism for recycling proteins of importance in apical growth.

1.15 Role of the TEA proteins during polarized growth in *Neurospora crassa*.

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Microtubule associated proteins is a group of heterogeneous motor and non-motor proteins. TEA complex is part of this group and has three members: Tea1 (Tip Elongation Aberrant protein) Tea4 and Mod5. This complex has been described to regulate the function of the formin that promotes actin nucleation and polymerization. In this study, we studied the orthologues of the Tea complex in *Neurospora crassa*. We named them as TEA-1, TEA-4 and TEA-5. We labeled each orthologue with GFP and analyzed their dynamics and localization, and produced the deletion mutants to characterize them. In conidia, TEA-1-GFP was localized in the cytoplasm until the germination site was selected. At that time, TEA-1-GFP accumulated as a bright spot before the germ tube emerged, and continue in the very tip as the tube grew. In mature hyphae was observed in the apical dome forming a fluorescent cap, avoiding the space of the Spitzenkörper, and also was found in septa forming a double ring flanking the cell wall. In the same way, TEA-4-GFP was observed at the apex forming a cap that occasionally avoids the central region. This protein was also observed in the septum. GFP-TEA-5 was observed similarly in the apex, occupying the area of the Spitzenkörper, and also was observed in septa. Experiments with Cytochalasin A and Benomyl showed that only Benomyl affects TEA-1 localization, whereas TEA-5 localization is affected when both cytoskeletons are depolymerized. Δtea-1 and Δtea-4 had growth rate reduction of 20% and produced few conidia. This evidence suggests that the TEA complex is involved in the selection of the germination site, conidiation and the organization of action filaments in the apex.

2.9 Cellulose Degradation is Regulated by cAMP-Dependent G Protein Signaling in *Neurospora crassa*

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Filamentous fungi perform a critical role as decomposers of lignocellulosic biomass into simpler compounds for carbon metabolism. Many fungal species use heterotrimeric G protein signaling to regulate metabolic processes, and G protein signaling has also been implicated in secretion of enzymes necessary for plant cell wall degradation in both pathogenic and nonpathogenic species. However, detailed mechanisms are lacking. One major downstream effector of G protein signaling is adenylyl cyclase, resulting in increased intracellular cAMP levels. This leads to activation of protein kinase A, which has a wide variety of target proteins. Preliminary experiments performed using mutants from *Neurospora crassa* show that G protein signaling is essential for hyphal growth when cellulose is the sole carbon source. Assays of secreted proteins from the G protein mutant strains grown in liquid culture containing cellulose show a decrease in protein concentration, as well as alterations in cellulolytic activity relative to the wild type strain. These results suggest that in *N. crassa,* cAMP-dependent signal transduction is required for secretion of cellulase enzymes for effective cellulose metabolism. Experiments are ongoing to determine whether these effects are at the level of mRNA synthesis or are post-transcriptional.

2.10 Probing roles for the RACK1 homolog CPC-2 during heterotrimeric G protein signaling in *Neurospora crassa*

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Receptor for Activated C Kinase-1 (RACK1) is a multifunctional eukaryotic scaffolding protein with a seven WD-repeat structure. Among their many cellular roles, RACK1 homologs have been shown to serve as alternative Gβ subunits during heterotrimeric G protein signaling in many systems. In this study, we are probing physical and genetic interactions between the RACK1 homolog *cpc-2* and components of the heterotrimeric G protein signaling pathway in the multicellular filamentous fungus *Neurospora crassa*. Results from cell fractionation studies and from imaging of a strain expressing CPC-2-GFP are consistent with CPC-2 as a cytosolic protein. Genetic epistasis experiments between *cpc-2*, the three Gα genes (*gna-1*, *gna-2* and *gna-3*) and the Gβ gene *gnb-1* demonstrated that *gna-2* is epistatic to *cpc-2* with regards to hyphal growth rate and aerial hyphae height, while deletion of *cpc-2* mitigates the increased macroconidiation on solid medium observed in Δ*gnb-1* mutants. Δ*cpc-2* mutants inappropriately produce conidiophores during growth in submerged culture and mutational activation of *gna-3* alleviates this trait. As reported previously, Δ*cpc-2* mutants are female-sterile, producing submerged perithecia, and fertility could not be restored by mutational activation of any of the three Gα genes. With the exception of macroconidiation on solid medium, double mutants lacking *cpc-2* and *gnb-1* exhibited more severe defects for all phenotypic traits, supporting a synergistic relationship between these two alternative Gβ subunits in *N. crassa*. Experiments testing physical interactions between CPC-2 and the G protein subunits are in progress.

2.11 Assigning putative functions to different regions of the RIC8 protein

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In all eukaryotic organisms, chemical and sensory stimuli are recognized by specialized cell-surface receptors called G-protein-coupled receptors (GPCRs). GPCRs act as Guanine nucleotide exchange factors (GEFs) for Gα subunits of Heterotrimeric (αβγ) G proteins. The receptors transduce information from environmental stimuli to intracellular signaling pathways. Recently, facilitated hydrolysis of GTP by non-GPCR GEFs, such as Resistance to Cholinesterase 8 (RIC8), has emerged as an important mechanism for Gα regulation in animals. RIC8 is present in animals and filamentous fungi, such as the model eukaryote *Neurospora crassa*, but is absent from the genomes of baker's yeast and plants. In *Neurospora*, RIC8 acts an upstream regulator of two Gα proteins (GNA-1 and GNA-3) and is necessary for normal growth as well as asexual and sexual development of the fungi. To better understand RIC8, we are investigating 10 amino acid residues that are highly conserved in *Homo sapiens, D. Melanogaster, C. elegans* and *N. crassa* which may be important for Gα binding and GEF activity. We focused on these highly conserved amino acids to obtain more information about the RIC8 structure and residues that are important for Gα binding, GEF activity and stability. Results from GEF activity assays indicates that point mutations in the N-terminus region yielded decreased GNA-1 GEF activity. The C-terminus region, the most conserved, had the most point mutations that resembled reactions without added RIC8 protein. Utilizing vector transformation, we are recombining the mutated *ric8* alleles into the *pan-2* locus via homologous recombination. Our progress in creating and isolating homokaryan mutant strains will allow for analyses of phenotypes during development (asexual and sexual) and growth. This work will help determine whether specific *ric8* point mutant alleles rescue Δ*ric8 p*henotypes.

2.12 Genetic and Biochemical Characterization of GH72 glucan transferases show they function in attaching glycoproteins into the fungal cell wall

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In this report we demonstrate that *Neurospora crassa* GH72 glucan transferases function to crosslink cell wall glycoproteins into the cell wall. With an *in vitro* assay, we show that the glucan transferases are able to attach lichenin, a cell wall glucan with a repeating β-1,4-glucose-β-1,4-glucose-β-1,3-glucose structure, to cell wall glycoproteins. We demonstrate that the pathway for attachment of lichenin to the glycoprotein has four steps. First, N-linked oligosaccharides present on the glycoproteins are modified by the addition of a galactomannan. As part of our research we have characterized the structure of the galactomannan, which consists of an α-1,6-mannose backbone with galactofuranose side chains. In the second step, the galactomannan is processed by members of the GH76 α-1,6-mannanases. In the third step, the glucan transferases cleave the lichenin and create substrate-enzyme intermediates. In the final step, the transferases transfer the lichenin to the processed galactomannan. We show that the glucan transferases demonstrate specificity for the processed galactomannan and for lichenin. The pathway effectively crosslinks glycoproteins into the fungal cell wall.

3.9 A Panel of Histone H3 Mutations to Investigate Centromere Maintenance and Gene Silencing

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Histones, as part of nucleosomes, are responsible for DNA packaging in chromosomes. They also affect gene expression by combinations of post-translational modifications (PTMs), collectively called the “histone code”. PTMs are especially prevalent on the amino terminus of histones. Co-activator or -repressor complexes “write”, “read”, or “erase” histone modifications, and in balance determine which DNA segments are free of histones and thus can be transcribed. The effects of histone H3 on gene silencing, DNA methylation, and centromere formation and maintenance has been studied in the model fungus, *Neurospora crassa*, for example, certain point mutations in the amino terminal tail of H3 can abrogate DNA methylation and have been shown to be lethal. Here we extended previous work by systematically mutating amino acids along the entire H3 protein, replacing the normal gene with the mutated copies in both *N. crassa* and the plant pathogen, *Fusarium graminearum*. We paid special attention to changing all lysines (K), which can be acetylated or methylated. Point mutations were introduced into plasmids by a modified “QuickChange” method, constructs were validated by sequencing, and transformed into each fungus by homologous recombination. Transformants were screened for proper integration, and strains were crossed to reporter strains to yield haploid progeny with mutated hH3 genes in combination with cytological markers, such as Red Fluorescent Protein (RFP)-tagged centromere proteins or Green Fluorescent Protein (GFP)-tagged proteins involved in gene silencing. Cytology is combined with molecular and morphological assays to determine how exactly point mutations affect centromere function in *Neurospora*, and gene silencing by H3K27 methylation in *Fusarium.*

3.10 Aberrant Heterochromatin in Neurospora Mutants Defective in Lysine-Specific Histone Demethylase

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Heterochromatin is a specialized form of chromatin responsible for silencing transcription and recombination of underlying DNA and that also participates in chromosome segregation and genome organization. In Neurospora, constitutive heterochromatin is largely found in centromeric and dispersed chromosomal regions exhibiting evidence of RIP, and is characterized by DNA methylation, trimethylation of lysine 9 on histone H3 (H3K9me3), and general hypoacetylation of histones. There is evidence that both H3K9me and DNA methylation are subject to spreading mechanisms to effectively cover incipient chromatin across heterochromatin domains and that this is stimulated by feedback between these two marks. Presumably, boundary elements and associated limiting factors are necessary to prevent heterochromatin from spreading into neighboring, gene-rich euchromatin. Thus, an important question is: how is excessive spreading prevented? One possibility is that histone demethylases somehow limit propagation of heterochromatin. One such candidate is lysine-specific demethylase 1 (LSD-1), which acts on H3K9me1 and me2, and is conserved from yeasts to mammals. We found that Neurospora LSD-1 mutants exhibit variable spreading of heterochromatin that is dependent on the catalytic activity of the histone deacetylase complex HCHC. Interestingly, DCDC, the complex responsible for all H3K9me3 in Neurospora, is present at regions susceptible to spreading both in the presence or absence of functional LSD-1. Although DNA methylation is directed by H3K9me3 in Neurospora and appears to have little effect on the proper establishment of H3K9me3, we found that spreading of H3K9me3 in LSD-1 mutants depends on DNA methylation, much like previously observed spreading of H3K9me3 in mutants defective for the putative histone demethylase DMM-1. Possible models will be discussed.

4.9 Characterization of higher order gene organization in the nucleus of *Neurospora crassa*

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Eukaryotic genomes are specifically compacted and organized in the nucleus, and current research has suggested that this non-stochastic organization may be essential for proper gene regulation. DNA is compacted through precise loops, which may correctly control intra-chromosomal transcription by bringing distal promoter / enhancer elements in close contact; loop placement may also control long-range inter-chromosomal contacts for proper gene expression. Presently, the dynamics and control of DNA looping, as well as the genetic factors necessary to establish loops, are not well understood. To mechanistically explore the establishment and regulation of intra-chromosomal loops and inter-chromosomal contacts, we utilized the genetically-tractable organism *Neurospora crassa*. The chromatin of Neurospora has similar properties to that of humans, yet the smaller genome of the genetically-tractable Neurospora is amenable to the high-throughput chromosome conformation capture sequencing (Hi-C) studies examining short- and long-range chromatin contacts. We have devised a bioinformatic protocol that allows us to examine the specific interactions of any euchromatic locus by mining published Hi-C datasets. We have started to employ this method to examine the genic interactions within the nucleus, which will be confirmed with traditional chromosome conformation capture (3C) methods. We present here our initial characterization of the organization of several genic loci in the nucleus of *Neurospora crassa*, which may elucidate mechanisms of transcriptional regulation critical for proper gene expression.

4.10 Elucidating low-temperature fitness in Louisiana populations of *Neurospora crassa*

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The search for genes that have been selected to promote adaptation using a “reverse ecology” approach detects regions of high differentiation referred to as “islands” of differentiation that arise by hybridization and introgression due to the presence of adaptive genes. Previous researchers used this approach to make and test hypotheses about genes that promote adaptation to low temperature in populations of *Neurospora crassa* from subtropical Louisiana. There, the average yearly minimum temperature is 9C cooler than the Caribbean basin. In that study, two genomic “islands” were detected, with genes related to cold temperature response located within. However, the reverse ecology approach cannot be used to identify genes with more subtle signatures of selection. To find additional genes involved in adaptation to growth at low temperature, we performed a linkage study using 250 progeny from a cross between Louisiana and Caribbean strains and found loci that significantly contributed to better growth at 10C, primarily situated in a region of Chromosome 5. We then performed an association study with Louisiana strains and found other genes of interest, with a similar high frequency of sites found within Chromosome 5. The resulting candidate genes were tested using the *Neurospora crassa* gene deletion collection, analyzing the phenotype of the ratio of growth rates at 10°C and 25°C as with previous studies.

4.11 Characterizing the genome organization of *Neurospora crassa* at high resolution

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Recent technological advances have allowed researchers to explore the organization of genomic DNA in the nucleus. Multiple studies from many labs in several model organisms have shown DNA makes non-stochastic contacts that appear to be critical for short-range DNA compaction and long-range gene regulation. Chromosomes are organized into local loops of DNA termed Topologically Associated Domains (TADs) that appear to be both structural and regulatory in nature. Long range promoter-enhancer contacts that are critical for proper gene expression have also been described. However, researchers are just “scratching the surface” in understanding the contacts that form and how these contacts change under different environmental conditions or genetic backgrounds. In a previous characterization of the chromosome conformation of the filamentous fungus *Neurospora crassa*, researchers found that silent (heterochromatic) regions of the genome strongly interacted to form a “heterochromatin bundle” with active (euchromatic) genomic regions looping out (Galazka*, Klocko* et al., 2016 *Genome Res*). However, much remains unexplored, including characterizing specific short- and long-range regulatory contacts that may be made by Neurospora genes at a high resolution. Here, we present our initial efforts to characterize the *Neurospora crassa* genome organization at a high resolution to more thoroughly understand short- and long-range DNA compaction.

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