
Neurospora
2023

Neurospora 2023

October 1-4
Camp Allen
Navasota, TX

Scientific Organizers

Luis M. Corrochano

Departamento de Genética
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Neurospora Policy Committee

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Brief Schedule:

	Morning	Afternoon	Evening
Sunday		3:00 Arrival and Check-in 5:00 Welcome. Perkins Award Winner Talks	6:30 Dinner 7:30 - 9:00 Mixer
Monday	8:00 Breakfast 9:00 - 12:00 Session I Fungal Circadian Clocks	12:00 Lunch 1:00 - 2:30 Afternoon off 2:30 - 3:30 Beadle and Tatum Award Lecture 3:30 - 6:30 Session II Fungal Cell Development and Morphogenesis	6:30 Dinner 7:30 Poster Session 1 and Mixer
Tuesday	8:00 Breakfast 9:00 - 12:00 Session III Fungal Cell Metabolism and Signaling	12:00 Lunch 1:00 - 3:00 Poster Session 2 3:00 *Neurospora* Business Meeting 4:00 - 6:00 pm Session IV Fungal Genetics and Gene Regulation	6:00 - 7:00 Dinner 7:00 Dodge Award Lecture 8:00 Closing Remarks and Mixer
Wednesday	8:00 Breakfast 9:00 - 12:00 Depart		

Complete Schedule of Activities:

Sunday, October 1st
(Central Daylight Time is GMT-5)

Time	Session	Chair	Speaker	Title
3:00 PM	Check-in			
5:00 PM	Opening Remarks	Luis M. Corrochano and Jennifer M. Hurley		
5:15 PM	Perkins Awards	Jackie Pelham and Tina Kelliher		
5:15 PM			Victor Sy	A DEAD-box RNA helicase mediates meiotic silencing by unpaired DNA
5:45 PM			Bin Wang	Multisite phosphorylation and dephosphorylation determine circadian transcriptional activity of the positive arm in the *Neurospora* clock
6:30 PM	**Dinner**			
7:30 PM	Welcome Reception			

Monday, October 2nd
(Central Daylight Time is GMT-5)

Time	Session	Chair	Speaker	Title
8:00 AM	**Breakfast**			
9:00 AM	Circadian Clocks and Light	Kathy Borkovich and Lori Huberman		
9:05 AM			Deb Bell-Pedersen	Using *Neurospora crassa* to mend a broken clock
9:25 AM			Christian Hong	Interconnected network of circadian rhythms, cell cycle, and DNA damage response
9:45 AM			Tina Kelliher	Genome-wide regulation of mRNA polyadenylation across nutrient environments and over circadian time
10:05 AM			Jia Hwei Cheong	The clock in growing hyphae and their synchronization in *Neurospora crassa*
10:25 AM	**Coffee Break**			
10:55 AM			Luis Larrondo	An ultradian oscillator ticks in *Neurospora*: from phenomena, to mechanisms and back to phenomena
11:15 AM			Michael Brunner	Phosphorylation of specific sites in WCC determines the phase of the circadian clock of *Neurospora*
11:35 AM			Jackie Pelham	The role of protein disorder in the temperature response of the *Neurospora* circadian clock
12:00 PM	**Lunch**			
1:00 PM	Afternoon Break			
2:30 PM	Beadle and Tatum Award Lecture	Jennifer M Hurley	Eugene Gladyshev	Towards understanding the initiation of homology-directed epigenetic silencing

3:30 PM	Fungal Cell Development and Morphogenesis	Deb Bell-Pedersen and Andrew Klocko	
3:35 PM		Steve Free	Cell wall biogenesis and functions
3:55 PM		Thomas Hammond	*Neurospora Sk-2* and *Sk-3*: recent advances towards understanding two complex spore killers
4:15 PM		Juan Manuel Martínez-Andrade	The unexplored apical and subapical organization of the endoplasmic reticulum in growing hyphae of *Neurospora crassa*
4:35 PM	**Coffee Break**		
5:05 PM		Yen-Wen Wang	Origins of lineage-specific elements via gene duplication, relocation, and regional rearrangement in *Neurospora crassa*
5:25 PM		Louise Glass	Elucidating mechanistic processes of the cell wall remodeling checkpoint in *Neurospora crassa*
5:45 PM		Abigail Deaven	Convergence to multicellularity: Understanding the contribution of chromatin modification to cell fate transitions in *Neurospora crassa*.
6:00 PM		Elizabeth Hutchison	Overexpression of the *Neurospora crassa* sexual development regulator *fsd-1* causes increased pigment production, reduced conidiation, and inhibits successful mating
6:15 PM		Teresa Lamb	Circadian clock control of ribosome composition leads to rhythms in translation

			and promotes rhythmic termination fidelity
6:30 PM	**Dinner**		
7:30 PM	Poster session 1 Posters 1-13		

Tuesday, October 3rd

Time	Session	Chair	Speaker	Title
8:00 AM	**Breakfast**			
9:00 AM	Fungal Cell Metabolism and Signaling	Thomas Hammond and Christian Hong		
9:05 AM			J. Phillip Benz	Identification of F-Box proteins involved in the switch to cellulolytic metabolism
9:25 AM			Lori Huberman	Characterizing the genetics of *Neurospora crassa* environmental sensing using functional genomics
9:45 AM			Cheng Wu	Translational regulation through the upstream open reading frame encoded inositol regulatory peptide
10:05 AM			Yagna Oza	Impact of G protein signaling on carbon catabolite repression and production of cellulases
10:25 AM	**Coffee Break**			
11:10 AM			Meaghan Jankowski	Distributed multivalent interactions in a protein complex in the circadian clock turns an hourglass into a persistent oscillator.
11:25 AM			David S. Roos	FungiDB: Tools for genomic-scale data exploration, analysis, integration and discovery
11:40 AM			Aaron Robinson	Differences in thermotolerance between ecotypes of *Neurospora discreta* are primarily due to only two genomic regions
12:00 PM	**Lunch**			
1:00 PM	Poster Session 2 Posters 14-27			

3:00 PM	*Neurospora* Business Meeting	Luis M. Corrochano and Jennifer M. Hurley		
4:00 PM	Fungal Genetics and Gene Regulation	J. Phillip Benz and Jonathan Arnold		
4:05 PM			Andrew Klocko	Histone deacetylation and cytosine methylation compartmentalize heterochromatic regions in the genome organization of *Neurospora crassa*
4:25 PM			Yi Liu	Codon usage control of translation efficiency: from initiation to elongation and termination
4:45 PM	**Coffee Break**			
5:15 PM			Scott Baker	Connecting genes and phenotypes by genome resequencing of *Neurospora crassa* mutant strains
5:35 PM			Oded Yarden	Wild and adventurous: *Neurospora crassa* leaves the lab
6:00 PM	**Dinner**			
7:00 PM	Dodge Award Lecture	Luis Larrondo	Jennifer Loros	Stories from a collegial life
8:00 PM	Closing Remarks and Mixer	Luis M. Corrochano and Jennifer M. Hurley		

Wednesday, October 4th

Time	Session	Chair	Speaker	Title
8:00 AM	Breakfast and depart			

Poster Presentations:

1. Griffin Best
   Circadian Clock Regulation of tRNA synthetases, mRNA translation, and translation fidelity in *Neurospora crassa*

2. Katherine Borkovich
   Characteristics of *Neurospora discreta* strains isolated from burnt Joshua trees after the Cima Dome fire in the Mojave Desert of California

3. Apurva Chatrath
   Characterization of the carbohydrate-binding domain in two *Neurospora crassa* cell wall proteins

4. Luis Corrochano
   VE-1 regulation of MAPK signaling controls sexual development in *Neurospora crassa*

5. Abigail Deaven
   Convergence to multicellularity: Understanding the contribution of chromatin modification to cell fate transitions in *Neurospora crassa*.

6. Protyusha Dey
   Characterization of the need for Galactofuranose during the *Neurospora crassa* life cycle

7. Clayton Hull-Crew
   Topological consequences of large-scale genome rearrangements in *Neurospora crassa*

8. Madhusree Gangopadhyay
   Rhythmic interaction between ZUOTIN and ribosomes may promote daily rhythms in protein folding and activity

9. Elizabeth Hutchison
   Overexpression of the *Neurospora crassa* sexual development regulator *fsd-1* causes increased pigment production, reduced conidiation, and inhibits successful mating.

10. Meaghan Jankowski
    Distributed multivalent interactions in a protein complex in the circadian clock turns an hourglass into a persistent oscillator.

11. Teresa Lamb
    Circadian clock control of ribosome composition leads to rhythms in translation and promotes rhythmic termination fidelity

12. Nickolas Lande
    Characterizing the landscape of histone post-translational modifications in strains of *Neurospora crassa* with single translocations.

13. Luis Larrondo
    Methylxanthines modulate circadian period length independently of the action of phosphodiesterase

14. Shahriar Mahmud
    Neurospora rfk-1: a Spore killer gene with an unusual intron

15. Jaideep Mallick
    Fungal Genetics Stock Center Status Report

16. Gabriela Mendoza-Rangel
    Neurospora rsk: a Spore killer resistance gene that functions in multiple tissue type

17. R. R. Mouriño-Pérez
    Toward Unraveling the Roles of TEA Proteins: Insights into Cytoskeletal Dynamics and Growth Symmetry in *Neurospora crassa*

18. Ebimobowei Preh
    Mechanism of circadian clock control of rhythmic translation in *Neurospora crassa*

19. Monique Quinn
    RNAseq and targeted metabolomics analyses implicate G protein signaling in regulation of arginine and ornithine metabolism and compartmentation in *Neurospora crassa*

20. Manuel Ramirez
    Neurospora crassa SCK1 is required for circadian clock control of translation initiation factor eIF2α activity

21. Aaron Robinson
    Differences in thermotolerance between ecotypes of *Neurospora discreta* are primarily due to only two genomic regions

22. David S. Roos
    FungiDB: Tools for genomic-scale data exploration, analysis, integration and discovery

23. Elizabeth-Lauren Stevenson
    A Case for the kinases: A role for CKI in temperature compensation of the *Neurospora crassa* circadian clock

24. Lucas Sutton
    Changes in core clock negative arm proteins reveal a relationship between the conformation of complexes and post-transcriptionally regulated proteins

25. Ziyan Wang
    Novel microscopy tools reveal dynamic sub-cellular distributions of core clock components in *Neurospora crassa*

26. Zheng Wang
    Origins of lineage-specific elements via gene duplication, relocation, and regional rearrangement in *Neurospora crassa*

27. Rochelle Yap
    Novel Candidates in the Polycomb repression network

Talk Abstracts:
Sunday, October 1 - Afternoon Session
Perkins Award Winners

A DEAD-box RNA helicase mediates meiotic silencing by unpaired DNA

Victor Sy

University of Missouri

In *Neurospora crassa*, a gene not having a pairing partner during meiosis is silenced by a mechanism called meiotic silencing by unpaired DNA (MSUD). MSUD targets the transcripts of an unpaired gene using common RNA interference factors, such as Dicer and Argonaute. By screening the *Neurospora* knockout library, we have identified a meiotic silencing gene called *sad-9,* which encodes a DEAD-box RNA helicase. While not essential for vegetative growth, SAD-9 plays a vital role in both sexual development and MSUD. SAD-9, together with the SAD-2 scaffold protein, recruits the SMS-2 Argonaute to the perinuclear region, where target mRNAs are recognized and silenced.

Multisite phosphorylation and dephosphorylation determine circadian transcriptional activity of the positive arm in the *Neurospora* clock

Bin Wang

Geisel School of Medicine at Dartmouth

In *Neurospora*, FRQ complexes with FRH and CK1 to form FFC, which represses WCC via phosphorylation promotion. Recently, we have mapped over 95 phosphosites on WCC and deciphered the phosphocode needed for rhythmic inhibition of WCC. However, it remains vague how WCC restores its circadian function. The present study shows that dephosphorylation leads to WCC activation, which proceeds vigorously in the dark and can sustain oscillations. FRQ-bound CK1 probably directly mediates WCC phosphorylation at the key residues.
The motif(s) on FRQ required for FFC and WCC interaction was poorly defined. New *frq* mutants confirm that multiple dispersed regions on FRQ are necessary for WCC interaction. Three Asp/Glu clusters in FRQ are found to be indispensable for FFC/WCC formation. Surprisingly, in several *frq* Asp/Glu-to-Ala mutants that vastly diminish FFC/WCC interaction, the core clock still oscillates robustly without a period change, indicating that stable interaction between the two arms does not determine period length.
A screen identified brd-8, loss of which reduces H4 acetylation and RNA polymerase (Pol) II occupancy, leading to a long circadian period, delayed phase, and defective overt circadian output. Taken together, our data identify auxiliary elements of the fungal NuA4 complex along with conventional NuA4 subunits are required for timely and dynamic *frq* expression and thereby a normal and persistent circadian rhythm.
It is elusive how all the phosphosites on FRQ control its activity. *frq* mutants covering all the identified 110 phosphosites were generated and examined for period changes. When phosphosites

in the N-terminal and middle regions were eliminated, longer periods were typically seen while removal of C-terminal phosphorylation resulted in extremely short periods and an overcompensated clock.

Monday, October 2 - Morning Session
Fungal Circadian Clocks

Using *Neurospora crassa* to mend a broken clock

Samuel Park, Samarjeet Singh, and Deborah Bell-Pedersen

Texas A&M University, College Station

Fungi serve as important models for circadian clock and aging research. Aging is associated with reduced circadian amplitude, and disruption of the circadian clock during aging is linked to increased risk of disease, including sleep disorders, metabolic disorder, and cancer. We set out to establish Neurospora crassa as a model for the development of therapeutic treatments for disorders associated with disrupted circadian rhythms, including aging, jet lag, and Seasonal Affective Disorder.
A high throughput screen of 2208 compounds from the National Cancer Institute FDA Approved Drug Library and the Target Mol Globally approved Drug Library identified several compounds that significantly increased circadian amplitude of the clock gene reporter FRQ::LUC in old *Neurospora* cultures, suggesting that these drugs may be useful to treat aging. Several drugs were also identified that increased the response of FRQ:LUC to light, suggesting that these drugs may be useful to phase shift the clock to treat jet lag and/or Seasonal Affective Disorder in humans that is characterized by reduced sensitivity of the clock to light. Detailed analyses of one of these compounds will be discussed, and evidence will be provided to support that at least some of the drugs function similarly in mammalian cells.

Interconnected network of circadian rhythms, cell cycle, and DNA damage response

Christian Hong

University of Cincinnati

Circadian rhythms coordinate temporal organization of cellular processes including cell cycle and DNA damage response (DDR) optimizing fitness and survival of organisms. Previously, it was demonstrated that DNA damage resets circadian rhythms in *Neurosora crassa* via DNA damage-activated checkpoint kinase, PRD-4, triggering phosphorylation and degradation of the core negative element, FRQ. It was also shown that the expression of *prd-4* is clock-controlled. However, detailed mechanisms regulating this bidirectional link remain largely unknown. In this work, we utilized mathematical modeling and experimental validations investigating interconnected networks of circadian rhythms, DNA damage response (DDR), and cell cycle where the circadian clock coordinates short- and long-term responses of DDR and cell cycle.

Genome-wide regulation of mRNA polyadenylation across nutrient environments and over circadian time

Tina Kelliher

University of Massachusetts Boston

The circadian clock is a ubiquitous biological process used by nearly every organism on the planet to anticipate daily fluctuations in the environment. Transcriptome profiling studies from cultured mammalian cells, insects, fungi, and many others have demonstrated rhythmic circadian regulation of 10-50% of all genes. Translational and proteomic assays have identified proteins that are expressed rhythmically but are not clock controlled at the level of mRNA abundance, suggesting extensive posttranscriptional regulation.
We identified two mutants in a Cleavage and Polyadenylation Specificity Factor complex through a genetic screen of the *Neurospora crassa* knockout collection. Single mutants Δ*cpsf5* (NCU09014) and Δ*cpsf6* (NCU02152) have a short circadian period length of 16 – 20 hours, depending on the nutrient growth conditions. Using genetic and biochemical approaches, we show that CPSF5 and CPSF6 form a heterodimeric complex in *Neurospora*, indicating functional and evolutionary homology to the mammalian CPSF complex involved in poly(A) tail placement and mRNA cleavage in the 3' UTR. We hypothesized that CPSF is required for normal polyadenylation and expression of a core clock transcript(s), leading to differential regulation and short period length in Δ*cpsf* mutants. Two core clock genes tested (*frequency*, *casein kinase I*) do not explain the short period defect, but two additional downstream genes will be discussed. To test for a pleotropic effect on multiple genes, we performed 3' End Sequencing of the Δ*cpsf5* Δ*cpsf6* double mutant compared to wild-type under various nutrient conditions to map poly(A) tail locations. We find that half of the polyadenylation landscape is altered in the CPSF mutants. Further, we find that hundreds of genes harbor differential polyadenylation depending on carbon source or on circadian time. Post-transcriptional regulation contributes significantly to proper circadian timekeeping, including mRNA polyadenylation.

The clock in growing hyphae and their synchronization in *Neurospora crassa*

Cheong, J. H. and J. Arnold

University of Georgia

Utilizing a microfluidic chip with serpentine channels, we inoculated the chip with an agar plug with *Neurospora crassa* mycelium and successfully captured individual hyphae in channels. For the first time, we report the presence of an autonomous clock in hyphae. Fluorescence of a mCherry reporter gene driven by a *clock-controlled gene-2* promoter (*ccg-2*p) was measured simultaneously along hyphae every half an hour for at least 6 days. We entrain single hyphae to light over a wide range of day lengths, including 6,12, 24, and 36 h days. Hyphae tracked in individual serpentine channels were highly synchronized (K=0.60-0.78). Furthermore, hyphae also displayed temperature compensation properties, where the oscillation period was stable over a physiological range of temperatures from 24 °C to 30 °C (Q10 = 1.00-1.10). A Clock Tube Model developed could mimic hyphal growth observed in the serpentine chip and provides a mechanism for the

stable banding patterns seen in race tubes at the macroscopic scale and synchronization through molecules riding the growth wave in a serpentine microfluidics device.

Mind The Gap: Identification of a common secondary mutation in the *Neurospora crassa* knockout collection conferring a cell fusion-defective phenotype

Alejandro Montenegro-Montero, Alejandra Goity, Paulo F. Canessa, Luis F. Larrondo

P. Universidad Catolica de Chile-iBio

Loss-of-function mutants represent a powerful tool to study gene function. The filamentous fungus *Neurospora crassa* is a well-established model organism, and features a comprehensive gene knockout strain collection. While these mutant strains have been used in numerous studies, resulting in the functional annotation of many Neurospora genes, direct confirmation of gene-phenotype relationships is often lacking, which is particularly relevant given the possibility of background mutations, sample contamination, and/or strain mislabeling. Indeed, spontaneous mutations resulting in phenotypes resembling many cell fusion mutants have long been known to occur at relatively high frequency in *N. crassa*, and these secondary mutations are common in the Neurospora deletion collection. The identity of these mutations, however, is largely unknown. Here, we report that the Δada-3 strain from the *N. crassa* knockout collection, which exhibits a cell fusion defect, harbors a secondary mutation responsible for this phenotype. Through whole-genome sequencing and genetic analyses, we found a ~30-kb deletion in this strain affecting a known cell fusion-related gene, soft/ham-1, and show that it is the absence of this gene -and not of *ada-3*- that underlies its cell fusion defect. We additionally found three other knockout strains harboring the same deletion, suggesting that this mutation may be common in the collection and may have impacted previous studies. Our findings provide a cautionary note and highlight the importance of proper functional validation of strains from mutant collections. We discuss our results in the context of the spread of cell fusion-defective cheater variants in *N. crassa* cultures.

Phosphorylation of specific sites in WCC determines the phase of the circadian clock of *Neurospora*

Anna Gatz, Axel Diernfellner, and Michael Brunner

Heidelberg University

Circadian clocks are cell-based molecular oscillators that generate rhythms with a period in the range of 24 hours. In free-running conditions, circadian clocks oscillate precisely in a self-sustaining and temperature-compensated manner, but the period length may deviate from 24 hours. Under most natural conditions, however, recurrent stimuli related to the geophysical cycle of the Earth's rotation, such as light and temperature, synchronize the circadian clock with the day-night cycle. Synchronization not only sets the length of the circadian period to exactly 24 hours, but also creates an internal phase that allows organisms to anticipate environmental changes. The circadian clock of Neurospora is based on negative feedback of FRQ on its circadian transcription activator WCC. We present evidence how phosphorylation of specific sites in WCC determines the phase of the *frq* transcription rhythm relative to light-dark transitions.

The role of protein disorder in the temperature response of the *Neurospora* circadian clock

Jackie Pelham

Washington University in St. Louis

The Earth's predictable light/dark cycle has led to the ubiquitous evolution of circadian rhythms, providing organisms with elevated survival and reproductive fitness. Investigations into the circadian core clock oscillator have revealed its control of an astounding amount of cellular physiology in numerous model organisms. However, the details of the molecular oscillator are still poorly understood. The oscillator comprises a transcription-translation feedback loop, and one of its intriguing and conserved features is the extensive level of intrinsic disorder in the negative arm. Given this observation, we sought to ask if these disordered regions contribute to clock timing or output, hypothesizing that plasticity could be a tunable mechanism for clock homeostasis. In the organism, Neurospora crassa, the negative arm of the clock is centered around the intrinsically disordered protein FREQUENCY (FRQ), which has two known isoforms produced via temperature-induced alternative splicing. To investigate the role of each isoform in timekeeping, we created mono-isoform FRQ strains of *N. crassa* to delineate any functionality they may individually impart to the clock. We discovered that the isoforms differ in resistance to protease treatment, cycloheximide half-life, and interactome. To explain these differences mechanistically, we surveyed the molecular grammar of the N-terminal intrinsically disordered region (IDR) of L-FRQ, revealing clock period affecting degrons. Mutation of a hydrophobic pair proximal to the degron alters L-FRQ IDR ensemble behavior and impacts clock function in vivo. Ensemble FRET and molecular simulations captured the biophysical behavior of the L-FRQ IDR, indicating that sequence-specific physiochemical properties govern the traits of the IDR. Summarizing these findings, we propose an isoform-specific mechanistic role for protein disorder in regulating the circadian clock under different temperatures.

Monday, October 2 - Afternoon Session
Beadle and Tatum Award Lecture
Fungal Cell Development and Morphogenesis

Towards understanding the initiation of homology-directed epigenetic silencing

Eugene Gladyshev

Institut Pasteur

Overall, our lab is interested in discovering and characterizing general mechanisms of homology-directed epigenetic regulation. In eukaryotes, such mechanisms may manifest themselves in phenomena as diverse as transvection, paramutation, random monoallelic gene expression, repeat-induced gene silencing, co-suppression, meiotic silencing and quelling. While many such phenomena have been already analyzed in great detail, the nature of the homology-dependent signal at their basis has remained mysterious. To better understand the initiation of homology-directed epigenetic silencing, we began by dissecting the homology requirements of the two genome-defense processes known as repeat-induced point mutation (RIP) and meiotic silencing by unpaired DNA (MSUD). There we have uncovered the existence of a recombination-independent mechanism of DNA homology recognition that appears to match double-stranded DNA molecules directly, without relying on single-stranded intermediates. While the extreme sensitivity of RIP and MSUD to DNA homology was absolutely critical for discovering the existence and key properties of the recombination-independent mechanism, RIP and MSUD themselves proved rather complicated for genetic and biochemical analysis, because these processes occur only in a few nuclei during the sexual stage (which requires mating between two different parental strains). Thus, we have designed a complementary system, in which perturbation of the nucleosome-rich chromatin state or Pol2-dependent transcription of a model repetitive DNA region trigger heterochromatin formation and siRNA expression in vegetative nuclei. A forward genetics screen for genes required for the initiation of silencing in this system identified several epigenetic factors conserved in higher eukaryotes, including the chromatin remodeler ATRX (also required for the expression of siRNAs during MSUD) and several components of the RPD3 (histone deacetylase) complex.

Cell wall biogenesis and function

Steve Free

SUNY University at Buffalo

The fungal cell wall is a glucan/chitin/glycoprotein matrix vital for the survival. Our laboratory has been particularly interested in the question of how proteins become incorporated into the wall. Our research has shown that a galactomannan attached to the N-linked oligosaccharides of cell wall proteins is used to covalently incorporate cell wall glycoproteins into the cell wall matrix. A full-length galactomannan is added to the proteins post-translationally and β-1,6-mannanases function to process the galactomannan on cell wall glycoproteins. Finally, we show that laminarin/lichenin transferases covalently attach laminarin (β -1,3-glucan) and lichenin (mixed β

-1,3-/ β -1,4-glucan) to the processed galactomannan covalently incorporate the glycoprotein into the cell wall matrix.

Neurospora Sk-2 and _Sk-3_: recent advances towards understanding two complex spore killers

Thomas Hammond

School of Biological Sciences, Illinois State University, Normal, IL, USA

The selfish genetic elements *Neurospora Spore killer-2* (*Sk-2*) and *Spore killer-3* (*Sk-3*) are transmitted to viable ascospores in a biased manner through spore killing. With respect to *Sk-2*, biased transmission depends on two genes: a poison gene and an antidote gene. Presumably, *Sk-3* also uses a poison-antidote system to achieve biased transmission to ascospores, and while the antidote gene has been identified, the identity of the poison gene remains unknown. Toward the goal of identifying the *Sk-3* poison gene, we have isolated $rfk\text{-}2^{UV}$, a mutation that disrupts spore killing. So far, we have determined that $rfk\text{-}2^{UV}$ lies 15.6 cM to the left of *mus-52* on Chromosome III (centromere proximal). Fortuitously, while creating genetic markers to further refine $rfk\text{-}2^{UV}$'s location, we identified a relatively short DNA interval that is required for spore killing in *Sk-3* × *SkS* (spore killing-sensitive) crosses. Our current goal is to understand the relationship between this DNA interval and $rfk\text{-}2^{UV}$.

The unexplored apical and subapical organization of the endoplasmic reticulum in growing hyphae of *Neurospora crassa*

Martínez-Andrade, J.M., Roberson, R.W., and Riquelme, M.

CICESE, Ensenada, ASU, Arizona, USA

The cytoplasmic order of the endoplasmic reticulum (ER) in relation to polarized protein secretion in fungal hyphae is largely unknown. To understand the domains and shapes of the ER in *Neurospora crassa*, we analyzed the organization of YOP-1, a predicted ER tubule-forming protein. Remarkably, YOP-1-GFP was found mostly at the peripheral ER (pER) at apical and near-apical regions devoided of nuclei (14.1 ± 2.2 μm), where the ER chaperone BiP-RFP was absent. In contrast, BiP-RFP was primarily found in hyphal regions II and III (~12 to 60 μm), which have a high number of nuclei. In addition, YOP-1-GFP was revealed at dynamic ER patches near the hyphal apex, similar to the membranes stained with ER-Tracker Blue-White DPX in a WT strain. To test the extension of the ER, we performed 3-D reconstructions and recordings in near real time with spinning disk confocal microscopy. We observed several pER membranes interconnected, emanating from the last cortical nuclei in the hyphal region II and extending toward the apex. A few YOP-1-GFP pER membranes, surrounded the SPK core. We confirmed the presence of smooth ER (sER) at the apex and rough ER (rER) at the subapex by transmission electron microscopy. Finally, ER organization was disrupted by exposure of the hyphae to dithiothreitol (DTT), tunicamycin (Tm) and latrunculin (LatA). After 1h of exposure to DTT (10 mM) and Tm (50 μg/mL), YOP-1-GFP was no longer localized at the apex. Instead, it co-localized fully with BiP-RFP in clusters of pER membranes near nuclei. Interestingly, only the localization of YOP-

1-GFP but not that of BiP-RFP was affected in the apical dome by LatA (10 μM). Altogether, our results suggest that YOP-1 positive ER membranes at the hyphal apex correspond to interconnected tubular ER (of sER), whereas the BiP positive ER membranes at the subapex correspond to flattened cisternae (of rER). All the ER domains and shapes seem to be interconnected and their localization appear to be governed by the nuclei.

Origins of lineage-specific elements via gene duplication, relocation, and regional rearrangement in *Neurospora crassa*

Yen-Wen Wang, Zheng Wang, Oded Yarden, and Jeffrey P. Townsend

Yale School of Public Health

The origin of new genes has long been a central interest of evolutionary biologists. However, their novelty evades reconstruction by the classical tools of evolutionary modeling. This evasion of insight from deep ancestral investigation necessitates intensive study of model species within well-sampled, recently diversified, clades. One such clade is the model genus *Neurospora*, members of which lack recent gene duplications, yet harbor clusters of lineage-specific genes (LSGs) adjacent to the telomeres. Several *Neurospora* species are comprehensively characterized organisms apt for studying the evolution of LSGs. Using gene synteny, we documented that 78% of *Neurospora* LSGs clusters are located in chromosomal regions featuring extensive tracts of non-coding DNA and duplicated genes. Here we report several instances of LSGs that are likely from regional rearrangements and potentially from gene rebirth. To broadly investigate functions of LSGs, we assembled transcriptomics data from 68 experimental data points and identified co-regulatory modules using Weighted Gene Correlation Network Analysis, revealing that LSGs are widely but peripherally involved in known regulatory machinery for diverse functions. The ancestral status of *mas-1* and its neighbors was investigated in detail, suggesting that it arose from an ancient lysophospholipase precursor that is ubiquitous in lineages of the Sordariomycetes; *mas-1* plays a role in cell-wall integrity and cellular sensitivity to antifungal toxins. Our discoveries illuminate a "rummage region" in the *N. crassa* genome that enables formation of new genes and functions to arise via gene duplication and relocation, followed by fast mutation and recombination facilitated by tandem repeats and deconstrained non-coding sequences.

Elucidating mechanistic processes of the cell wall remodeling checkpoint in *Neurospora crassa*

Adriana M. Rico-Ramírez and N. Louise Glass

University of California, Berkeley

Allorecognition is the ability of cells to differentiate self from non-self. In *Neurospora crassa*, three allorecognition systems function as checkpoints during germling and hyphal fusion by restricting cell fusion between genetically incompatible strains. The cell wall remodeling (*cwr*) checkpoint acts upon cell contact, regulating cell wall dissolution and subsequent cell fusion of cells. The *cwr* region consists of two linked genes that are under severe linkage disequilibrium, *cwr-1* (NCU01380) and *cwr-2* (NCU01382). Phylogenetic analysis of a wild *N. crassa* population

showed that *cwr-1/cwr-2* alleles fall into six different haplogroups (HG). Previously we found that the CWR-1 encodes a protein with a polysaccharide monooxygenase (PMO) domain that oxidatively degrades chitin. The PMO domain is sufficient to cause cell fusion arrest and confers specificity by interacting in trans with CWR-2, a predicted transmembrane protein, but PMO catalytic activity is not required for allorecognition. PMO domain modeling from the six different CWR-1 haplogroups revealed two regions, L2 and LC, with high levels of structural variability. By constructing CWR-1 chimeras, we determined that the LC region is important for allorecognition, and confers specificity when interacting in trans with CWR-2. However, the LC region alone is not sufficient to confer allorecognition and cell fusion arrest. Modeling of the six different HGs of the transmembrane protein CWR-2 revealed variability in the two major domains (D2/D4) that are oriented towards the cell wall. CWR-2 chimeras were constructed that swapped the D2 or D4 regions, which altered the allelic specificity of CWR-2, although the cell fusion block was not as penetrant as with wild type CWR-2. Further analyses of CWR-2 chimeras from the different haplogroups will define the regions responsible for allorecognition and the cell wall remodeling checkpoint during cell fusion.

Convergence to Multicellularity: Understanding the contribution of chromatin modification to cell fate transitions in *Neurospora crassa*.

Abigail Deaven, Abigail Ameri, Zachary Lewis

University of Georgia

Multicellularity is a complex process that arose independently in the plant, animal, and fungal kingdoms, and typically requires intricate genetic and epigenetic regulation. In most eukaryotes, Polycomb Repressive Complex 2 (PRC2) regulates multicellular development by establishing domains of histone H3 lysine 27 tri-methylation (H3K27me3) across conditionally activated genes. In the model filamentous fungus *Neurospora crassa*, the majority of PRC2-target genes are exclusively expressed in perithecia; however, the general mechanisms that control specific gene activation in order to promote perithecial fate are poorly understood. Using ChIP-seq to profile the distribution of H3K27me3 during sexual development, we observed that the chromatin state was remarkably stable in sexual tissue despite gene upregulation. However, our analyses uncovered a predicted forkhead domain transcription factor (*vsd-1*), which is upregulated with a subtle loss of H3K27me3 across its gene body during sexual development. *vsd-1* is necessary for female fertility, and may be sufficient to drive perithecial induction. Thus, we propose that *vsd-1* acts as a pioneer factor to promote expression of genes in inaccessible chromatin domains during sexual development. Future work will profile the gene regulatory network controlling sexual development and identify regions of differential chromatin accessibility in vegetative and sexual tissue. This work will uncover novel mechanisms of epigenetic regulation of cell fate transitions in the fungal kingdom, which has important implications for the control of devastating fungal pathogens and the production of valuable secondary metabolites in medically and industrially relevant fungi.

Overexpression of the *Neurospora crassa* sexual development regulator *fsd-1* causes increased pigment production, reduced conidiation, and inhibits successful mating

Elizabeth Hutchison, Hannah Smith, Katherine Cotten, Kaitlyn Gera, and Margaux Hales

SUNY Geneseo

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. In addition, *fsd-1* is transcribed into three different transcripts, encoding for a similar protein but differing in their intron/exon structure. We constructed overexpression strains of all three transcripts, labeled with an N-terminal GFP tag. Overexpression strains have increased pigmentation, produce fewer conidia, and have slower growth compared to wild type. In addition, nuclei overexpressing *fsd-1* cannot be recovered from a cross. In order to gain insight into the potential *fsd-1* regulon, we conducted Illumina RNAseq to measure transcriptional changes during vegetative growth (for an overexpression strain) or during mating (for the f*sd-1* deletion strain). Gene expression changes matched the strain phenotypes we observed. For example, many of the significantly enriched gene ontology categories of differentially expressed genes between mutant and wild type strains belonged to the categories of melanin biosynthesis, cell wall synthesis, conidiation, and mating. Future directions include analysis of the phenotype and transcriptional profile of an *fsd-1* DNA binding domain mutant allele.

Circadian clock control of ribosome composition leads to rhythms in translation and promotes rhythmic termination fidelity

Teresa M. Lamb, Kathrina D. Castillo, Rachel Porter, Ebimobowei Preh, Sam O. Purvine, Cheng Wu, Matt S. Sachs, and Deborah Bell-Pedersen

Texas A&M University, College Station

Ribosome composition can vary depending on cell type, developmental or environmental state, translation rate or mRNA target. Here we describe changes in ribosome composition dependent on the circadian clock in *Neurospora crassa*. Mass spectrometry of ribosomes pelleted from wild type and clock mutant cells at different times of day identified six ribosomal proteins, and one ribosome-associated protein, that cycled in abundance dependent on clock. We focused on a non-essential eukaryotic specific ribosomal component, eL31, and independently confirmed clock control of the abundance of eL31-HA in purified ribosomes. To examine the role eL31 plays in circadian regulation of translation, ribosome-protected footprint (RPF)- and RNA- sequencing were performed on circadian-timed cells deleted for the gene encoding eL31, *rpl-31*. Deletion of *rpl-31* inhibited translation rhythms for 42% of rhythmically translated mRNAs (562/1328) and altered the translation of many mRNAs encoding trans-membrane domains, including ion transporters. Clock-dependent and eL31-enhanced rhythms in translation termination fidelity (stop codon read-through (SCR)) were observed in vivo. However, WT and Δ*rpl-31* cells had similar levels of SCR in vitro, where the ionic conditions are experimentally controlled. We predicted that altered translation of ion channels in Δrpl-31 might lead to increased Mg2+ levels, which would in turn drive SCR in vivo. ICP-MS elemental analyses supported this idea. Taken together these studies demonstrate that the circadian clock governs daily changes in ribosome composition that control rhythms in translation and impact translation fidelity through changes in intracellular Mg2+ levels.

Monday, October 2 - Evening Session

Posters Session 1

Circadian Clock Regulation of tRNA synthetases, mRNA translation, and translation fidelity in *Neurospora crassa*

Griffin Best, Kathrina Castillo, Emily Chapa, Deborah Bell-Pedersen

Texas A&M University, College Station

About half of the proteins synthesized in eukaryotic cells under control of the circadian clock arise from arrhythmic mRNAs, supporting a role for clock control of posttranscriptional mechanisms. In *Neurospora crassa*, the circadian clock controls rhythmic mRNA translation, in part, through regulation of the eIF2α kinase CPC-3 (the homolog of yeast and mammalian GCN2). CPC-3 phosphorylates and inactivates the translation initiation factor eIF2α during the subjective day, leading to rhythmic translation initiation of select mRNAs. We discovered that clock control of CPC-3 activity requires the rhythmic charging of tRNAval and rhythmic levels of the valyl-tRNA synthetase (ValRS). These data supported that circadian rhythms in tRNA synthetases (RS) lead to rhythms in the ratio of charged versus uncharged tRNAs that drive rhythmic CPC-3 activity and P-eIF2α levels. To determine if rhythmic CPC-3 regulation is specific to ValRS, we examined five other RS's: MetRS, PheRS, GlnRS, LeuRS and AspRS, and found that they are clock-controlled, all peaking in the subjective night. Understanding clock regulation of MetRS is of particular interest due to its function in charging initiator tRNAmet. Additionally, in mammalian cells, phosphorylation of MetRS by the clock-controlled MAP kinases ERK1 and ERK2 reduces MetRS specificity for tRNAmet which leads to methionylation of specific nonmethionyl tRNAs (Met-misacylation) and Met misincorporation into polypeptides that provides a sink for reactive oxygen species. These data support the intriguing idea that clock control of MAK1 and MAK2 (*N. crassa* homologs of ERK1 and ERK2) activity in *N. crassa* promotes Met-misacylation and increased Met incorporation in polypeptides during the day to protect the organism from circadian oscillations in ROS levels and to produce protein variants beyond what is encoded in the genome.

Characteristics of *Neurospora discreta* strains isolated from burnt Joshua trees after the Cima Dome fire in the Mojave Desert of California

Yagna Oza, Rubab Sheikh, Alexander J. Carrillo, M. Fabiola Pulido-Chavez, Dylan J. Enright, Sydney I. Glassman, Katherine A. Borkovich

University of California, Riverside

A lightning strike sparked the Cima Dome fire that burned more than 43,000 acres of the Mojave National Monument east of Barstow, California, in August 2020. The charred area included a large Joshua tree woodland. Patches of orange fungal growth were spotted on many Joshua trees within a few weeks after the fire. Samples were collected and geographic coordinates recorded for each tree. Single colony isolates from each of 21 samples were purified by streaking three times on sorbose medium. The 21 strains were subjected to ITS sequencing, with the results demonstrating

a close phylogenetic relationship to *N. discreta*. Plate growth assays at 25-45 degrees Celsius revealed differences between subgroups of strains, in growth rate and peak growth temperatures and overall morphology. The same was true for measurements of macroconidia production at different temperatures. A pairwise mating test between all 21 strains and wild type *N. discreta* strains showed evidence for two mating types in the population. Statistical clustering of the phenotypic data and RNAseq analysis are in progress.

Characterization of the carbohydrate-binding domain in two *Neurospora crassa* cell wall proteins

Apurva Chatrath, Kevin Greeley, Gabriela Maciel, and Stephen J. Free

SUNY University at Buffalo

NCW-1 and NCW-3 are major non-GPI anchored cell wall glycoproteins with carbohydrate-binding domains that are expressed in vegetative hyphae. The main goal of this study was to identify the substrate polysaccharide they bind to and determine what functions they might provide to the cell wall. We characterized mutants lacking NCW-1 and NCW-3 and found them to be morphologically indistinguishable from the wild type. The proteins were cloned and expressed in wild type Neurospora cells with a HIS6 tag (both proteins) and with a mCherry fluorescent tag (NCW-3 only). The proteins were purified on nickel columns and characterized. NCW-3 was found to bind to chitin magnetic beads and specifically recognizes N-acetyl-glucosamine. NCW-3 may play a role in the incorporation of chitin into the fungal cell wall. NCW-1 is homologous to a protein from Aspergillus that deacetylates galactosaminogalactan, a cell wall polysaccharide. An analysis of wild type and NCW-1 mutant polysaccharides showed that the NCW-1 mutant is unable to deacetylate an N-acetylgalactosamine-containing polysaccharide. The genes for galactosaminogalactan synthesis and deacetylation are found throughout the fungal kingdom and the function of galactosaminogalactan has not yet been fully elucidated. We found the NCW-1 mutant generates a hyphal matrix with less tensile strength than the wildtype and the mutant hyphae are defective in their ability to adhere to a glass surface. We hypothesize that the NCW-1 deacetylated galactosaminogalactan could serve as intercellular glue by binding to hydrophilic surfaces and/or to negatively charged polysaccharides found in neighboring cells and in plant cell walls.

VE-1 regulation of MAPK signaling controls sexual development in *Neurospora crassa*

Sara Cea-Sánchez, Sara Martín-Villanueva, Gabriel Gutiérrez, David Cánovas and Luis M. Corrochano

University of Seville, Spain

The velvet complex is a fungal-specific protein complex that participates in the regulation of gene expression during development, pathogenesis, and secondary metabolism in response to environmental signals such as light. In *Neurospora crassa* the velvet complex is composed of VE-1, VE-2, and LAE-1. Strains with deletions in *ve-1* or *ve-2* have increased conidiation, and a delayed and reduced sexual development. Alterations in the development of female structures

(protoperithecia) in the *ve-1* and *ve-2* mutants suggested that a protein complex composed of VE-1/VE-2 regulates transcription during sexual development. The transcriptome of wild-type and *ve-1* mutant strains was characterized in a time-course experiment during sexual development in dark and light. We have identified 2,117 genes with different transcriptional profiles between the wild-type and the mutant strain in cultures kept in the dark, and 4,364 genes when cultures were kept in the light with an overlap of 1,648 genes. Among the misregulated genes, we detected genes that are known for their regulatory roles in sexual development, including genes in the mitogen-activated protein kinase (MAPK) signaling pathways, cell-cell fusion genes (ham genes) and transcription factor genes involved in fruiting body development. We have detected in vitro binding of VE-1 and VE-2 to the promoter sequences of *mak-1*, *mak-2*, *mek-2* and *os-4*, suggesting that VE-1/VE-2 plays a direct regulatory role in the transcription of MAPK genes. Furthermore, we detected transcription of *ve-1*, *ve-2*, and *lae-1* during all stages of sexual development, but the three proteins were not detected in the later stages of development (96 and 144 hours after fertilization). Our results suggest that the absence of VE-1 results in transcriptional changes that disrupt the signal transduction cascade regulating sexual development in *N. crassa*. Grant PID2021-128001OB-I00 funded by MCIN/AEI/ 10.13039/501100011033 and by "ERDF A way of making Europe".

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Characterization of the need for Galactofuranose during the *Neurospora crassa* life cycle

Protyusha Dey, Hayden Schaff, Christian Heiss, Griffin Keiser, Tatiana Rojo Moro, Parastoo Azadi, Pavan Patel and Stephen. J Free

SUNY University at Buffalo

Galactofuranose is found as a constituent in the cell walls of filamentous fungi. The galactofuranose residues are an integral part of the N-linked oligosaccharides, O-linked oligosaccharides, and GPI- anchored galactomannan. The biosynthetic pathway of galactofuranose has three consecutive steps which are catalyzed by three groups of proteins: UDP-galactose mutase, UDP-galactofuranose translocases and galactofuranose transferases. The Neurospora genome contains a single UDP-galactose mutase gene (ugm-1 /NCU01824) and two UDP-galactofuranose translocases, used to import UDP-galactofuranose into the lumen of the Golgi apparatus (ugt-1/NCU01826 and ugt-2/NCU01456). The results of our study demonstrate that loss of galactofuranose synthesis or its translocation into the lumen of the secretory pathway affects the morphology and growth rate of the vegetative hyphae, the production of conidia, and dramatically affects the perithecia development. In mutants that are unable to make galactofuranose or transport it into the lumen of the Golgi apparatus, ascospore development is aborted soon after fertilization and perithecium maturation is aborted prior to the formation of the neck and ostiole. Cloning and expression of the ugm-1 gene in ‚àÜugm-1 mutant restores the wild type phenotypes.

Topological Consequences of Large-Scale Genome Rearrangements in *Neurospora crassa*

Clayton Hull-Crew, Farha Kaddar, Sara Rodriguez, Yulia Shtanko, Debbie Dellacroce, Victoria Toscano, Andrew D. Klocko

University of Colorado Colorado Springs (UCCS)

Populations of a single species can accumulate vast genetic diversity over time, frequently resulting from alterations that change the order of the underlying DNA in an organism’s genome (syntenic changes). Known impacts that can affect the synteny of a genome include duplications, deletions, and translocations (large genome rearrangements), all of which have previously been shown as causative to genetic disease, particularly cancer in humans. These structural variants are known to impact genome function, including the regulation of transcription, as translocations have been observed to increase the expression of oncogenes. However, many syntenic changes are harmless and/or externally invisible, implying that some structural variants minimally impact genome function, or the existence of compensatory mechanisms to preserve gene expression and cell line viability. Importantly, any structural variant would alter genome organization in a eukaryotic nucleus. Previous work has shown that epigenetic factors, including histone post-translational modifications delineating heterochromatin (e.g., H3K9me3) are major determinants for organizing the eukaryotic genome: silent regions enriched with H3K9me3 interact, and are important for the regulation of gene expression. However, current research lacks an adequate understanding of the relationship between genome structure and epigenetic control for

coordinating eukaryotic gene expression. To address this, I will examine strains of the filamentous fungus *Neurospora crassa* that contain single translocations for distribution of key histone post-translational modifications, chromosome conformation, and gene expression. Through comparison to well established wildtype Neurospora strains, this comprehensive approach should elucidate the impact of large syntenic alterations on genome organization and function.

Rhythmic interaction between ZUOTIN and ribosomes may promote daily rhythms in protein folding and activity

Madhusree Gangopadhyay, Teresa Lamb, Kathrina D. Castillo, Tamika Harford and Deborah Bell-Pedersen

Texas A&M University, College Station

Several ribosome-associated chaperones bind nascent polypeptide chains to mediate co-translational folding. Zuotin (ZUO) is a part of the ribosome-associated complex (RAC) that functions as a ribosome co-chaperone. In yeast cells, ~ 30% of ribosomes associate with the RAC, suggesting some level of specificity. Quantitative mass spectrometry across circadian time in *Neurospora crassa* revealed that the interaction between ZUO and ribosomes is clock-controlled. Thus, we hypothesized that the rhythmic ZUO/ribosome interaction may lead to rhythms in the folding of specific protein targets. Protein aggregation assays confirmed that ZUO promotes proper protein folding in *N. crassa*. Identification of misfolded proteins by mass spectrometry revealed that ZUO-dependent folding targets included protein trafficking, translation and spliceosome components. Experiments are currently underway to determine if cellular protein folding is rhythmic and dependent on ZUO and/or FRQ. Interestingly, while the interaction of ZUO with ribosomes is clock-controlled, ZUO protein levels do not cycle. Thus, the daily rhythm in folding of specific protein targets, potentially including ZUO itself, likely contributes to protein activity rhythms in the absence of rhythmic abundance.

Overexpression of the *Neurospora crassa* sexual development regulator *fsd-1* causes increased pigment production, reduced conidiation, and inhibits successful mating

Elizabeth Hutchison, Hannah Smith, Katherine Cotten, Kaitlyn Gera, and Margaux Hales

SUNY Geneseo

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phenotypes we observed. For example, many of the significantly enriched gene ontology categories of differentially expressed genes between mutant and wild type strains belonged to the categories of melanin biosynthesis, cell wall synthesis, conidiation, and mating. Future directions include analysis of the phenotype and transcriptional profile of an fsd-1 DNA binding domain mutant allele.

Distributed multivalent interactions in a protein complex in the circadian clock turns an hourglass into a persistent oscillator.

Meaghan Jankowski, Daniel Griffith, Divya Shastry, Jacqueline Pelham, Garrett Ginell, Joshua Thomas, Pankaj Karande, Alex Holehouse, and Jennifer Hurley.

Rensselaer Polytechnic Institute

The circadian clock organizes a wide range of cellular physiology through a molecular circuit composed of a Transcription-Translation Feedback Loop. To study the mechanisms underlying clock regulation, we need to know more about clock protein interactions both within the core loop, as well as with their downstream targets that determine clock output. Interestingly, many core clock proteins are enriched in intrinsically disordered regions (IDRs) that lack a fixed three-dimensional structure. IDRs often contain Short Linear binding Motifs (SLiMs) that mediate specific interactions with other proteins, so it is of interest to identify which IDRs might be important for the formation of clock protein complexes. However, these more flexible proteins are more difficult to express and purify for typical biophysical methods. To address this issue, we employed a synthetic peptide microarray approach to the disordered clock protein FREQUENCY (FRQ) from *N. crassa*. We were able to recapitulate a previously-known SLiM for its well-known interactor, FRQ-interacting RNA Helicase (FRH), but also extend this SLiM to include novel critical residues. We also discovered positive-charge clusters or "blocks" of residues within FRQ that interact non-specifically with FRH via electrostatic interactions. Mutating a positive-charge block in FRQ led to a lengthening of clock period and loss of physiological clock output, while binding with FRH was maintained. We cannot currently explain the mechanism leading to the extended clock period in this mutant. Our results overall support a fuzzy-like complex model for FRQ and FRH that enables the persistence of circadian oscillations and appropriate downstream interactions, rather than being necessary for transcriptional repression. We found these positively charged clusters were a consistent molecular feature throughout circadian clocks in eukaryotes, suggesting that dynamic fuzzy-like complexes may more broadly support clock function.

Circadian clock control of ribosome composition leads to rhythms in translation and promotes rhythmic termination fidelity

Teresa M. Lamb, Kathrina D. Castillo, Rachel Porter, Ebimobowei Preh, Sam O. Purvine, Cheng Wu, Matt S. Sachs, and Deborah Bell-Pedersen

Texas A&M University, College Station

Ribosome composition can vary depending on cell type, developmental or environmental state, translation rate or mRNA target. Here we describe changes in ribosome composition dependent on

the circadian clock in *Neurospora crassa*. Mass spectrometry of ribosomes pelleted from wild type and clock mutant cells at different times of day identified six ribosomal proteins, and one ribosome-associated protein, that cycled in abundance dependent on clock. We focused on a non-essential eukaryotic specific ribosomal component, eL31, and independently confirmed clock control of the abundance of eL31-HA in purified ribosomes. To examine the role eL31 plays in circadian regulation of translation, ribosome-protected footprint (RPF)- and RNA- sequencing were performed on circadian-timed cells deleted for the gene encoding eL31, *rpl-31*. Deletion of *rpl-31* inhibited translation rhythms for 42% of rhythmically translated mRNAs (562/1328) and altered the translation of many mRNAs encoding trans-membrane domains, including ion transporters. Clock-dependent and eL31-enhanced rhythms in translation termination fidelity (stop codon read-through (SCR)) were observed in vivo. However, WT and Δ*rpl-31* cells had similar levels of SCR in vitro, where the ionic conditions are experimentally controlled. We predicted that altered translation of ion channels in Δrpl-31 might lead to increased Mg2+ levels, which would in turn drive SCR in vivo. ICP-MS elemental analyses supported this idea. Taken together these studies demonstrate that the circadian clock governs daily changes in ribosome composition that control rhythms in translation and impact translation fidelity through changes in intracellular Mg2+ levels.

Characterizing the landscape of histone post-translational modifications in strains of *Neurospora crassa* with single translocations.

Farh Kaddar, Nickolas M. Lande, Clayton Hull-Crew, Sara Rodriguez, Andrew D. Klocko

University of Colorado Colorado Springs (UCCS)

Large genome rearrangements (e.g., “translocations”), are where a double-strand DNA break is improperly repaired, causing the movement of a large section of DNA from a donor chromosome into a different genomic location. Translocations are often seen in human cancers, but the effect of these large genome rearrangements on genome function are not well understood. In particular, little information exists about how translocations affect the deposition of post-translational modifications (PTMs) on histones, including the methylation of lysine residues, many of which are considered “epigenetic” in nature. To understand how large genome rearrangements affect eukaryotic genome function, we examined the enrichment of histone PTMs in strains of the filamentous fungus *Neurospora crassa*, each of which contain a single translocation. Previous work has shown that the di- or tri-methylation of lysine 27 on histone H3 (H3K27me2/3), which demarcates facultative heterochromatin in Neurospora, can be altered in translocation strains. Here, we examined the enrichment of unique PTMs and their relation to translocation breaks in *Neurospora crassa*. We performed Chromatin Immunoprecipitation-sequencing (ChIP-seq) to analyze enrichment levels of the tri-methylation of lysine 9 on histone H3 (H3K9me3), which demarcates silent heterochromatic regions, as well as the tri-methylation on lysine 4 on histone H3 (H3K4me3), a histone mark that is associated with actively transcribed genomic loci. Comparative analysis of the ChIP-seq data between a wildtype (WT) strain and several translocation strains could highlight changes in the silent or active histone PTMs, which might elucidate how translocations impact genome function. We will present our most recent analysis of the deposition of histone PTMs in single-translocation strains, which may provide more insight into how translocations influence epigenetic genome function in higher eukaryotes.

Tuesday, October 3 - Morning Session
Fungal Cell Metabolism and Signaling

Identification of F-Box proteins involved in the switch to cellulolytic metabolism

Lisa T. Meyer, Maria A. Crivelente Horta, Gustavo H. Goldman, J. Philipp Benz

TUM School of Life Sciences, Technical University of Munich

Fungi are of particular interest for the circular bioeconomy to decompose renewable plant biomass. However, natural "brakes" exist, preventing the fungi to waste energy. One of these is carbon catabolite repression (CCR), a highly conserved and multi-faceted signaling process leading to the repression of lignocellulose utilization in the presence of preferred carbon sources. Therefore, there is a strong interest to overcome this internal "brake". The proteins involved in CCR are known to be regulated, among other things, by targeted degradation. Key factors are the so-called F-Box proteins, which destine proteins for the proteasomal degradation process. F-Box proteins are found in all eukaryotes and are known to be involved e.g. in carbohydrate sensing and sulfur assimilation. Nevertheless, the specific function of the majority of the F-Box proteins present in filamentous fungi remains enigmatic. We employed Neurospora crassa to identify F-Box proteins involved in the switch between CCR and lignocellulose utilization. N. crassa deletion strains of 40 genes with putative F-Box protein function were screened to identify aberrant phenotypes related to CCR. Several deletion strains were found to display significant CCR-repressed or de-repressed phenotypes. Four candidate F-Box proteins with a strong potential for regulatory importance in lignocellulose signaling pathways were subjected to GFP pull-down experiments to elucidate potential interaction partners. In parallel, the transcriptomic changes in the corresponding *fbx* gene deletion strains during the switch from repressed to de-repressed states were assayed by RNAseq to identify their regulatory influence on carbon utilization. The results of these experiments allow for a better understanding of the function of F-Box proteins during carbohydrate metabolic switches in general and will be essential to allow rational strain modifications, leading to improved enzyme-producing strains of high interest for the industry.

Characterizing the genetics of *Neurospora crassa* environmental sensing using functional genomics

Lori B. Huberman, Renato Carvalho, Joshua D. Kerkaert, Brandon Reyes-Chavez

Cornell University

Fungi have evolved signaling networks to respond to chemical signals in their environment. These chemical signals include toxins, such as fungicides, that fungi must avoid physically, biologically, or chemically. To investigate the signaling and transcriptional networks that respond to these chemical signals, we are using a combination of massively parallel screens of barcoded mutant libraries and transcriptional profiling in *Neurospora crassa* and a distantly related basidiomycete yeast, *Rhodosporidium toruloides*. By comparing the genes necessary to respond to environmental chemical signals in these distantly related fungi, we can identify conserved and divergent mechanisms of environmental sensing.

Translational regulation through the upstream open reading frame encoded inositol regulatory peptide

Cheng Wu, Ivaylo P Ivanov, Ananya Dasgupta, Thomas E Dever, Matthew S Sachs

Texas A&M University

Up to 50% of eukaryotic mRNAs contain upstream open reading frames (uORFs). In some cases, these uORFs are evolutionarily conserved and serve critical roles in controlling gene expression. An important emerging class of regulatory uORF-encoded peptides cause ribosomes to stall in response to metabolites. We discovered a uORF in the 5'-leaders of fungal mRNAs specifying the first enzyme necessary to synthesize the central metabolic molecule inositol, inositol-3-phosphate synthase. This uORF's initiation codon is often a near-cognate start codon in good context, as in Neurospora, but can be an AUG in poor context. Based on experimental data, we have named the conserved uORF-encoded peptide the inositol regulatory peptide (IRP). Analyses of IRP function in *N. crassa* in vivo provided evidence that the endogenous gene (*inl*, NCU06666) is controlled at the translation level by exogenous inositol. In vivo and in vitro analyses showed that inositol regulation of reporter genes is sensitive to mutations in conserved IRP residues. In fungal and mammalian cell-free translation systems, the IRP functioned either as a uORF or when fused in-frame with a reporter gene. Ribosome profiling using the *N. crassa* cell-free system showed inositol caused ribosome stalling at the wild-type uORF but not mutated uORF stop codons. The wild-type but not mutated IRP also regulated the expression of reporter genes in transfected mammalian cells in response to exogenous inositol. These data are consistent with models for IRP regulation in which inositol or a closely related molecule interacts directly with ribosomes, the nascent IRP, or both, to interfere with peptidyltransferase center activity. Stalling would act directly to reduce leaky scanning. Inositol-regulated ribosome stalling would thus regulate inositol-3-phosphate synthase biosynthesis and inositol metabolism.

Impact of G protein signaling on carbon catabolite repression and production of cellulases

Yagna Oza, Logan Collier, Katherine Borkovich

University of California, Riverside

Filamentous fungi utilize lignocellulolytic biomass through the secretion of cellulases that decompose cellulose into simpler molecules such as cellobiose and glucose. Our lab has previously demonstrated the crucial role of G-protein signaling in cellulose degradation in *Neurospora crassa*, with no detectable glucose release activity detected in some G-protein subunit deletion mutants after pre-growth in glucose and then transfer to cellulose medium. Building on these results, we are looking further into the regulation of specific classes of cellulase enzymes by G-proteins and also at how multiple G-protein subunits work together to control the degradation of cellulose. We are also exploring the possible involvement of G-protein signaling in controlling carbon catabolite repression and utilization of non-preferred carbon sources in N. crassa. Results will be presented from testing the functionality of CCR in G-protein mutants. Quantification of the activity of specific cellulases secreted by each gene deletion mutant will further elucidate the role of each G-protein subunit in cellulose degradation in *N. crassa*.

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Meaghan Jankowski, Daniel Griffith, Divya Shastry, Jacqueline Pelham, Garrett Ginell, Joshua Thomas, Pankaj Karande, Alex Holehouse, and Jennifer Hurley.

Rensselaer Polytechnic Institute (RPI)

The circadian clock organizes a wide range of cellular physiology through a molecular circuit composed of a Transcription-Translation Feedback Loop. To study the mechanisms underlying clock regulation, we need to know more about clock protein interactions both within the core loop, as well as with their downstream targets that determine clock output. Interestingly, many core clock proteins are enriched in intrinsically disordered regions (IDRs) that lack a fixed three-dimensional structure. IDRs often contain Short Linear binding Motifs (SLiMs) that mediate specific interactions with other proteins, so it is of interest to identify which IDRs might be important for the formation of clock protein complexes. However, these more flexible proteins are more difficult to express and purify for typical biophysical methods. To address this issue, we employed a synthetic peptide microarray approach to the disordered clock protein FREQUENCY (FRQ) from *N. crassa*. We were able to recapitulate a previously-known SLiM for its well-known interactor, FRQ-interacting RNA Helicase (FRH), but also extend this SLiM to include novel critical residues. We also discovered positive-charge clusters or "blocks" of residues within FRQ that interact non-specifically with FRH via electrostatic interactions. Mutating a positive-charge block in FRQ led to a lengthening of clock period and loss of physiological clock output, while binding with FRH was maintained. We cannot currently explain the mechanism leading to the extended clock period in this mutant. Our results overall support a fuzzy-like complex model for FRQ and FRH that enables the persistence of circadian oscillations and appropriate downstream interactions, rather than being necessary for transcriptional repression. We found these positively charged clusters were a consistent molecular feature throughout circadian clocks in eukaryotes, suggesting that dynamic fuzzy-like complexes may more broadly support clock function.

Differences in thermotolerance between ecotypes of *Neurospora discreta* are primarily due to only two genomic regions

Aaron J. Robinson, Donald O. Natvig, John W. Taylor, Igor Grigoriev, Kerrie Barry, Patrick Chain

Los Alamos National Laboratory

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 35C, but isolates from New Mexico grow much better above 40C. 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 (CSP), we obtained complete genome sequences for 82 progeny from crosses with parents from NM and AK. Progeny were selected to exhibit 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 and Oxford Nanopore MinION platforms. Quantitative trait locus (QTL) analysis was performed with Illumina data and bulked-segregant analysis was performed with MinION long-read data. These analyses, while failing to find clear global differences in genome content or amino acid composition, demonstrated two genomic regions associated with thermotolerance. A region of linkage group III overlaps with a region previously identified in *N. crassa* that may be under selection with respect to temperature adaptation, and a region on linkage group I appears to play a secondary role in the thermotolerant phenotype. Observed amino acid modifications in this region could contribute to thermotolerance, and we have identified several genes of unknown function which are conserved across the fungal tree of life which we are working on annotating. All isolates have been deposited at the Fungal Genetic Stock Center and serve as valuable resources for other Neurospora research.

FungiDB: Tools for Genomic-Scale Data Exploration, Analysis, Integration and Discovery

David S Roos,

University of Pennsylvania, Philadelphia PA 19104 USA … on behalf of the FungiDB team (a component of the VEuPathDB Bioinformatics Resource Center)

Biomedical research is increasingly driven by Big Data: genome sequences & diversity data, all manner of multi-Omics datasets, etc. How can we effectively collect, store, maintain and integrate this information to ensure FAIR (Findable, Accessible, Interoperable, Reusable) data access, advancing biological under¬standing and defining targets for further study in the lab, field & clinic? The Eukaryotic Pathogen & Vector Genomics Resource (VEuPathDB.org) – including FungiDB.org – provides a robust, sustain¬able data-mining resource, accessed by 1000s of researchers daily, and demonstrably expediting disco¬very & translational research on diverse eukaryotic microbes (fungi & protists). Staff will be available at Neurospora 2023 to demon¬strate database functionality and discuss topics of community interest, e.g.:

» accessing & interpreting information on genes, genomes, population diversity, comparative genom¬ics, DNA/protein motifs, protein structures, interactomes, epigenetics, transcript¬omes, proteomes, meta¬bolomes, pathways, subcellular localization, phenotypic character¬ization, orthology-based functional inference, automated & curated annotation, etc

» strategies for integrating & interrogating diverse datasets … and analyzing & sharing the results obtained

» assessing & improving the quality and accuracy of available annotation … capturing expert knowledge from the community

» analyzing your own (or any public) datasets via the free, private, easy-to-use, cloud-based VEuPathDB Galaxy instance … and integrating/querying results in the context of other data in FungiDB

» identification and prioritization of new Neurospora (and other) datasets for integration, and develop¬ment of new database features; prospects for sustainable coordination with other resources
» new datasets & functionalities currently under development at VEuPathDB/FungiDB … what to expect over the coming years
» additional help & assistance with database mining & FAIR data access/sharing

Tuesday, October 3 - Afternoon Session

Posters Session 2

Methylxanthines modulate circadian period length independently of the action of phosphodiesterase

Consuelo Olivares-Yañez, María P. Alessandri, Loreto Salas, Luis F. Larrondo

P. Universidad Catolica de Chile-iBio

In *Neurospora crassa*, caffeine and other methylxanthines are known to inhibit phosphodiesterase (PDE) activity, leading to augmented cAMP levels. In this organism it has also been shown that the addition of these drugs significantly lengthens circadian period, as seen by conidiation rhythms. Utilizing in vivo bioluminescence reporters, pharmacological inhibitors as well as cAMP analogs, we revisited the effect of methylxanthines and the role of cAMP signaling in the *Neurospora* clockworks. We observed that caffeine, as all the tested methylxanthines, led to significant period lengthening, visualized with both core-clock transcriptional and translational reporters. Remarkably, this phenotype is still observed when phosphodiesterase (PDE) activity is genetically or chemically (IBMX) abrogated. Likewise, methylxanthines still exert a period effect in several cAMP signaling pathway mutants, including mutants in Adenylate Cyclase (*cr-1*) or PKA (Δ*pkac-1*), suggesting that these drugs lead to circadian phenotypes through mechanisms different from the canonical PDE-cAMP-PKA signaling axis. Thus, this study highlights the strong impact of methylxanthines on circadian period in Neurospora, albeit the exact mechanisms still remain somehow elusive.

Neurospora rfk-1: a Spore killer gene with an unusual intron

Shahriar Mahmud, Gabriela Mendoza-Rangel, Nicholas Rhoades, Tom Hammond

School of Biological Sciences, Illinois State University, Normal, IL, USA

The selfish genetic element *Spore killer-2* (*Sk-2)* is transmitted to viable *Neurospora* ascospores in a biased manner through spore killing. Spore killing is dependent on a gene called *required for killing-1* (*rfk-1*). This gene contains four exons and three introns. Intron 1 is unusual because it contains seven repeats of a 46-48 bp sequence. While the importance of Intron 1 to r*fk-1* function is unclear, our preliminary data suggests that Intron 1 is critical for phenotypic expression of *rfk-1*. Here, we present our current results concerning the roles of *rfk-1* introns in *rfk-1* phenotypic expression.

Fungal Genetics Stock Center Status Report

Jaideep Mallick and John Leslie

Fungal Genetic Stock Center, Kansas State University, Manhattan, Kansas

The Fungal Genetics Stock Center has expanded from 400 strains in 1960 to well over 90,000 strains from 40 species and almost a thousand plasmids. Our project with the K-State library to make all of the FGSC strain deposit sheets available through an on-line archive is nearly complete and will enable researchers to have access to strain details previously available only to FGSC staff. The most recent species addition is *Candida auris*, an emerging health risk. Orders run around 500 per year and the revenue generated funds the salary for a half-time technician. With Kevin McCluskey's departure, FGSC now has a new curator, Dr. Jaideep Mallick, who took up the position in the 4th quarter of 2022. Support for the curator is partially from K-State, a grant from Open Philanthropy, and a DoD grant that began in October 2022 and has a five-year term. The DoD project focuses on *Agrobacterium tumefaciens* and plasmids that can be used with it to transform fungi with genes that can be used to degrade wastes found in post-military environmental settings. The goal is to optimize transformation protocols that use *A. tumefaciens* for fungal transformations and to make strains, plasmids and protocols readily available to the fungal research community. Please continue to send your materials (and deposit sheets!) to us so that we can expand the collection further and keep it relevant!

Neurospora rsk: a Spore killer resistance gene that functions in multiple tissue types

Gabriela Mendoza-Rangel, Shahriar Mahmud, Nicholas Rhoades, and Tom Hammond

School of Biological Sciences, Illinois State University, Normal, IL, USA

Neurospora Spore killer-2 (*Sk-2*) is a complex genetic element found within a recombination-suppressed region of Chromosome III. *Sk-2* is unusual because it is transmitted to the surviving offspring of an *Sk-2* x *Sk-2*-sensitive cross in a biased manner. This bias is dependent on a gene called *resistance to Spore killer* (*rsk*). We have recently found that *rsk*-mediated ascospore survival can be studied in vegetative tissue. This finding should help us elucidate *rsk*'s resistance mechanism. Here, we present our experimental plan and recent findings toward the goal of understanding rsk-mediated ascospore survival.

Toward Unraveling the Roles of TEA Proteins: Insights into Cytoskeletal Dynamics and Growth Symmetry in Neurospora crassa

Callejas-Negrete, O. A. and Mouriño-Pérez R. R.

Departamento de Microbiología. Centro de Investigación Científica y Educación Superior de Ensenada. México

The coordinated movement of cellular components required for polarized growth through actin and microtubules is very important. Despite this, our understanding of the proteins responsible for organizing and associating both cytoskeletal elements in the tip of *Neurospora crassa* cells remains limited. Notably, the TEA (Tip Elongation Aberrant protein) complex has previously been linked to both actin and microtubules in *Schizosaccharomyces pombe* and *Aspergillus nidulans*. In our study, we conducted a detailed analysis of the dynamic behavior of TEA-1, TEA-4, and TEA-5 proteins tagged with either GFP or mCHFP. We also characterized deletion mutants to investigate their roles in relation to the actin and microtubular cytoskeleton. Surprisingly, our findings

indicated that neither *tea-1* nor *tea-4* were essential for cell viability, except tea-5. However, Δ*tea-1* and Δ*tea-4* mutants exhibited a slight reduction in growth rate, while the heterokaryotic Δ*tea-5* mutant was significantly more affected, particularly in terms of biomass production compared to the wild-type strain. Interestingly, the hyphal morphology in both Δ*tea-1* and Δ*tea-4* mutants remained largely unaffected, although there was a noticeable increase in hyphal diameter in the Δ*tea-4* mutant. Branching was unaffected in Δ*tea-1*, while the Δ*tea-4* mutant displayed a higher number of branches compared to the wild type. Conidiation, the formation of asexual spores, was severely impacted in all the mutants. Notably, TEA-1-GFP and TEA-4-GFP proteins were found to accumulate as bright spots in the location where the germ tube emerges. After germination, both proteins were predominantly present in the apical dome, avoiding the Spitzenkörper region. Meanwhile, GFP-TEA-5 was primarily localized in the apical dome of mature hyphae. In addition, TEA-1-GFP, TEA-4-GFP, and GFP-TEA-5 were observed in forming septa, appearing as double rings flanking the plasma membrane. These findings suggest that this group of proteins may not play a direct role in determining the directionality of polarized growth. Instead, they appear to be involved in breaking symmetry during conidial germination, conidial formation, and the organization of actin filaments in the apical region of *N. crassa*

Mechanism of circadian clock control of rhythmic translation in *Neurospora crassa*

Ebimobowei Preh and Deborah Bell-Pedersen

Texas A&M University, College Station

The circadian clock in *Neurospora crassa* regulates daily rhythms in the phosphorylation and daytime inactivation of the conserved translation initiation factor eIF2α which is responsible for the rhythmic translation of ~15% of mRNAs. Cycling phosphorylated eIF2α levels require rhythmic activation of the eIF2α kinase CPC-3 (the homolog of yeast and mammalian GCN2). However, how the clock controls the activity of CPC-3 is not known. To be activated, CPC-3 forms a complex with GCN1, which helps to bring uncharged tRNAs to the tRNA binding domain on CPC-3. In Saccharomyces cerevisiae, activation of GCN2 requires direct interaction of GCN1 and GCN2 with ribosomes. Based on these data, I hypothesized that *N. crassa* GCN1 and CPC-3 rhythmically interact with the ribosome, and that this interaction is necessary for rhythmic CPC-3 activity. To test this hypothesis, the interaction of CPC-3::V5 and GCN1 with ribosomes was examined. Ribosomes were purified by sucrose density gradient ultracentrifugation from cultures grown in constant dark (DD) and harvested every 4 hours in a circadian time course. While CPC-3::V5 and GCN1 were found to co-sediment with monosomes and polysomes, the interaction of CPC-3::V5 and GCN1 with ribosomes was arrhythmic in DD. Data showed that CPC-3 interaction with the ribosome is necessary for acute stress induction of eIF2α phosphorylation, but not for circadian clock regulation of eIF2α phosphorylation. Mutating GCN1 ribosome binding sites reduced the levels of phosphorylated eIF2α, but had no effect on its rhythmic accumulation. Together, these data suggest that GCN1 and CPC-3 interactions with the ribosome are not rhythmic and are not necessary for the rhythmic activity of CPC-3. Work is in progress to investigate the alternative hypothesis that the clock regulates the interaction between uncharged tRNA and GCN1, leading to rhythmic activity of CPC-3, and ultimately driving rhythms in the translation of select mRNAs.

RNAseq and targeted metabolomics analyses implicate G protein signaling in regulation of arginine and ornithine metabolism and compartmentation in *Neurospora crassa*

Monique Quinn, Alexander Carrillo, Lida Halilovic, Katherine A. Borkovich

University of California, Riverside

Resistance to inhibitors of cholinesterase 8 (RIC8) has been demonstrated to function as a non-receptor guanine nucleotide exchange factor (GEF) and protein chaperone for heterotrimeric Gα subunits. In *Neurospora crassa* RIC8 has been shown to act as a GEF for the Gα subunits GNA-1 and GNA-3 in vitro and to affect the stability of Gα subunits in vivo. Δ*ric8* strains possess numerous morphological defects, including macroconidiation in submerged cultures. Our laboratory performed RNAseq and targeted LC-MS to analyze the Δ*ric8* mutant strain for possible effects on metabolism in *N. crassa*. In the targeted metabolomics dataset, we observed that a number of metabolites involved in the arginine biosynthesis pathway, were present at different levels in the Δ*ric8* and wild type strains. However, RNAseq analysis revealed that the genes encoding enzymes involved in arginine biosynthesis were not differentially expressed. This result suggested that the enzymes may be regulated at the post-transcriptional level. Enzymatic assays for several pathway enzymes did not reveal differences between Δ*ric8* and wild type strains. Previous work in *N. crassa* has shown that some arginine biosynthetic metabolite levels are lower in conidia compared to hyphae, and that the majority of the cellular arginine and some arginine biosynthetic metabolite pools are contained in the vacuole. We are currently investigating expression of amino acid transporters in our RNAseq dataset, and current results will be presented.

***Neurospora crassa* SCK1 is required for circadian clock control of translation initiation factor eIF2α activity**

Manuel Ramirez, Deborah Bell-Pedersen

Texas A&M University, College Station

The circadian clock is an endogenous time keeping mechanism that controls rhythms in up to half of the eukaryotic genome at the level of transcript and/or protein abundance. The *Neurospora crassa* clock controls mRNA translation, in part, through the rhythmic and inhibitory phosphorylation of eukaryotic translation initiation factor subunit, eIF2α. In wild type *N. crassa*, phosphorylated eIF2α (P-eIF2α) peaks during the day and troughs at night, leading to increased mRNA translation at night. N. crassa CPC-3, the homolog of yeast and mammalian GCN-2, is the kinase responsible for rhythmic eIF2α phosphorylation, and CPC-3 activity is regulated by the clock. Several proteins are known to control GCN-2 activity in yeast and mammalian cells. To determine which, if any, of these proteins had a similar regulatory effect on rhythmic CPC-3 activity in *N. crassa*, rhythmic P-eIF2α levels were assayed in strains deleted for the putative CPC-3 regulators. *N. crassa* strains deleted for *stk-10*, the gene encoding SCK1 protein, abolished the rhythms in P-eIF2α levels. SCK1 is a serine/threonine kinase that may be regulated by the nutrient sensing TOR pathway. These data suggested a model in which TOR activates SCK1 and active SCK1 phosphorylates and inhibits CPC-3 kinase activity at night. Consistent with this model, when WT cells are treated with rapamycin, an inhibitor of TORC1, the levels of P-eIF2α are

significantly higher than in untreated WT cells and treated Δ*stk-10* cells. Experiments are in progress to determine if rhythms in SCK1 activity or abundance are necessary for CPC-3 rhythmic activity.

Differences in thermotolerance between ecotypes of *Neurospora discreta* are primarily due to only two genomic regions

Aaron J. Robinson, Donald O. Natvig, John W. Taylor, Igor Grigoriev, Kerrie Barry, Patrick Chain

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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 35C, but isolates from New Mexico grow much better above 40C. 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 (CSP), we obtained complete genome sequences for 82 progeny from crosses with parents from NM and AK. Progeny were selected to exhibit 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 and Oxford Nanopore MinION platforms. Quantitative trait locus (QTL) analysis was performed with Illumina data and bulked-segregant analysis was performed with MinION long-read data. These analyses, while failing to find clear global differences in genome content or amino acid composition, demonstrated two genomic regions associated with thermotolerance. A region of linkage group III overlaps with a region previously identified in *N. crassa* that may be under selection with respect to temperature adaptation, and a region on linkage group I appears to play a secondary role in the thermotolerant phenotype. Observed amino acid modifications in this region could contribute to thermotolerance, and we have identified several genes of unknown function which are conserved across the fungal tree of life which we are working on annotating. All isolates have been deposited at the Fungal Genetic Stock Center and serve as valuable resources for other *Neurospora* research.

A case for the Kinases: A Role for CKI in temperature compensation of the *Neurospora crassa* circadian clock

Elizabeth-Lauren Stevenson, Christina M. Kelliher, Jennifer J. Loros, Jay C. Dunlap

Geisel School of Medicine at Dartmouth

The molecular circadian clock in animals and fungi consists of a transcription-translation feedback loop that is regulated post-translationally throughout the circadian day by phosphorylation events. In the classic clock model *Neurospora crassa*, the positive arm of the clock, a heterodimeric complex of transcription factors, activates the transcription of the negative arm of the clock, Frequency (FRQ), which complexes with Casein Kinase I (CKI) to inactivate the positive arm via

phosphorylation, thereby inhibiting their own transcription. Several key features define circadian rhythms, including the maintenance of a consistent period across temperatures (temperature compensation/TC). This essential clock property is found in all organisms with circadian clocks, yet its mechanism remains undefined.
We discovered a novel mutation in CKI that confers a long period and severe undercompensation such that period shortens as temperature increases, suggesting a role for CKI in the TC mechanism of *Neurospora*. We find that reduction in CKI levels or activity causes the clock to be undercompensated, compared to a reduction in Casein Kinase II (CKII) activity conferring overcompensation. Inhibiting CKI in a CKII hypomorph still dose dependently altered its normally overcompensated TC profile, suggesting CKI is downstream of CKII in the TC mechanism. Hypothesizing that TC may be achieved by temperature-dependent differential phosphorylation of clock components, we performed phosphoproteomics in WT and CKI mutant backgrounds across a range of temperatures and identified phosphosites whose phospho-occupancy changes significantly with both temperature and genotype. These data provide support for a kinase-based model of temperature compensation.

Changes in core clock negative arm proteins reveal a relationship between the conformation of complexes and post-transcriptionally regulated proteins

Jackie Pelham, Alexander Mosier, Lucas Sutton, Samuel C Altshuler, Christopher Kirchhoff1, William Fall, Lisa Baik, Joanna Chiu, and Jennifer Hurley

Rensselaer Polytechnic Institute

The circadian clock consists of a transcription/translation negative feedback loop (TTFL) in order to anticipate changes in environmental queues and is conserved throughout species. Within this feedback loop, we would expect to see corresponding rhythmic protein and mRNA levels, however up to 80% of rhythmic proteins stem from non-rhythmic mRNA. This suggests the presence of an unknown source of post-transcriptional regulation. Additionally, the negative arm of this TTFL contains a conservation of intrinsically disordered proteins across phyla. Specifically, the negative arm proteins, FREQUENCY (FRQ) in *Neurospora crassa*, PERIODs in humans (hPER2) and *Drosophila melanogaster* (dPER) are conserved intrinsically disordered, flexible proteins that can occupy multiple conformations. We hypothesize that these negative arm proteins create time specific conformational changes that coordinate protein-protein interactions. Furthermore, conformational plasticity would facilitate the formation of time-specific protein "Hubs" imparting circadian post-transcriptional regulation. In *N. crassa*, we utilized a limited protease digestion approach, we demonstrated that FRQ shifts to a more open conformation correlating to an increase in phosphorylation. Furthermore, using FRQ targeting Co-immunoprecipitation mass spectrometry we showed that temporal conformational fluidity alters in accordance to changes in FRQ-centered macromolecular complexes. In *D. melanogaster*, we observed dPER's interactions are spatiotemporally regulated. Using computational techniques, we determined Short Linear Motifs (SLiMs) localize to disordered regions in FRQ, dPER and hPER. Computationally, we illustrated that SLiMs have a high tendency to occur in regions of phosphorylation and regions shifting from disordered to ordered. Interactors of FRQ and dPER oscillated in macromolecular complexes in a time specific manner; many of which correlated with post transcriptionally regulated proteins. Although the mechanism for post-transcriptional regulation remains unknown,

we suggest that the conserved intrinsic disorder of the negative arm proteins allow for conformational changes to correlate with the variation observed in protein-protein interactions of the core clock.

Novel microscopy tools reveal dynamic sub-cellular distributions of core clock components in *Neurospora crassa*

Ziyan Wang, Bradley M. Bartholomai, Jennifer J. Loros, Jay C. Dunlap

Geisel School of Medicine at Dartmouth

Circadian rhythms are endogenous daily oscillations driven by a molecular clock that helps organisms better coordinate with the environment. In *Neurospora crassa*, this clock regulates the expression of over 40% of the genome and affects spores' development and liberation. The core clock mechanism involves a phosphorylation-driven transcription/translation negative feedback loop with positive and negative elements. The transcription factor White Collar Complex (WCC) serves as the positive element driving the transcription of frequency (*frq*). The intrinsically disordered protein Frequency (FRQ), with other regulators, forms the negative element complex that inhibits the function of WCC and stops *frq* transcription. Molecular components of the circadian clock have been described over decades of genetic and molecular biological studies. However, little is known about their dynamics and regulation at the subcellular level.
Live-cell imaging has emerged as a valuable tool in circadian research. We implemented novel strategies and microscopy tools for Neurospora, including 4-color imaging and microfluidics compatible with multi-day growth, to facilitate live-cell imaging of low-abundance circadian proteins. Through multi-color live-cell imaging in single cells, we tracked the circadian dynamics of the subcellular localization of WCC and FRQ in high spatiotemporal resolution. We also observed *in vivo* highly dynamic LLPS-like behaviors of FRQ using the super-resolution SoRa microscope. Furthermore, by employing FRAP, we have unraveled the circadian-regulated nuclear import of FRQ and its underlying mechanism. We also optimized photoconvertible fluorescent proteins to facilitate further exploration of the nucleocytoplasmic transport of clock proteins.
Our work showcases the successful application of advanced microscopy techniques to gain insights into the intricate subcellular dynamics of circadian proteins, paving the way for a deeper understanding of circadian rhythms.

Origins of lineage-specific elements via gene duplication, relocation, and regional rearrangement in *Neurospora crassa*

Zheng Wang, Yen-Wen Wang, Oded Yarden, and Jeffrey P. Townsend

Yale School of Public Health

The origin of new genes has long been a central interest of evolutionary biologists. However, their novelty evades reconstruction by the classical tools of evolutionary modeling. This evasion of insight from deep ancestral investigation necessitates intensive study of model species within well-sampled, recently diversified, clades. One such clade is the model genus Neurospora, members of which lack recent gene duplications, yet harbor clusters of lineage-specific genes (LSGs) adjacent

to the telomeres. Several Neurospora species are comprehensively characterized organisms apt for studying the evolution of LSGs. Using gene synteny, we documented that 78% of Neurospora LSGs clusters are located in chromosomal regions featuring extensive tracts of non-coding DNA and duplicated genes. Here we report several instances of LSGs that are likely from regional rearrangements and potentially from gene rebirth. To broadly investigate functions of LSGs, we assembled transcriptomics data from 68 experimental data points and identified co-regulatory modules using Weighted Gene Correlation Network Analysis, revealing that LSGs are widely but peripherally involved in known regulatory machinery for diverse functions. The ancestral status of *mas-1* and its neighbors was investigated in detail, suggesting that it arose from an ancient lysophospholipase precursor that is ubiquitous in lineages of the Sordariomycetes; mas-1 plays a role in cell-wall integrity and cellular sensitivity to antifungal toxins. Our discoveries illuminate a "rummage region" in the *N. crassa* genome that enables formation of new genes and functions to arise via gene duplication and relocation, followed by fast mutation and recombination facilitated by tandem repeats and deconstrained non-coding sequences.

Novel Candidates in the Polycomb repression network

Rochelle Yap, Aileen Ferraro, Zachary Lewis

University of Georgia

Chromatin structure and its impact on coordinated gene expression is crucial for the development of healthy cells. Polycomb proteins, a class of chromatin modifying complexes, play a pivotal role in maintaining balanced gene expression across many eukaryotes. Polycomb Repressive Complex 2 (PRC2) catalyzes trimethylation of Lysine 27 on Histone H3 (H3K27me3) and establishes H3K27me3 associated gene-silencing. Any disruption to H3K27me3-mediated gene silencing can lead to unbalanced transcriptional profiles, disorganized chromatin, and disease. We aim to expand our understanding of how Polycomb proteins maintain transcriptionally repressed heterochromatin and contribute to genome stability, using the fungal organism, *Neurospora crassa*.
The discovery that most fungi encode Polycomb components provides an opportunity to apply powerful fungal genetics approaches to study the control and function of this conserved gene regulatory pathway. PRC2 targeted domains are characterized as facultative heterochromatin and include key sets of fungal genes, including cellular differentiation and secondary metabolite SM production. However, how PRC2 is controlled and promotes silencing of these gene clusters remains a mystery.
To identify candidates involved in the Polycomb Repression Network, we performed an RNAseq screen on over 370 deletion strains of genes with known or predicted roles in chromatin structure and regulation. Through bioinformatic analysis, I have identified novel candidate genes associated with derepression of facultative heterochromatin genes and involvement in the Polycomb Repression Network. I will present my latest data from genetic and biochemical experiments to identify candidates and deduce their role in the Polycomb Repression Network.

Fungal Genetics and Gene Regulation

Histone deacetylation and cytosine methylation compartmentalize heterochromatic regions in the genome organization of *Neurospora crassa*

Ashley W. Scadden, Alayne S. Graybill, Clayton Hull-Crew, Tiffany J. Lundberg, Nickolas M. Lande, Andrew D. Klocko

University of Colorado Colorado Springs (UCCS)

Chromosomes must correctly fold in eukaryotic nuclei for proper genome function. Eukaryotic organisms hierarchically organize their genomes: in the fungus *Neurospora crassa*, chromatin fiber loops compact into Topologically Associated Domain (TAD)-like structures that are anchored by the aggregation of silent heterochromatic regions. However, insufficient information exists on how histone post-translational modifications, including acetylation, impact genome organization. In *Neurospora*, the HCHC complex (comprised of the proteins HDA-1, CDP-2, HP1, and CHAP) deacetylates heterochromatic regions, including centromeres: loss of individual HCHC members increases centromeric acetylation and alters cytosine methylation. Here, we evaluate the role of the HCHC complex on genome organization using chromosome conformation capture with high-throughput sequencing (Hi-C) in strains deleted of the cdp-2 or chap genes. CDP-2 loss increases interactions between intra- and inter-chromosomal heterochromatic regions, while loss of CHAP decreases heterochromatic region compaction. Individual HCHC mutants exhibit different patterns of histone post-translational modifications genome-wide: without CDP-2, heterochromatic H4K16 acetylation is increased, yet some heterochromatic regions lose H3K9 trimethylation, which increases interactions between heterochromatic regions; CHAP loss produces minimal acetylation changes but increases heterochromatic H3K9me3 enrichment. Interestingly, loss of both CDP-2 and the DIM-2 DNA methyltransferase causes extensive genome disorder, as heterochromatic-euchromatic contacts increase despite additional levels of H3K9me3. Our results highlight how the increased cytosine methylation in HCHC mutants ensures heterochromatic compartmentalization when silenced regions become hyperacetylated with loss of HDAC activity.

Codon usage control of translation efficiency: from initiation to elongation and termination

Yi Liu

UT Southwestern

Preference for certain synonymous codons, a phenomenon called codon usage bias, is a universal feature of all genomes. By using *Neurospora*, *Drosophila* and mammalian systems, we demonstrate that codon usage is a major determinant of translation elongation rate and regulates protein folding by affecting the time available for co-translational process. On the other hand, codon usage is a major determinant of gene expression levels by controlling transcription and translation efficiency. Its effect on translation efficiency regulates ribosome stalling and premature termination. Rare codons promote premature termination, a mechanism mediated by the translation termination factor eRF1, which recognizes ribosomes stalled on rare sense codons. Furthermore,

translation elongation process can feedback to regulate initiation efficiency. Translation of mRNAs with more rare codons induces phosphorylation of initiation factor eIF2α, which inhibits translation initiation efficiency. Together, these results establish codon usage as a major mechanism that control translation efficiency.

Connecting genes and phenotypes by genome resequencing of *Neurospora crassa* mutant strains

Kevin McCluskey, Scott E. Baker

DOE Joint BioEnergy Institute and Environmental Molecular Sciences Division, Pacific Northwest National Laboratory

Many genes identified via classical genetic approaches in *Neurospora crassa* are "anonymous"; these genes have a phenotype mapped to a genetic region, but have not been associated with physical location in the genome. As such there is no correlation between the genetic locus and any open reading frame in the genome. We have resequenced over 500 strains of *N. crassa* representing over 300 classically described but otherwise anonymous genes and identified candidate loci for many of these genes. Efforts at the DOE national user facility, the Environmental Molecular Sciences Laboratory, to develop deeper connections between genes and phenotypes will also be highlighted.

Wild and adventurous: *Neurospora crassa* leaves the lab

Oded Yarden, Katherine A. Borkovich, Luis F. Larrondo, Scott E. Baker

The Hebrew University of Jerusalem

Neurospora crassa has been studied under laboratory conditions for nearly a century. The common, standard, wild type strain used by many members of the *N. crassa* research community is the Oak Ridge background strain 74-OR23-1VA (FGSC 2489). As cultures of this strain have been obtained, maintained, shared and recultured in many labs, the question of possible accumulation of genetic variability among wild type strains, its degree and the possible implications of such changes has surfaced. Increased practices involving transfer and sharing of strains between labs and the possible effects such transfers (including exposure to en-route radiation) may have on the genetic integrity and uniformity of such strains adds an additional aspect to the question raised. Lastly, the increased use and sharing of mutants defective in nonhomologous end-joining DNA repair for research purposes may accentuate the possible accumulation of changes during culturing/transfer of strains, and when en route. We have initiated a community-based effort to resequence wild type strains from different labs using the mentioned wild type in order to determine the degree of variability among our supposedly identical strains. In addition, we are embarking on an international strain transfer experiment using the wild type and the commonly used Δmus-51 strain to study the stepwise accumulation of genetic variation during subsequent transfer and reculturing of these strains. We hope the outcome of this venture will help establish guidelines for future use of fungal, as well as other cultured cells, in an optimized manner that is suited for scientific communities sharing and transferring live biological

material. We foresee that the results will be of broad interest also to colleagues working with cell culture and model organisms.

Tuesday, October 3 - Evening Session
Dodge Award Lecture

Stories from a collegial life

Jennifer Loros

Geisel School of Medicine at Dartmouth

I met *Neurospora* somewhere around 1979. This organism has been a faithful friend now for over 40 years and has introduced me to great scientists, smart and interesting people and life-long companionship. The type of person you become is a result of what you are surrounded by. I am a better person because of *Neurospora*.

Meeting Information:

Virtual Participants - Zoom Webinar Instructions

We will use the zoom 'webinar' format to broadcast platform talks. Virtual participants must register to attend each session using the link below! Once registered, you will be provided with instructions and a zoom link for logging in to view the virtual talks.

1. Each zoom webinar is scheduled to open ~20 minutes before each session begins. This is done so that virtual speakers may log on and test their slides
2. Only panelists (session speakers) can share audio and video, This is done to ensure high quality audio/video performance during talks.
3. During the question and answer period, audience members may raise their hand or enter questions into the chat box. At this time, you will be given temporary "panelist" status. You may turn on your microphone and camera and ask your question.
4. In-person attendees do not need to register for the webinar.

Register to view the Neurospora 2023 Webinar here:

https://zoom.us/webinar/register/WN_U5DnuOqpRCyhQDddAp5n8g

Archived Platform Talks

Talks will be recorded and uploaded to a private YouTube channel. We will send links for viewing archived talks via email as soon as these are available for viewing.

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