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Neurospora 2021 - Program and Abstracts

Neurospora Conference

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Neurospora 2021 - Program and Abstracts

Abstract

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Keywords

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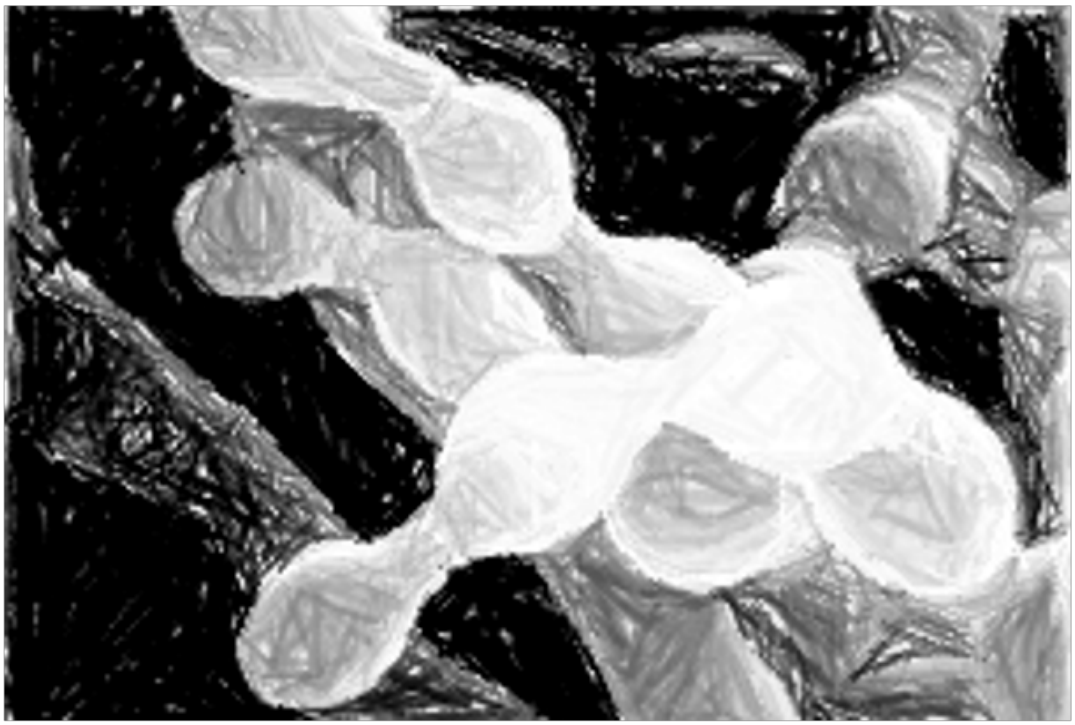

Neurospora 2021

Image credit: Rosa R. Mouriño Pérez

Neurospora 2021

October 17-20
Camp Allen
Navasota, TX

Scientific Organizers

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Brief Schedule

	Morning	Afternoon	Evening
Sunday		3:00 PM Arrival 5:00 - Welcome 5:0 - Talks from Perkins Award Winners	6:30 - 7:30 Dinner 7:30 - 9:00 Mixer
Monday	7:30 Breakfast 8:30 - 12:30 Session I Dodge Award Lecture The Fungal Cell Beadle and Tatum Lecture	12:10 Lunch 1:30 - 3:30 Virtual Poster Session 3:30 - 6:30 Session II Genomics and Genome Function	6:30 - 7:30 Dinner 7:30 In person Poster session and Mixer
Tuesday	7:30 Breakfast 8:30 - Reflection on the career and contributions of Nick Read 9:00 - 11:30 Session III - Talks from Europe and Asia 11:30 - Metzenberg Award Lecture	12:30 Lunch 1:30 - 3:30 Virtual Poster Session 3:30 Neurospora Business Meeting 4:00 - 6:10 pm Session IV Circadian Clocks 6:10 - Closing Remarks	6:30 - Dinner 7:30 - In person Poster session and Mixer
Wednesday	7:30 AM Breakfast 8:30 - 12:00 Depart		

Complete Schedule of Activities

Sunday, October 17th
(Central Daylight Time is GMT-5)

3:00 PM	Check in
Neurospora 2021 Zoom Webinar Session 1 Register: *link disabled*	
5:00 PM	**Opening Remarks** Zachary A. Lewis and Rosa R. Mouriño-Pérez
Perkins Award Winners	
Chair: **Meritxell Riquelme**	
5:10 PM	**Marisela Garduno-Rosales, CICESE** *Actin dynamics following hyphal mechanical injury in Neurospora crassa and Trichoderma atroviride* Video: *Recording Error
5:30 PM	**Jacqueline Pelham, RPI** *The intrinsically disordered FREQUENCY isoforms play a dynamic role in negative arm clock regulation in Neurospora crassa.* Video: *link disabled*
5:50 PM	**Tina Kelliher, Geisel School of Medicine at Dartmouth** *Nutritional compensation of the Neurospora circadian clock is achieved at the levels of transcription and mRNA regulation* Video: *link disabled*
6:30 PM	**Dinner**
7:30 PM	**Welcome Reception at Camp Allen**

Monday, October 18th
(Central Daylight Time is GMT-5)

7:30 AM	**Breakfast**
Neurospora 2021 Zoom Webinar Session 2 Register: *link disabled*	
8:30 AM	**Dodge Award Presentation**
	Chair: **Salomon Bartnicki**
8:40 AM	**Dr. Barry Bowman, University of California, Santa Cruz** *Why Neurospora? – The Science and The Community* Video: *link disabled*
The fungal cell	
Chair: **Rosa R. Mouriño-Pérez**	
9:30 AM	**Bradley M. Bartholomai, Geisel School of Medicine at Dartmouth** *Localization of frequency mRNA by PERIOD-2 contributes to period length determination in the Neurospora crassa circadian clock* Video: *link disabled*
9:50 AM	**Meritxell Riquelme, CICESE** *Neurospora crassa hyphae secrete extracellular vesicles during vegetative growth*
10:10 AM	**Coffee Break**
10:40 AM	**Brian Shaw, Texas A&M University** *The Conidial Coin Toss* Video: *link disabled*
11:00 AM	**Dan Ebbole, Texas A&M University** *FRQish epigenetic transcriptional silencing of Pyricularia oryzae effector gene expression* Video: *link disabled*

Beadle and Tatum Award Lecture	
Chair: **Jennifer Loros**	
11:20 AM	**Jennifer Hurley, Rensselaer Polytechnic Institute** *Demonstrating Flexibility in the Rigid Circadian Clock Using Neurospora crassa* Video: *link disabled*
12:30 PM	**Lunch**

1:30 PM	**Gather Town Virtual Poster Session** Join: *link disabled* Instructions on page 51

Monday, October 18th
(Central Daylight Time is GMT-5)

Neurospora 2021 Zoom Webinar Session 3 Register: *link disabled*	
Genomics and Genome Function	
Chair: **Zachary A. Lewis**	
3:30 PM	**Andrew Klocko, University of Colorado, Colorado Springs** *The genome organization of Neurospora crassa at high-resolution uncovers principles of fungal chromosome topology* Video: *link disabled*
3:50 PM	**Lori Huberman, Cornell University** *Filamentous fungi at the buffet: The regulatory and transcriptional landscape of nutrient sensing* Video: *link disabled*
4:10 PM	**Nick Rhoades, Illinois State University** *RNA Editing Controls the Toxicity of a Neurospora Spore Killer* Video: *link disabled*
4:30 PM	**Coffee Break**

5:00 PM	**Kathy Borkovich, University of California, Riverside** *Statistical clustering analysis of phenotypic data from Neurospora crassa* Video: *link disabled*
5:20 PM	**Michael Freitag, Oregon State University** *The curse of being first meets the need to be complete - update on Neurospora genome and transcriptome sequencing.* Video: *link disabled*
5:40 PM	**Matt Sachs, Texas A&M University** *Structure of the translating Neurospora ribosome arrested by cycloheximide* Video: *link disabled*
6:30 PM	**Dinner**
7:40 PM	**Mixer and in-person poster session**

Tuesday, October 19th
(Central Daylight Time is GMT-5)

7:30 AM	**Breakfast**
Neurospora 2021 Zoom Webinar Session 4 Register: *link disabled*	
8:30 AM	**Reflection on the career of Nick Read (Brian Shaw and Michael Freitag)** Video: *link disabled*
Talks from Europe and Asia	
Chair: **Thomas M. Hammond**	
9:00 AM	**Luis Corrochano, Universidad de Sevilla** *Regulation of asexual and sexual development by the velvet complex in Neurospora crassa* Video: *link disabled*

9:20 AM	**Zhipeng Zhou, Huazhong Agricultural University** *RNA 2'-O-methyltransferase MRM1 regulates Neurospora circadian clock by inhibiting the translation of clock protein FRQ*
9:40 AM	**Oded Yarden, The Hebrew University of Jerusalem** *The GUL-1 protein binds multiple RNAs involved in cell wall remodeling and affects the MAK-1 pathway in Neurospora crassa* Video: *link disabled*
10:00 AM	**J. Philipp Benz, Technical University of Munich** *F-box proteins as novel targets for engineering of lignocellulase hypersecretion in filamentous fungi* Video: *link disabled*
10:30 AM	**Coffee Break**
10:50 AM	**Andre Fleissner, Technische Universitaet Braunschweig** The vesicle enigma: Cell fusion relies on a subset of vesicular structures, which also mediate a novel type of hyphal fusion Video: *link disabled*
11:10 AM	**Eugene Gladyshev, Institut Pasteur** *Modulation of C-to-T mutation by recombination-independent pairing of closely-positioned DNA repeats* Video: *link disabled*
Metzenberg Award Lecture	
Chair: **Jay Dunlap**	
11:30 AM	**Louise Glass, University of California, Berkeley** *My life as a fungus, Part II. How Robert Metzenberg taught me to be curious about the biology of Neurospora crassa.* Video: *link disabled*
12:30 PM	**Lunch**

1:30 PM	**Gather Town Virtual Poster Session** Join: *link disabled* Instructions on page 51

Tuesday, October 19th
(Central Daylight Time is GMT-5)

Neurospora 2021 Zoom Webinar Session 5 Register: *link disabled*	
3:30 PM	**Neurospora Business Meeting**
Circadian clocks	
Chair: **Jenn Hurley**	
4:00 PM	**Deb Bell-Pedersen, Texas A&M University** *Circadian Clock Control of mRNA Translation* Video: *link disabled*
4:20 PM	**Yi Liu, University of Texas Southwestern Medical School** *Mechanism of circadian negative feedback process from Neurospora to mammals: conservation and surprises* Video: *link disabled*
4:40 PM	**Bin Wang, Geisel School of Medicine at Dartmouth** *New NuA4 subunits reveal a crucial role of dynamic expression of the negative arm in the Neurospora clock* Video: *link disabled*
5:00 PM	**Coffee Break**
5:30 PM	**Christian Hong, University of Cincinnati** *Molecular mechanisms regulating frequency demultiplication of circadian rhythms in Neurospora crassa* Video: *link disabled*

5:50 PM	**Luis Larrondo, Millennium Institute for Integrative Biology (iBio)** **Pontificia Universidad Católica de Chile** *A semi-synthetic circadian oscillator revealing the emergence of a "lights on timer" behavior.* Video: *link disabled*
6:10 PM	**Closing Remarks**
6:30 PM	**Dinner**
7:30 PM	**Mixer and Poster Session**

Wednesday, October 20th
(Central Daylight Time is GMT-5)

8:00 AM	**Breakfast**
	Depart

Poster Session

1. **Understanding the molecular functions of phospholipase C-1 and secretory phospholipase A2 in *Neurospora crassa***
   Darshana Baruah, Prof. Ranjan Tamuli

2. **FungiDB: Tools for omics scale data exploration, analysis, integration and discovery**
   Evelina Basenko on behalf of the entire VEuPathDB Bioinformatics Resource Center

3. **Circadian Clock Control of tRNA Synthetases in *Neurospora crassa***
   Griffin Best, Emily Chapa, Kathrina Castillo, and Deborah Bell-Pedersen

4. **Cellular Assessment of the Antifungal Effects of Sertraline in the Human Pathogen *Cryptococcus neoformans***
   Matthew R Breuer, Ananya Dasgupta, Joseph Vasselli, Brian Shaw, Matthew S Sachs

5. **Characterization of the Δ*tea-5* mutant in the filamentous fungi *Neurospora crassa***
   Pedro A. Lopez-Garcia, Olga A. Callejas-Negrete, Fausto M. Villavicencio-Aguilar, Rosa R. Mouriño-Pérez

6. **Characterizing Genetic Mechanisms for Measuring Day-Length in *Neurospora crassa***
   Sienna Casciato, Kwangwon Lee

7. **Circadian Clock-Controlled Translation of Specific *Neurospora crassa* mRNAs Requires Rhythmic eIF2α Activity and P-body Sequestration**
   Kathrina D. Castillo, Cheng Wu, Zhaolan Ding, Matthew S. Sachs, and Deborah Bell-Pedersen

8. **Determining genetic signatures of the cryptococcal response to Zoloft (Sertraline) by an integrated approach combining transcriptome and translatome**
   Ananya Dasgupta, Cheng Wu, Tim A. Dahlmann, Minou Nowrousian, Ulrich Kück, Xiaorong Lin, Matthew S. Sachs

9. **Dynamics of the nanoscopic remodeling of cortical actin during hyphal growth of *Neurosopra Crassa***
   Diego Luis Delgado-Álvarez, Adán Oswaldo Guerrero-Cárdenas, Marisela Garduño-Rosales, Rosa Reyna Mouriño-Pérez

10. **Adapting CUT&RUN to Map DNA-protein interactions in *Neurospora***
Felicia Ebot-Ojong, Masayuki Kamei, and Zachary A. Lewis

11. **Protein-protein interactions at the Microtubule Organizing Centers (MTOCs) of *Neurospora crassa***
Espino-Vázquez Astrid N., Hernández Nahum V., Callejas-Negrete Olga Alicia, Rosa R. Mouriño-Pérez

12. **Exploring the topological genetic plasticity of circadian oscillators through transcriptional rewiring**
Alejandra Goity and Luis F. Larrondo

13. **BRO1 localizes to a specific subpopulation of vesicles which contribute to cell-cell fusion in *N. crassa***
Hamzeh H.Hammadeh, Ulrike Brandt, André Fleißner

14. **Dividing, joining and fixing- The chitin synthase regulator CSR-3 contributes to cell-cell fusion, septation and cell wall repair during the vegetative development of *Neurospora crassa***
Stephanie Herzog, Tanja Nicole Sedlacek, Kristian Daniel Ralf Roth, Manuel Reuning, Laura Köpping, Ulrike Brandt and André Fleißner

15. **Molecular mechanisms regulating frequency demultiplication of circadian rhythms in *Neurospora crassa***
Nayana Wanasingha, Antonio Pregueiro, Mari Kim, Suengwon Lee, Luis F. Larrondo, Jennifer J. Loros, Jay C. Dunlap, Sookkyung Lim, and Christian I. Hong

16. **LOCATE reveals electrostatic “islands” and hotspots are involved in FRQ/FRH binding that regulates robustness but not feedback in the circadian clock**
Meaghan S. Jankowski, Divya G. Shastry, Jacqueline F. Pelham, Joshua Thomas, Dan Griffith, Alex Holehouse, Pankaj Karande, Jennifer M. Hurley

17. **A novel transmission ratio distortion, and a possible trans-species polymorphism affecting MSUD in *Neurospora*.**
Durgadas P Kasbekar

18. **Circadian clock control of ribosome heterogeneity and function: the role of RPL-31, a non-essential ribosomal component, in rhythmic translation**
Teresa M. Lamb, Kathrina D. Castillo, Rachel Porter, Amanda Petty, Tamika Harford, Ebimobiwei Preh, Sam O. Purvine, Cheng Wu, Matt S. Sachs, and Deborah Bell-Pedersen

19. **A Unified Model for Entrainment by Circadian Clocks: Dynamic Circadian Integrated Response Characteristic (dCiRC)**
Zheming An, Benedetto Piccoli, Martha Merrow, Kwangwon Lee

20. **The substituting role of ANXC4 during Ca2+-dependent membrane damage response systems in *Neurospora crassa***
Linda Matz, Marcel Schumann, André Fleißner

21. **Reverse and Forward genetic screens identify new and old elements impacting the *Neurospora crassa* Circadian Clock.**
Felipe Muñoz, Valeria Caballero, Luis F. Larrondo

22. **The role of DFG-5 in targeting *Neurospora crassa* glycoproteins for cell wall incorporation and for extracellular secretion.**
Pavan K Patel, Stephen J. Free

23. **Mechanisms of circadian clock control of CPC-3 activity in *Neurospora crassa***
Ebimobowei Preh, Deborah Bell-Pedersen

24. **Elucidating the Fungal immune system and testing the potential role of Nucleotide-binding domain Leucine-rich repeat-like proteins (NLR-like) against bacterial antagonists.**
Frances Grace Stark, Ksenia Krasileva

25. **FREQUENCY Phosphosite Mutations Perturb Temperature Compensation of the *Neurospora* Circadian Clock**
Elizabeth-Lauren Stevenson, Christina M. Kelliher, Jennifer J. Loros, Jay C. Dunlap

26. **Interspecies interactions in filamentous ascomycete fungi are mediated by a highly conserved cell-cell communication mechanism**
Natascha Stomberg, Hamzeh H. Hammadeh, Antonio Serrano, Ulrike Brandt, André Fleißner

27. **Generating an accurate map of temperature responses mediated by hsp promoter elements**
Cyndi Tabilo, Veronica Del Rio and Luis F. Larrondo

28. **Accounting for the Collar: Quantification of the Endocytic Collar in Three Fungal Species**
Joseph G. Vasselli, Ellen Kainer, and Brian D. Shaw

29. **3-color live-cell imaging reveals dynamic sub-cellular distributions of core clock components**
Ziyan Wang, Bradley M. Bartholomai, Jennifer J. Loros, Jay C. Dunlap

30. **New NuA4 subunits reveal a crucial role of dynamic expression of the negative arm in the *Neurospora* clock**
Bin Wang, Xiaoying Zhou, Arminja N. Kettenbach, Hugh D. Mitchell, Jennifer J. Loros, and Jay C. Dunlap

31. **Examining changes to Eukaryotic Genome Organization Upon Altering Epigenetic Mark Levels**
Ashley Ward, Andrew D. Klocko

32. **The kinase activity of COT-1 is essential for proper polarization and interaction of germinating conidiospores in *Neurospora crassa***
Lucas Well, Ulrike Brandt, Oded Yarden, André Fleißner

33. **Does *Neurospora crassa* need different MTOCs?**
Rosa Ramírez Cota, Rocío Evelyn Macias Díaz, Olga Alicia Callejas-Negrete, Michael Freitag, Reinhard Fischer, Robert W. Roberson, Rosa R. Mouriño-Pérez

34. **RNA Editing Controls the Toxicity of a *Neurospora* Spore Killer**
Nicholas A. Rhoades and Thomas M. Hammond

35. **The intrinsically disordered FREQUENCY isoforms play a dynamic role in negative arm clock regulation in *Neurospora crassa***
Jacqueline F. Pelham, Joshua Thomas, Alexander E. Mosier, Alex S. Holehouse, Jennifer M. Hurley

Abstracts

Session 1: Perkins Award Winners

Actin dynamics following hyphal mechanical injury in *Trichoderma atroviride* and *Neurospora crassa*

Marisela Garduño-Rosales, Olga A. Callejas-Negrete, Elizabeth Medina-Castellanos, Alfredo Herrera-Estrella, Salomón Bartnicki-García, Rosa R. Mouriño-Pérez

The actin cytoskeleton is essential for all eukaryotic cells, and it participates in different subcellular structures and processes. In filamentous fungi, actin is involved in supporting polarized growth, septa development, and endocytosis. We investigated the regeneration of hyphae of *Trichoderma atroviride* and *Neurospora crassa* after mechanical injury with special attention to the role of the actin cytoskeleton. Actin dynamics was followed by confocal microscopy in strains of *T. atroviride* and *N. crassa* tagged with Lifeact-GFP. In growing hyphae of both fungi, actin appears in three structural forms: patches, cables and actomyosin rings. Most patches are conspicuously arranged in a collar in the hyphal subapex. A strong actin signal colocalized with the core of the Spitzenkörper. Cables and patches of actin appear along the cortex throughout the hyphae. Following mechanical damage at the margin of growing mycelia of *T. atroviride* and *N. crassa*, the severed hyphae lost their cytoplasmic contents but owing to septum pore plugging by a Woronin body, the rest of the hyphal tube remained whole. In both fungi, patches of actin began accumulating next to the plugged septum. Regeneration was attained by the emergence of a new hyphal tube as an extension of the plugged septum wall. The septum wall was gradually remodeled into the apical wall of the emerging hypha. Whereas in *T. atroviride* the reboot of polarized growth took about ~1 h, in *N. crassa,* actin patch accumulation began almost immediately, and new growing hyphae were observed ~30 min after injury. By confocal microscopy, we found that chitin synthase 1 (CHS-1), a chitosome component, accumulates next to the plugged septum in regenerating hyphae of *N. crassa*. We concluded that the actin cytoskeleton plays a key role in hyphal regeneration by supporting membrane remodeling and serving as the conveyor of the vesicles responsible for new wall growth and organization of the new tip-growth apparatus.

The intrinsically disordered FREQUENCY isoforms play a dynamic role in negative arm clock regulation in *Neurospora crassa*

Jacqueline F. Pelham, Joshua Thomas, Alexander E. Mosier, Alex S. Holehouse, Jennifer M. Hurley

The Earths predictable light/dark cycle has led to the ubiquitous evolution of circadian rhythms which provide 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 is comprised of 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 serve as 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 of the isoforms 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 provide a mechanistic explanation for these differences, we surveyed the disordered N-terminal region of L-FRQ, revealing potential degrons, which we hypothesize dictate the differential half-lives of the different isoforms. To further characterize the N-terminus we performed all-atom simulations of both the native and phosphomimetic variants. Our analysis revealed N-terminal phospho-dependent transient helices which could serve as a binding motif phosphoswitch to regulate the timing of clock output or L-FRQ half-life. In summary, we propose an isoform-specific mechanistic role for protein disorder in period regulation of the circadian clock under different temperatures.

Nutritional compensation of the *Neurospora* circadian clock is achieved at the levels of transcription and mRNA regulation

Christina M. Kelliher, Jennifer J. Loros, and Jay C. Dunlap

Circadian clocks maintain a period length of approximately 24 hours at a range of physiologically relevant external conditions, such as temperature or nutrient levels, in a defining principle called compensation. Compensation effectors play an active role in buffering the core circadian network from the external environment, and compensation mechanisms have been primarily solved using the *Neurospora* circadian model system. Phosphorylation of the core negative elements of the clock, Frequency (FRQ) in *Neurospora* or PER in insects and mammals, contributes to Temperature Compensation (TC). However, Nutritional Compensation (NC) is understudied. Here, we show that kinase mutants with altered TC do not also affect the clock's ability to compensate period length across nutrient levels in *Neurospora*. Because TC effectors are distinct from NC effectors, we performed a reverse genetic screen for alterations in the circadian period length upon glucose and amino acid starvation using ~500 single gene knockouts from the *Neurospora* deletion collection. Novel regulators were identified with disrupted or complete lack of nutritional compensation. Taken together with four NC mutants that have been characterized in previous work (*csp-1*, *rco-1*, *prd-1*, and *ras-2*), a model emerges where normal NC of the circadian clock is maintained by regulation at the levels of transcription, chromatin regulation, and mRNA stability, which is distinct from the current phosphorylation-centric model for TC mechanism. Fascinatingly, our genetic screen uncovered one hit where NC is altered under amino acid starvation but unaffected by changes in glucose availability, consistent with multiple independent nutritional controls for period length. Previous studies and preliminary data suggest that both the clock's

positive arm (the White Collar Complex) and negative arm (FRQ/FRH/CKIa) are regulated to achieve nutritional compensation of the ~24-hour circadian period length.

Monday, October 17 - Morning Session

Dodge Award Lecture

Why *Neurospora*? – The Science and The Community

Barry Bowman

In my 48 years of working with *Neurospor*a I have seen it serve as an excellent model organism for investigating many important topics in biology. Circadian rhythms, the function of mitochondria, gene silencing and population biology are just a few examples. Much of the progress we have made can be attributed to the outstanding *Neurospora* Community. It has been said that “we play well with others.” As a long-time witness to these interactions, I have some tales to tell and photographs to show. I will also present some of my recent work with super resolution microscopy to show why I think *Neurospora* is a superb system for the study of organelles and cell biology.

The fungal cell

Localization of frequency mRNA by PERIOD-2 contributes to period length determination in the *Neurospora crassa* circadian clock.

Bradley M. Bartholomai, Amy S. Gladfelter, Jennifer J. Loros, Jay C. Dunlap

Core molecular mechanisms of circadian rhythms have been elucidated in animals, plants, fungi, and some prokaryotes over the past several decades. Animal and fungal clocks are remarkably similar in their molecular architecture, and although much is understood about their central mechanism, little is known about the spatiotemporal dynamics of the gene products involved. Studies in the filamentous fungus, *Neurospora crassa* have revealed significant temporal delays between rhythmic accumulation of mRNAs and the proteins they give rise to, indicating a role for complex post-transcriptional regulation. We have employed single molecule RNA-FISH (smFISH) to show that the mRNA of frequency (*frq*), which encodes the primary component of the negative arm of the oscillator, cycles in abundance throughout the circadian day with a single digit ratio of transcripts to nuclei. Furthermore, the number of nuclei actively transcribing *frq* changes in a circadian manner and is also generally quite low compared with the total number of nuclei in each hyphal compartment. Using spatial point patterning statistics, we show that frq is spatially clustered near to nuclei in a time-of-day dependent manner. This clustering is abolished in the absence of the RNA-binding protein PRD-2 that was recently shown also to bind to mRNA encoding Casein Kinase 1. PRD-2 is an intrinsically disordered protein that displays cellular behavior and in vitro characteristics

that are consistent with liquid-liquid phase separation. Taken together these data suggest that *frq* is sequestered in biomolecular condensates by PRD-2 near to nuclei, consistent with a role for RNA-binding proteins and phase-separated regions in spatiotemporally organizing clock mRNAs to facilitate local translation and assembly of clock protein complexes.

Neurospora crassa hyphae secrete extracellular vesicles during vegetative growth

Elizabeth Medina-Castellanos, Juan Manuel Martínez-Andrade, Ruben Dario Cadena-Nava, Meritxell Riquelme

Extracellular vesicles (EVs) are nano-sized membranous carriers released by living cells to the surrounding medium that participate in diverse processes, such as intercellular communication, virulence, and disease. In pathogenic fungi, EVs contain a variety of lipids, nucleic acids, and specific enzymes that allow them to invade the host or undergo environmental adaptation successfully. In *Neurospora crassa*, the vesicle-dependent secretory mechanisms that lead to polarized growth are well studied. In contrast, the biosynthesis of EVs has been practically unexplored. In the present work, we analyzed *N. crassa*'s cultures supernatant for the presence of EVs. We combined the use of dynamic light scattering (DLS) and transmission electron microscopy (TEM). Samples for TEM analyses were fixed and stained with OsO4 vapors. This approach provided a better selective contrast to stain the lipidic membranes of the EVs than in other reports and avoided introducing artifacts. We identified EVs of spherical shape, with a predominant subpopulation of EVs averaging a size distribution of 27 nm in diameter and another less abundant subpopulation of 47 nm in diameter. The content of *N. crassa* EVs, as revealed by mass spectrometry analysis, included enzymes involved in carbohydrate metabolic processes, oxidative stress response, cell wall organization/remodeling, and circadian clock proteins. Some of these proteins have not been previously reported in exosomes from human cells or in EVs of other fungi. Notably, some other proteins (containing a CFEM domain or clock-controlled) have not been previously identified, suggesting a putative new role for EVs in intercellular communication and development in non-pathogenic fungi.

The Conidial Coin Toss

Brian D. Shaw, Joseph G. Vasselli, Hope Hancock, Ellen Kainer, and Thomas M. Chappell

Colletotrichum graminicola is an economically significant fungal pathogen of maize. The primary infective conidia of the fungus, the macroconidia, are splash dispersed during rain events. The adhesion of the macroconidia is required for the development of infection structures. Macroconidia are capable of immediate adhesion due to hydrophobic interactions with the substrate. We report that rapid adhesion in *C. graminicola* is polarized, with a single-sided strip of adhesive material running the length of a single side of the conidium to the tips. This strip of adhesive is co-localized with dynamic transverse actin cables, and both the adhesive strip and actin cables are formed prior to adhesion

on the infection court. These polarized adhesives determine early adhesion and increases in adhesion rates can be induced by applying force to flip conidia onto their adhesive faces.

FRQish epigenetic transcriptional silencing of Pyricularia oryzae effector gene expression

Dan Ebbole

Fungal plant pathogen effector genes typically display in planta-specific expression. The *HAG* (Host Adapted Gene) family of the rice blast pathogen is regulated in this manner and antisense RNA is transcribed in antiphase with peak effector expression. The circadian clock regulator frq is also regulated in part by an antisense transcript. Histone 3 lysine acetylation and methylation states reflect chromatin status, and a comparison of HAG effector histone status and expression in vitro will be discussed.

Beadle and Tatum Award Lecture

Demonstrating Flexibility in the Rigid Circadian Clock Using *Neurospora crassa*

Jennifer Hurley

Circadian rhythms are highly conserved, roughly 24-hour, physiological cycles that, through the ideal programming of behavior, are believed to enhance fitness by ensuring organismal functions are optimally synchronized with the appropriate phase of the circadian day. Circadian rhythms are controlled via a highly regulated transcription-translation based negative feedback loop, or clock. The current paradigm for clock regulation over cellular physiology is that transcriptional activity from the positive arm of the transcription– translation negative feedback loop drives the expression of a host of gene promoters that modulate organismal behavior. However, mounting evidence suggests that circadian regulation is imparted on cellular physiology beyond the level of transcription. We have analyzed the output of the clock on many levels in *Neurospora crassa* over circadian time, demonstrating evidence for extensive post-transcriptional regulation. While confirmation of circadian post-transcriptional regulation has now been demonstrated throughout clock organisms, the source of this regulation remains unclear. However, we have found that the negative arm proteins of the *Neurospora* clock are intrinsically disordered and appear to form an electrostatically driven, temporally changing, interaction that may regulate the formation of macromolecular complexes centered around the *Neurospora* clock protein FRQ. Therefore, we hypothesize that the negative arm is a source of post-transcriptional regulation and that this regulatory capability is enabled by the disordered nature of negative-arm clock proteins.

Monday, October 17 - Afternoon Session

Genomics and Genome Function

The genome organization of *Neurospora crassa* at high-resolution uncovers principles of fungal chromosome topology

Sara Rodriguez, Ashley Ward, Andrew T. Reckard, Yulia Shtanko, Clayton Hull-Crew, and Andrew D. Klocko

Filamentous fungi at the buffet: The regulatory and transcriptional landscape of nutrient sensing

Lori B. Huberman, Vincent W. Wu, David J. Kowbel, Juna Lee, Chris Daum, Igor V. Grigoriev, Ronan C. O'Malley, and N. Louise Glass

Sensing available nutrients and efficiently utilizing them is a challenge common to all organisms. The model filamentous fungus *Neurospora crassa* catabolizes a variety of carbohydrates: from simple sugars to the complex carbohydrates found in plant cell walls. Additionally, *N. crassa* utilizes a variety of organic and inorganic nitrogen sources. The transcriptional network regulating these interconnected metabolic pathways is an interplay of transcriptional activators and repressors that ensure expression of only the genes required to utilize the most preferred nutrients available. We used RNA sequencing to profile the transcriptional response of *N. crassa* to over 50 different nutrient conditions to explore this transcriptional network and identified several instances of interactions between seemingly unconnected regulatory pathways. Combining our observations of coordinately expressed genes with promoter-binding data from DNA affinity purification sequencing of transcription factors allowed us to assign roles to uncharacterized transcription factors and improved our understanding of the role of a number of transcription factors involved in nutrient sensing. One of these uncharacterized transcription factors regulates genes involved in both mannose and amino acid utilization, suggesting an integration of nitrogen and carbon metabolism. The genes involved in nutrient sensing are highly conserved amongst fungi, so we expect that the role of these genes in *N. crassa* may have implications for the roles of orthologous pathways in pathogenic fungi, where accurate nutrient sensing is important for disease progression.

RNA Editing Controls the Toxicity of a *Neurospora* Spore Killer

Nicholas A. Rhoades and Thomas M. Hammond

Sk-2 is a complex meiotic drive element found in Neurospora that is transmitted to offspring through sexual reproduction in a non-Mendelian manner. Typical Mendelian genetics dictates that each allele in a sexual cross should have an equal probability of being inherited by the proceeding generation; however, Sk-2 is able to transmit itself through sexual reproduction in a biased manner by eliminating any meiotic product that does not inherit Sk-2 via spore killing. In Sk-2 SkS (Spore killer-sensitive) crosses, asci with four black,

viable ascospores and four white, inviable (“killed”) ascospores are produced. The four surviving ascospores almost always inherit the Sk-2 element, resulting in a >99% biased transmission of Sk-2 to the surviving population. Previous work has identified two genes that are crucial for successful meiotic drive by spore killing, rsk (resistance to Spore killer), and rfk-1 (required for killing-1). These genes are located on opposite arms of chromosome III but are genetically linked due to several genomic rearrangements within Sk-2, suppressing recombination in this region and ensuring that both rfk-1 and rsk are always inherited together. Here, we present evidence that the killing gene, rfk-1, encodes two protein variants: an innocuous 102 amino acid product (RFK-1A) and a toxic 130 amino acid product (RFK-1B). We also show that expression of RFK-1B requires an early stop codon in rfk-1 mRNA to undergo adenosine-to-inosine (A-to-I) mRNA editing, which occurs exclusively during the sexual cycle of Neurospora. In addition, we demonstrate that RFK-1B is toxic when expressed within vegetative tissue of SkS strains, and that this vegetative toxicity can be suppressed by co-expressing the Sk-2 allele of RSK, suggesting the resistance activity of RSK is functional in vegetative tissues. Currently, we are examining the possibility that the secondary structure of the rfk-1 transcript is critical for RNA editing to occur.

Statistical clustering analysis of phenotypic data from *Neurospora crassa*

Alexander J Carrillo, Ilva E Cabrera, Marko J Spasojevic, Patrick Schacht, Jason E Stajich, Katherine A Borkovich

We analyzed data for 10 different growth or developmental phenotypes that have been obtained for 1168 *N. crassa* knockout mutants. Of these mutants, 265 (23%) are in the normal range, while 903 (77%) possess at least one mutant phenotype. With the exception of unclassified functions, the distribution of functional categories for genes in the mutant dataset mirrors that of the *N. crassa* genome. In contrast, most genes do not possess a yeast ortholog, suggesting that our analysis will reveal functions that are not conserved in Saccharomyces cerevisiae. To leverage the phenotypic data to identify pathways, we used weighted Partitioning Around Medoids (PAM) approach with 40 clusters. We found that genes encoding metabolic, transmembrane and protein phosphorylation-related genes are concentrated in subsets of clusters. Results from K-Means clustering of transcriptomic datasets showed that most phenotypic clusters contain multiple expression profiles, suggesting that co-expression is not generally observed for genes with shared phenotypes. Analysis of yeast orthologs of genes that co-clustered in MAPK signaling cascades revealed potential networks of interacting proteins in *N. crassa*.

The curse of being first meets the need to be complete - update on *Neurospora* genome and transcriptome sequencing.

Michael Freitag*, Robert Riley, Alan Kuo, Sajeet Haridas, Anna Lipzen, Kerry Barry, and Igor Grigoriev

A Community Sequencing Project (CSP504417, approved in 2019) has as its goal the assembly of telomere-to-telomere genome sequences of 49 strains of 32 different filamentous fungi. The strains were selected to yield the highest possible leverage of already existing JGI datasets. Thus far, seven *Neurospora* genomes have been assembled, and transcriptome assemblies have been finished; annotation is still in progress. Four *N. crassa* genomes have been completed, from FGSC2489 (the reference strain, OR74A), FGSC4200 (the most frequently used *mat a* parent, ORS6a), FGSC2225 (Mauriceville, a SNP-rich strain frequently used for mapping), and FGSC8790 (Homestead, D30, a strain used for population genetics and QTL analyses). Two *N. discreta* genomes (FGSC8570, PS4; FGSC9959, PS6) and one *N. perkinsii* (FGSC10406, PS3) genome are also near finished. Here, we will report results from our preliminary analyses of these telomere-to-telomere assemblies.

Structure of the translating *Neurospora* ribosome arrested by cycloheximide

Lunda Shen, Zhaoming Su, Kailu Yang, Cheng Wu, Thomas Becker, Deborah Bell-Pedersen, Junjie Zhang, Matthew S. Sachs

Ribosomes translate RNA into proteins. The protein synthesis inhibitor cycloheximide (CHX) is widely used to inhibit eukaryotic ribosomes engaged in translation elongation. However, the lack of structural data for actively translating polyribosomes stalled by CHX leaves unanswered the question of which elongation step is inhibited. We elucidated CHX's mechanism of action based on the cryo-electron microscopic structure of actively translating *Neurospora crassa* ribosomes bound with CHX at 2.7 Å resolution. The ribosome structure from this filamentous fungus contains clearly resolved ribosomal protein eL28, like higher eukaryotes but unlike budding yeast, which lacks eL28. Despite some differences in overall structures, the ribosomes from *Neurospora*, yeast, and humans all contain a highly conserved CHX-binding site. We also sequenced classic *Neurospora* CHX-resistant alleles. These mutations, including one at a residue not previously observed to affect CHX-resistance in eukaryotes, were in large subunit proteins uL15 and eL42 that are part of the CHX binding pocket. In addition to A-site tRNA, P-site tRNA, mRNA, and CHX that are associated with the translating *N. crassa* ribosome, spermidine (SPD) is also present near the CHX-binding site close to the E site on the large subunit. The tRNAs in the peptidyl transferase center are in the A/A site and P/P site. The nascent peptide is attached to the A-site tRNA and not the P-site tRNA. The structural and functional data obtained show that CHX arrests the ribosome in the classical PRE translocation state and does not interfere with A-site reactivity.

Tuesday, October 18 - Morning Session

Talks from Europe and Asia

Regulation of asexual and sexual development by the velvet complex in *Neurospora crassa*

Sara Cea-Sánchez, Sara Martín Villanueva, María Corrochano-Luque, María Jose García-Marcelo, Gabriel Gutiérrez, David Cánovas and Luis M. Corrochano.

Regulation of development is an essential biological process for many fungal species with great impact in Agriculture, Industry, and Medicine. Several proteins are involved in the regulation of fungal development, such as the heterotrimeric velvet complex. The velvet complex participates in the regulation of gene expression in response to environmental signals such as light, the regulation of development, and secondary metabolism. The velvet family of regulatory proteins is defined by the velvet domain, a DNA binding domain that is required for gene regulation and protein-protein interactions. The velvet proteins interact with each other forming regulatory complexes. *N. crassa* has three velvet proteins (VE-1, VE-2 and VOS-1) and a fourth truncated version (VEL-3), belonging to a paraphyletic subfamily of velvet proteins. VE-1 and VE-2, together with LAE-1 interact to form a velvet complex. Deletion of ve-1 and ve-2 in *N. crassa* cause similar but not identical alterations in both sexual and asexual development. Strains lacking VE-1 and/or VE-2 display increased conidiation as well as a notably delayed and reduced sexual development with fewer fruiting bodies when compared to the wild-type strain. We have shown previously the presence of the velvet complex in vegetative mycelia, and we have now characterized the presence of the components of the velvet complex during different stages of development in *N. crassa*. We have shown that all the components of the velvet complex are detected in aerial hyphae, the early stages of asexual development, and in protoperithecia, the initial stages of sexual development. In order to understand the role of VE-1 as a transcriptional regulator during development, we have performed two sets of RNA-seq experiments. We have characterized the transcriptome of the *N. crassa* wild type and Δ*ve-1* mutant, as they progress from a vegetative growth to conidiation in dark and light. In addition, we have characterized the transcriptome of the wild.

RNA 2'-O-methyltransferase MRM1 regulates *Neurospora* circadian clock by inhibiting the translation of clock protein FRQ

Zhipeng Zhou

The GUL-1 protein binds multiple RNAs involved in cell wall remodeling and affects the MAK-1 pathway in *Neurospora crassa*

Inbal Herold, Anne Yenewodage, Avihai Zolti1, Marisela Garduño-Rosales, Zheng Wang, Francesc López-Giráldez, Rosa R. Mouriño-Pérez, Jeffrey P. Townsend, Igor Ulitsky, Oded Yarden

The *Neurospora crassa* GUL-1 is part of the COT-1 pathway, which plays key roles in regulating polar hyphal growth and cell wall remodeling. We show that GUL-1 is a bona fide RNA-binding protein (RBP) that can associate with 828 “core” mRNA species. When cell wall integrity (CWI) is challenged, expression of over 25% of genomic RNA species are modulated. GUL-1 also interacts with over 100 different proteins, including stress-granule and P-body proteins, components of the MAPK, COT-1, and STRIPAK complexes. Several additional RBPs were also shown to physically interact with GUL-1, some of them with apparently-overlapping functions. Under stress conditions, GUL-1 affects the CWI pathway via altered phosphorylation levels of MAK-1, interaction with mak-1 transcript, and involvement in the expression level of the transcription factor adv-1.

F-box proteins as novel targets for engineering of lignocellulase hypersecretion in filamentous fungi

J. Philipp Benz, Nils Thieme, Raphael Gabriel, Timo Schuerg, Scott E. Baker, Steve W. Singer, Chaoguang Tian, Lisa T. Kohler, Gustavo H. Goldman, André Fleißner

Because of their environmental role as decomposers, fungi are the most valuable sources of carbohydrate active enzymes (CAZymes). Current genome editing methods can create enzyme hypersecreting strains by design. However, the identification of candidate genes for targeted engineering remains a bottleneck due to the inherent complexity and cross-talk of signaling pathways. Our knowledge of the gene induction processes is steadily increasing. However, to rewire cellular metabolic processes for a switch to new environmental conditions, the termination of preexisting programs is necessary to avoid interference. This reprogramming involves the targeted degradation of regulators, enzymes and other proteins via the ubiquitin-proteasome system. Of central importance for this process are F-box domain containing proteins, which recruit specific target proteins to the major E3 ubiquitin ligase, the Skp-Cullin-F-box (SCF) complex, for poly-ubiquitination.Using systems biology and genetic studies of F-box mutants, we are aiming to shed new light on the regulation of enzyme secretion in filamentous fungi, allowing to identify new targets for reverse-engineering of industrially employed fungi. To this end, we recently uncovered that mutation of a gene encoding the F-box protein EXO-1 (representing a classical *N. crassa* mutant) causes inducer-independent hypersecretion of amylases, invertase and pectinases under carbon catabolite de-repressing conditions. A genetic screen of all available F-box gene deletion strains in *N. crassa* furthermore identified a number of additional candidates with cellulolytic phenotypes. Finally, several

F-box proteins could be shown to display carbon source-specific phosphorylation patterns, suggesting an active role in lignocellulose signaling pathways. Overall, these results demonstrate that F-box proteins are not only important factors in cellular protein turnover, but also highly interesting targets for advanced fungal engineering strategies.

The vesicle enigma: Cell fusion relies on a subset of vesicular structures, which also mediate a novel type of hyphal fusion

Hamzeh H. Hammadeh, Ulrike Brandt, and André Fleißner

Cell-cell fusion occurs at different developmental stages of *Neurospora crassa* and promotes colony establishment, growth and functioning. Germinating spores of this fungus mutually attract each other and fuse into supracellular units, which further develop into the mycelial colony. Within mature colonies, hyphal branches merge, thereby forming cross connections between the leading hyphae. While the molecular basis of the cellular communication preceding cell-cell contact has started to emerge, the processes mediating the actual cell merger remain mostly cryptic. Earlier studies in different model organisms, including the fruit fly and baker´s yeast, but also in *N. crassa,* reported the presence of vesicle accumulations at the cell fusion point. The role and function of these structures has, however, been the subject of intense debates. Recently, we identified a fusion-specific subset of vesicular structures, which are involved in fusion related cell-cell communication and very strongly accumulate at the fusion point after physical contact of the fusion partners has been established. A hallmark of these vesicles is the presence of BRO1, which is homologous to the conserved scaffolding protein ALIX of animals. ALIX mediates numerous cellular processes, including membrane deformation and fission. While studying BRO1 dynamics, we identified a new mode of hyphal fusion, which takes place at randomly occurring hyphal contact sites. We hypothesize that the BRO1 carrying vesicles represent the earlier reported structures that accumulate at the fusion side. These vesicles probably contain the cell-cell communication signal and might also carry the molecular machinery mediating plasma membrane merger. Our current investigations aim at isolating these organelles to determine their molecular cargo.

Modulation of C-to-T mutation by recombination-independent pairing of closely-positioned DNA repeats

Florian Carlier, Tinh-Suong Nguyen, Alexey K. Mazur, Eugene Gladyshev

Repeat-induced point mutation (RIP) is a genetic process that creates cytosine-to-thymine (C-to-T) transitions in duplicated genomic sequences in fungi. RIP detects duplications (irrespective of their origin, specific sequence, coding capacity and genomic positions) by a recombination-independent mechanism that likely matches intact DNA double helices directly, without relying on the annealing of complementary single strands. In the fungus *Neurospora crassa*, closely-positioned repeats can induce mutation of the adjoining non-repetitive regions. This process is related to heterochromatin assembly and requires the cytosine methyltransferase DIM-2. Using DIM-2-dependent mutation as a

readout of homologous pairing, we find that GC-rich repeats produce a much stronger response than AT-rich repeats, independently of their intrinsic propensity to become mutated. We also report that direct repeats trigger much stronger DIM-2-dependent mutation than inverted repeats. These results can be rationalized in the light of a recently proposed model of homologous DNA pairing, in which DNA double helices associate by forming sequence-specific quadruplex-based contacts with a concomitant release of supercoiling. A similar process featuring pairing-induced supercoiling may initiate epigenetic silencing of repetitive DNA in other organisms, including humans.

Metzenberg Award Lecture

My life as a fungus, Part II. How Robert Metzenberg taught me to be curious about the biology of *Neurospora crassa*

Louise Glass

When I was a post-doctoral associate in Bob Metzberg's laboratory at the University of Wisconsin, Madison, our laboratory meetings never focused on researcher's data and experiments, but on unusual observations made by Bob on aspects of the biology of *Neurospora crassa* and how these might be addressed experimentally, with a focus on genetics and strain building. These conversations were an incredible gift. Since that time, I have been drawn to the weird, wonderful and understudied aspects of the biology of *N. crassa*, taking advantage of populations of Neurospora collected by another incredible researcher, David Perkins. A number of significant questions remain and will be considered. How is development, metabolism and the circadian clock coordinated in multinucleate syncytia? What is the role of septa in development and movement of organelles in the syncytium and does septal gating play a role? What is the spatial relationship between mRNA, protein and nucleus of origin in a syncytia and how is that regulated, for example, during asynchronous mitoses? How does Neurospora compete and coordinate its growth, metabolism, substrate utilization with other organisms in its environment, including other fungi, bacteria and insects? What are the underlying molecular mechanisms of cooperative versus competitive behavior? And many unanswered questions regarding the ecology and adaptation of Neurospora—is it an endophyte? Is it associated with insects for dispersal? There are still many unknown and undiscovered aspects of the *Neurospora* biology that would intrigue Bob Metzenberg. I often ask myself "How would Bob address this biological question to identify fundamental mechanisms?". His thought processes and experimental approaches to Neurospora biology were so elegant.

Tuesday, October 18 - Afternoon Session

Circadian clocks

Circadian Clock Control of mRNA Translation

Teresa M. Lamb, Kathrina Castillo, Ebimobowei Preh, Tamika Harford, and Deborah Bell-Pedersen

A longstanding puzzle in circadian biology is the existence of proteins that show a robust rhythm despite no rhythm in the levels of the corresponding mRNA. Although some of this effect may be due to circadian regulation of protein degradation, much of this circadian regulation of protein levels is due to rhythmic mRNA translation. In *Neurospora crassa*, at least 15% of mRNAs are rhythmically translated from mRNAs that don't cycle in abundance. We discovered that clock control of translation can be regulated in at least three different ways.
In one mechanism, the clock regulates the phosphorylation, and thus inactivation, of up to half of the conserved translation initiation factor eIF2a. Rhythms in the levels of phosphorylated eIF2a are caused by daytime activation of the eIF2a kinase CPC-3, and nighttime activation of PPP1 phosphatase. This mechanism mediates rhythmic translation initiation of up to half of rhythmically translated mRNAs.
The second mechanism involves targeting mRNAs to evolutionarily conserved cytoplasmic ribonucleoprotein granules, called p-bodies, which contain translationally repressed mRNAs. Among the 67 most robust rhythmically translated mRNAs, 93% contain a sequence motif that targets these mRNAs to p-bodies to repress translation. Loss of either the p-body component SNR-1, or deletion of the p-body target sequence, validated that this process is necessary for rhythmic translation of specific mRNAs.
The third mechanism involves clock control of ribosome composition. In *N. crassa*, the clock regulates levels of at least six ribosomal proteins, and 1 ribosome interacting protein, in intact ribosomes, some of which are not essential for growth. One of the non-essential clock-controlled ribosomal proteins is RPL-31. Loss of RPL31 in ribosomes abolished rhythms a large fraction of the rhythmically translated mRNAs and led to an increase in translation stop codon readthrough. Disruption of clock control of mRNA translation reduces the fitness of *N. crassa* cells. Clock control of mRNA translation, as opposed to mRNA levels, provides several opportunities to increase fitness, including partitioning the energy demanding process of translation for highly expressed genes to times of day when energy levels are high, and sequestering mRNAs to p-bodies to preserve mRNAs that encode proteins needed to overcome stress to allow for a rapid response to an acute stress. Because rhythmic translation is a powerful way for the clock to control biological functions, it is not surprising that several different mechanisms have evolved to carry out this regulation.

Mechanism of circadian negative feedback process from *Neurospora* to mammals: conservation and surprises

Yi Liu

The eukaryotic circadian clocks are based on autoregulatory negative feedback loops to generate endogenous rhythmic gene expression. Unlike typical kinase and its substrates, the formation of a stable complex between core clock components and casein kinase 1

is a conserved feature of circadian clock mechanisms from *Neurospora* to mammals. In *Neurospora*, we demonstrated that the formation of FRQ-CK1 complex is required for circadian rhythmicity and the affinity between FRQ and CK1 is the main determinant of circadian period length. The disruption of the FRQ-CK1 interaction abolishes FRQ phosphorylation and the FRQ-dependent WC phosphorylation, indicating that phosphorylation of FRQ must first require its tight association with CK1 and FRQ acts as a CK1 substrate-recruiting subunit. The FRQ-dependent WC phosphorylation by CK1 inhibits its transcriptional activation activity, resulting in the closing of the negative feedback loop. To determine whether the circadian negative feedback mechanism is conserved in mammals, we determined the Per-CK1 interaction site on Per2 and created knock-in Per2 mouse models in which the Per-CK1 interaction site is mutated by a point mutation. Cellular and genetic results establish a conserved mechanism in the core circadian negative feedback loops from fungi to mammals.

New NuA4 subunits reveal a crucial role of dynamic expression of the negative arm in the *Neurospora* clock

Bin Wang, Xiaoying Zhou, Arminja N. Kettenbach, Hugh D. Mitchell, Jennifer J. Loros, and Jay C. Dunlap

Post-translational modifications (PTMs) on histones have been found to play diverse functions in regulating chromatin events and gene expression. The operation of circadian clocks heavily replies on finely and timely tuned expression of the proteins comprising core oscillators. However, most studies of PTMs' effects on circadian clocks were conducted using static systems in which circadian clocks were terminated due to the essential role of PTMs on gene expression. In the *Neurospora* circadian system, the White Collar Complex (WCC), a heterodimeric transcription factor formed from White Collar-1 (WC-1) and White Collar-2 (WC-2), induces transcription of the circadian pacemaker gene frequency (frq). FRQ, the gene product of frq, interacts with FRH (FRQ-interacting helicase) and CK-1 to repress its own transcription by inhibiting WCC. In this study, genetic screening identified a new gene, designated as *eaf-8*, required for the normal circadian period determination in *Neurospora*. Absence of *eaf-8* leads to a long circadian period, delayed phase, defective overt circadian output under certain temperatures, and compromised temperature compensation. EAF-8 strongly associates with the NuA4 histone acetyltransferase complex, as well as with the transcription elongation factor BYE-1, and loss of eaf-8 reduces H4 acetylation and RNA polymerase (Pol) II occupancy at frq and other known circadian genes. Reciprocally, expression of *eaf-8*, *bye-1*, histone hH2Az, and several NuA subunits is controlled by the circadian clock, indicating that the molecular clock regulates the basic chromatin status. Taken together, our data identify a new type of the NuA4 complex formed by EAF-8 and BYE-1 with conventional NuA4 subunits identified in yeast, which is required for timely and dynamic *frq* expression and thereby a normal circadian period.

Molecular mechanisms regulating frequency demultiplication of circadian rhythms in *Neurospora crassa*

Nayana Wanasingha, Antonio Pregueiro, Mari Kim, Suengwon Lee, Luis F. Larrondo, Jennifer J. Loros, Jay C. Dunlap, Sookkyung Lim, and Christian I. Hong

A semi-synthetic circadian oscillator revealing the emergence of a "lights on timer" behavior.

Luis F. Larrondo

In the fungus *Neurospora crassa*, as in other model organisms, synthetic biology based-strategies have been seldom adopted for the study of circadian oscillators. Our current efforts on this matter have focused on examining the topological plasticity of the *Neurospora* circadian clock to inquire if the evolutionary conserved "core-oscillator design" present in different phyla, could work if a different circuitry is imposed. By transcriptional rewiring, we built a semi-synthetic oscillator where the expression of the negative element is no longer directly controlled by the canonical positive element, and that instead depends on at least five transcriptional regulators, that by definition are now part of the core circadian oscillator. Thus, this hybrid-oscillator, displays an extended TTFL architecture, which is an admixture of canonical core-clock elements and multiple new components. Importantly, this circuit behaves as a bona fide clock yet, remarkably, it also displays new unexpected properties. Thus, in contrast with the WT *Neurospora* clock that acts as a lights-off timer, this hybrid oscillator behaves as a "lights on timer", fixing the phase of the clock when the lights are turned on. Thus, this approach contributes with important insights regarding clock properties, such as phase determination and the processing of light cues, as well as on the evolution of clock designs. FONDECYT 1211715. iBio and HHMI International Research Scholar program.

Poster Sessions

1. Understanding the molecular functions of phospholipase C-1 and secretory phospholipase A2 in *Neurospora crassa*

Darshana Baruah, Prof. Ranjan Tamuli

The model filamentous fungus, *Neurospora crassa*, possesses a unique Calcium (Ca2+) signaling machinery with 48 Ca2+ signaling proteins involving in the pathway, which is more in number than those identified in rice blast fungus *M. grisea* and the most extensively studied model organism *S. cerevisea*. However, little is known about the physiology of Ca2+ signaling machinery in *N. crassa* or other related fungi compared to plants and animals. In *N. crassa*, several Ca2+ signaling proteins, including the phospholipase C (PLC) and secretory phospholipase A2 (sPLA2), are involved in sensing the increase in [Ca2+]c. The PLC and sPLA2 belong to the phospholipase superfamily of proteins. Previous studies showed phospholipase C-1 (PLC-1), and secretory phospholipase A2 (sPLA2) are essential for survival under multiple stress conditions. This study showed that a strain lacking plc-1 exhibited defects in the circadian clock, and plc-1 regulates the clock function by maintaining a proper frq and wc-1. Recently, *N. crassa* is gaining importance as a model organism to obtain a more detailed understanding of the physiology of lignocellulose degradation across fungi as a nearly complete genome deletion set is available. Surprisingly, when grown on crystalline cellulose (Avicel) as the sole carbon source, the Ca2+ signaling mutant ∆*splA2* consumed cellulose faster than the wild type, exhibited significantly increased protein secretion, endoglucanase activity, and displayed ER response. ∆*splA2* mutant also showed increased expression of cellulolytic genes during growth on crystalline cellulose.

2. FungiDB: Tools for omics scale data exploration, analysis, integration and discovery

Evelina Basenko on behalf of the entire VEuPathDB Bioinformatics Resource Center

"FungiDB (fungidb.org) is a free online resource for data mining and functional genomics analysis for fungal and oomycete species. FungiDB is part of The Eukaryotic Pathogen & Vector Genomics Resource (VEuPathDB.org) that provides a robust, sustainable data-mining resource, expediting discovery and translational research on diverse eukaryotic microbes, including pathogenic and nonpathogenic model organisms, hosts and vectors of the disease. The easy-to-use web-based interface offers means to explore various genomes and associated annotation, functional data (transcriptomics or proteomics), automatic analyses (ie. InterPro scan, signal peptide and transmembrane domain predictions, orthology, etc.), community-submitted data (e.g. *Neurospora* Genome Project Phenotype Image Collection, Phenotypic analysis of *Neurospora crassa* knockout mutants, etc.) and more. The interactive interface facilitates hypothesis-driven data exploration via the search query system and evidence tracks in JBrowse. Furthermore, omics scale dataset analysis can be performed on the VEuPathDB Galaxy, while private FungiDB My workspace allows for viewing and interrogating private data against data

sets already integrated into FungiDB. The resource infrastructure also facilitates capturing expert knowledge via the user comment system and Apollo, real-time collaborative genome annotation and curation platform. FungiDB is a component of the NIAID Bioinformatics Resource Centers and is supported in part by NIH HHSN75N93019C00077 and the Wellcome Biomedical Resources #212929/Z/18/Z grants.

3. Circadian Clock Control of tRNA Synthetases in *Neurospora crassa*

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

About half of proteins synthesized in eukaryotic cells under control of the endogenous circadian clock arise from mRNAs that are not rhythmic, supporting a role for clock control of posttranscriptional mechanisms. In *Neurospora crassa*, the circadian clock controls rhythmic mRNA translation through regulation of the eIF2α kinase CPC-3 (the homolog of yeast and mammalian GCN2). Active CPC-3 phosphorylates and inactivates eIF2α, leading to reduced translation initiation. In yeast, GCN2 is activated by binding to uncharged tRNA, which accumulates in cells during amino acid starvation. Consistent with these data, we found that clock control of CPC-3 activity requires the rhythmic accumulation of the valyl-tRNA synthetase (ValRS), and hypothesized that clock control of tRNA synthetases (RS's) promotes the rhythmic accumulation of uncharged tRNAs to activate CPC-3 during the day. To address this hypothesis, we selected four clock-controlled RS's, AspRS, GlnRS, MetRS and PheRS, for further study. Translational fusions of AspRS, GlnRS, and PheRS to LUC confirmed that their protein levels are clock-controlled and with peak levels at night. These data support that uncharged tRNA levels peak during the day to activate CPC-3, leading to an increase in the levels of phosphorylated eIF2α and reduced translation initiation. Experiments are underway to constitutively express the RS's to determine if loss of RS rhythmicity alters rhythmic eIF2α phosphorylation and mRNA translation. This work will provide key insights into the mechanisms of protein production by the circadian clock, and because RS's have roles outside of mRNA translation, may also provide a mechanism to connect clock control of protein synthesis to other clock regulated cellular processes, including nutrient metabolism, cell division, and development.

4. Cellular Assessment of the Antifungal Effects of Sertraline in the Human Pathogen *Cryptococcus neoformans*

Matthew R Breuer, Ananya Dasgupta, Joseph Vasselli, Brian Shaw, Matthew S Sachs

5. Characterization of the Δ*tea-5* mutant in the filamentous fungi *Neurospora crassa*

Pedro A. Lopez-Garcia, Olga A. Callejas-Negrete, Fausto M. Villavicencio-Aguilar, Rosa R. Mouriño-Pérez

The orchestrated movement of elements needed for polarized growth via actin or microtubules is crucial for growth and morphogenesis in filamentous fungi. The TEA complex (Tip Elongation Aberrant protein; TEA-1, TEA-4 and TEA-5) is associated with actin as well as microtubules in cell growing poles in both *Schizosaccharomyces pombe* and *Aspergillus nidulans*. In *Neurospora crassa*, the association of both cytoskeletal components with the TEA complex is still not well understood. To study the role of the TEA complex in polarize growth, we analyzed Δtea-5 mutant, where we observed that the lack of tea-5 was ascospore lethal. The heterokaryon strain showed a decrease of 43% in the elongation rate, 40% in biomass production and 75% in conidia production (p<0.05). Branching rate was two-fold higher in the Δ*tea-5* mutant ($p<0.05$) in comparison with de WT. Mature hyphae showed a small and unstable Spitzenkörper. *tea-5* is essential in *N. crassa* and the partial silencing of the gene strongly affects the growth rate, increases the frequency of branching, reduces significantly conidial production and participates breaking the symmetry during hyphal development.

6. Characterizing Genetic Mechanisms for Measuring Day-Length in *Neurospora crassa*

Sienna Casciato, Kwangwon Lee

Photoperiodism is a physiological response of an organism to changes of the ambient environment over a year and plays a major role in fitness of an organism in nature. It has been proposed that photoperiodism is in part associated with the circadian rhythm. Unlike the mechanism of photoperiodism, that of the circadian rhythm (24-hour cycles within an organism) is well characterized. We hypothesized that there are multiple genes that are involved in photoperiodism, and that the genes involved in the circadian clock might also be involved in photoperiodism. To test our hypotheses, we developed the protoperithecia assay (PPA). Protoperithecia is a female sexual reproductive structure in *N. crassa* and is known to be responsive to different photoperiods. This data was used as the readout for an organism’s ability to determine the day-length. We performed Quantitative Trait Loci (QTL) analysis on the number of protoperithecia at different photoperiods using 91 F1 progeny of *N. crassa*. We found a major QTL on chromosome (Chr) 1, and multiple minor QTLs on other Chr including Chr 5. We characterized 17 knockout mutants on the major QTL in Chr 1 and 21 knockout mutants in the target region of Chr 5. We calculated the effect size, or Cohen’s d values, for each mutant and used this value to determine candidate genes for photoperiodism in *N. crassa*. Using this method, we identified candidate genes on major QTL in Chr 1 and minor QTL in Chr 5. We also identified clock/photoreceptor mutants that are defective in measuring the day length. Our data

support the view that the circadian clock is a part of the day-length measuring mechanism, thus influencing the photoperiodism. Our data also supported the hypothesis that there are multiple genes involved in photoperiodism. The current study will provide a comprehensive view on the possible genetic mechanisms of photoperiodism.

7. Circadian Clock-Controlled Translation of Specific *Neurospora crassa* mRNAs Requires Rhythmic eIF2α Activity and P-body Sequestration

Kathrina D. Castillo, Cheng Wu, Zhaolan Ding, Matthew S. Sachs, and Deborah Bell-Pedersen

At least half of proteins that accumulate with a circadian rhythm in *Neurospora crassa* are produced from mRNAs whose levels are not clock-controlled, indicating a prominent role for clock regulation of post-transcriptional processes. Phosphorylation of at least 30% of available *N. crassa* eIF2α, a conserved translation initiation factor, is clock-controlled, peaking during the subjective day. To determine the impact of rhythmic eIF2α phosphorylation on rhythmic translation, we performed temporal ribosome profiling and RNA-seq in WT, clock mutant Δfrq, eIF2α kinase mutant Δcpc-3, and constitutively active cpc-3c cells. We discovered that ~14% of *N. crassa* mRNAs are rhythmically translated in WT cells, and translation rhythms for ~30% of these mRNAs were dependent on the clock and CPC-3. Most circadian translation initiation-controlled genes (cTICs) are expressed from non-rhythmic mRNAs, and contain a cytoplasmic P-body localization motif present in the 5' untranslated region (UTR). Deletion of the P-body component SNR-1, and deletion of the P-body motif in the 5' UTR of one of these mRNAs zip-1, significantly altered rhythmic translation of zip-1 mRNA. Furthermore, the deletion of P-body components SNR-1 or SNR-7 led to reduced linear growth rates in constant conditions, to a level comparable to cells lacking rhythmic eIF2α phosphorylation. Together, these results revealed a mechanism by which the circadian clock regulates rhythmic translation of specific mRNAs, through rhythmic eIF2α activity and P-body metabolism.

8. Determining genetic signatures of the cryptococcal response to Zoloft (Sertraline) by an integrated approach combining transcriptome and translatome

Ananya Dasgupta, Cheng Wu, Tim A. Dahlmann, Minou Nowrousian, Ulrich Kück, Xiaorong Lin, Matthew S. Sachs

Cryptococcus neoformans, a heterothallic basidiomycete fungus, is one of the most common invasive, opportunistic pathogens. It predominantly affects immunocompromised patients and is the most common cause of fungal meningitis worldwide. It is difficult to treat with current antifungal therapies because of the emerging resistance to azoles such as fluconazole (FLC). Zoloft (sertraline or SRT) is a widely prescribed drug to treat mood disorders and depression; we and others have shown it kills *C. neoformans*. Additionally, SRT and FLC act synergistically against *C. neoformans*. How SRT kills *C. neoformans* remains unclear. Therefore, we chose as a strategy to dissect the antifungal action of SRT to identify changes in gene expression following treatment of *C. neoformans* with SRT, FLC, and SRT+FLC using strand-specific RNA-

seq and ribosome profiling. Our differential gene expression results indicate that SRT kills the pathogen by a different mechanism than FLC and suggest that SRT may primarily exert its effect by altering membrane transport and lipid metabolism in *C. neoformans*.

9. Dynamics of the nanoscopic remodeling of cortical actin during hyphal growth of *Neurosopra crassa*

Diego Luis Delgado-Álvarez, Adán Oswaldo Guerrero-Cárdenas, Marisela Garduño-Rosales, Rosa Reyna Mouriño-Pérez

The actin cytoskeleton in filamentous fungi is a key component in both exocytosis and endocytosis, fundamental processes that determine hyphal morphogenesis. Endocytic actin patches are composed of cortical F-actin filaments bound together by a variety of Actin Binding Proteins such as fimbrin, coronin, and the Arp2/3 complex. The precise size, origin, and transport mechanisms of the endocytic vesicles are still a matter of interest but its study has been hindered by the resolving capabilities of confocal and TIRF microscopy. Through nanoscopic live-cell imaging of GFP-labeled actin structures and performing super-resolution/nanoscopic analysis, we can vastly improve the quality of information and are able to perform single-particle tracking and velocimetry. Though actin patches are sufficiently scattered in the subapical and basal regions of the hypha, they are highly concentrated in the subapical ring, which makes the observation of individual patches difficult. Studying actin patches in this region is difficult with diffraction-limited microscopy. Thus, nanoscopy offers the opportunity to describe changes in size, shape, and motility of individual structures. This work utilizes live-cell nanoscopy, which will help to provide detailed information on the origin and fate of actin patches to further understand their role in hyphal morphogenesis.

10. Adapting CUT&RUN to Map DNA-protein interactions in *Neurospora*

Felicia Ebot-Ojong, Masayuki Kamei, and Zachary A. Lewis

Chromatin immunoprecipitation coupled with high-throughput DNA sequencing (ChIP-seq) has been the gold standard for genome-wide analysis of DNA-protein interactions but suffers from several limitations that restrict its resolution and sensitivity. The standard ChIP assay is time intensive, has low resolution, and requires a large input of cells. To address the limitations of ChIP-seq, the Henikoff lab developed a new approach known as Cleavage Under Targets and Release Using Nuclease (CUT&RUN) which uses the DNA cutting activity of micrococcal nuclease (MNase) fused to protein A/G to specifically isolate DNA that is bound by a protein of interest or a modified histone. Here we show that the method CUT&RUN can be adapted to work in the model fungi *Neurospora crassa*. We expressed and purified the Protein A/G-MNase fusion construct in E. coli, carried out CUT&RUN to assay chromatin modifications with known distributions and tested for localization of protein complexes that have been difficult to “CHIP”. The CUT&RUN technique enabled us to achieve single nucleosome resolution not seen in previous *Neurospora crassa* ChIP-seq data.

11. Protein-protein interactions at the Microtubule Organizing Centers (MTOCs) of *Neurospora crassa*

Espino-Vázquez Astrid N., Hernández Nahum V., Callejas-Negrete Olga Alicia, Rosa R. Mouriño-Pérez

Nucleation and anchoring of Microtubules (MTs) take place at specific sites of the cells known as the MTOCs, where the γ-tubulin and proteins of the CGP family are the main components. In fungi, the best-studied MTOCs are the Spindle Pole Bodies (SBPs), also referred to as centrosomal MTOCs. However, in some fungal species, non-centrosomal MTOCs have been identified at the cytoplasm, hyphal tips, or septa. In order to explore the composition and location of the MTOCs of *Neurospora crassa*, we analyzed the protein-protein interactions of four proteins of interest: γ-tubulin, MZT-1 (a predicted MTOC component), APS-2 (predicted septal and MTOC component), and SPA-10 (pore septal protein). De novo interactions were identified by Co-Immunoprecipitation (Co-IP) followed by nano LC-MS/MS. The targeted analysis confirmed not only the interaction between γ-tubulin and GCP proteins but also with MZT-1 and APS-2, although in a lower proportion. Additionally, the histone chaperone FACT had a significant interaction with our proteins of interest. On the other hand, SPA10 does not physically interact with γ-tubulin, MZT-1, or APS2, but during the functional enrichment analysis demonstrated to share several partners with them. In this exploratory analysis was confirmed the role of γ-tubulin and MZT-1 in microtubule nucleation and spindle assembly and also that all four target proteins sustain physical interactions to many primary metabolism proteins.

12. Exploring the topological genetic plasticity of circadian oscillators through transcriptional rewiring

Alejandra Goity and Luis F. Larrondo

Circadian clocks are present in a wide range of organisms, being fundamental in temporally coordinating myriad biological processes in a daily fashion. Although these clocks independently appeared several times throughout evolution, the molecular bases governing them are conserved among eukaryotes: a central circadian oscillator is composed of a transcriptional-translational feedback loop (TTFL), where a negative element directly inhibits a positive one that promotes its expression. The latter is also responsible for transmitting the temporal information through the output pathways, a transcriptional cascade leading to the rhythmic expression of thousands of clock-controlled genes (ccgs). To challenge whether a classical TTFL architecture is the only way to achieve a functional circadian clock, we explored the genetic topological plasticity of circadian systems by adopting a transcriptional rewiring approach. Thus, we generated semi-synthetic oscillators by exchanging the promoter of the negative element with the promoters of selected ccgs, bringing part of the regulation of output pathways to the core mechanism. Using this approach, we evaluated the ability of a modified clock to sustain rhythms, even when the evolutionarily conserved TTFL design has been fundamentally modified. Herein we describe one of these semi-synthetic clocks which shows all basic circadian features: period of about 24 h, entrainment and temperature compensation.

Genetic analyses, reveal that this alternative topology includes the presence of at least 5 new core-clock components, including both transcriptional activators and repressors which can -nevertheless- coordinately work to allow a highly intricated process such as circadian timing to occur. FONDECYT 1211715. iBio and HHMI International Research Scholar program

13. BRO1 localizes to a specific subpopulation of vesicles which contribute to cell-cell fusion in *N. crassa*

Hamzeh H.Hammadeh, Ulrike Brandt, André Fleißner

Colony initiation of filamentous fungi commonly involves fusion of germinating vegetative spores. Studies in *Neurospora crassa* revealed an unusual cell-cell communication mechanism mediating this process, in which the fusion partners coordinately alternate between two physiological stages, probably related to signal sending and receiving. This “cell dialog” involves the alternating, oscillatory recruitment of the SO protein and the MAK-2 MAP kinase module to the apical plasma membrane of growing fusion tips. We recently identified BRO1 as a new factor essential for germling interaction and fusion. In *N. crassa*, BRO1 is essential. Down-regulation of bro1 gene expression results in a ∆so-like phenotype, including the lack of chemotropic cell-cell interactions and subsequent fusion. Subcellular localization and live-cell imaging revealed that BRO1-GFP localizes to the cytoplasm and in vesicles in non-interacting germlings and in mature hyphae. BRO1-GFP accumulates at the tips of the interacting germlings in a dynamic, oscillating manner, such that high signal intensity of BRO1-GFP in one tip correlates with low signal intensity at the tip of the fusion partner. The co-localization of BRO1-GFP and different vesicles markers indicated that BRO1 marks a sub- population of vesicles, which specifically accumulate at cell fusion sites. Downregulation of bro1 partially relieves the phenotype of a plasma membrane fusion mutant, in which the membranes frequently fail to merge and aberrant engagement of the fusion machinery results in cell lysis. When bro-1 is partially repressed, membrane fusion is still blocked but cell lysis is absent. We therefore hypothesize, that BRO1 might be involved in transporting the fusion machinery to the plasma membrane of fusing cell tips. Together these data indicate that BRO1 plays distinct roles in cell-cell communication and plasma membrane fusion. Future analysis of its molecular bases of fungal cellular communication, and membrane fusion.

14. Dividing, joining and fixing- The chitin synthase regulator CSR-3 contributes to cell-cell fusion, septation and cell wall repair during the vegetative development of *Neurospora crassa*

Stephanie Herzog, Tanja Nicole Sedlacek, Kristian Daniel Ralf Roth, Manuel Reuning, Laura Köpping, Ulrike Brandt and André Fleißner

In fungi, the cell wall represents the outermost cellular layer, which sculpts and protects the cell, but also mediates signal exchange with the environment. During colony development, the cell wall is remodeled in response to various external and internal signals in order to promote cellular growth, variable morphologies and resistance towards

different types of stresses. The molecular composition of this flexible barrier varies within the fungal kingdom but most species possess the polysaccharide chitin. Its synthesis includes the fine-tuned orchestrated activity of numerous chitin synthases and corresponding regulators with partially redundant but also highly defined tasks. Although the involved proteins are conserved, their specific tasks differ between species and are not fully understood yet. Here, we show that the chitin synthase regulator CSR-3 responds to various environmental and internal signals by subcellular translocation towards the cell surface, where it likely mediates chitin synthesis of its target synthase enzyme. In the filamentous fungus *Neurospora crassa*, germinating conidia and mature hyphae build highly interconnected networks via cell-cell fusion. CSR-3 possesses a novel and distinct function in the formation of stable fusion pores during these developmental processes. We provide evidence that phosphorylation and the CaaX motif at the C-terminus of the protein control CSR-3's activity. Additionally, it contributes to septation, that sustains and subdivides the hyphal architecture, and formation of vegetative spores. Furthermore, CSR-3 responds to drug induced membrane or cell wall wounding as putative part of a cell wall repair system and might promote protection against mycoparasites. Taken together, these data highlight the complex regulation of cell wall synthesis and indicate that an individual factor is involved in a plethora of cellular functions including growth, compartmentation, cell fusion and stress induced repair."

15. Molecular mechanisms regulating frequency demultiplication of circadian rhythms in *Neurospora crassa*

Nayana Wanasingha, Antonio Pregueiro, Mari Kim, Suengwon Lee, Luis F. Larrondo, Jennifer J. Loros, Jay C. Dunlap, Sookkyung Lim, and Christian I. Hong

16. LOCATE reveals electrostatic "islands" and hotspots are involved in FRQ/FRH binding that regulates robustness but not feedback in the circadian clock

Meaghan S. Jankowski, Divya G. Shastry, Jacqueline F. Pelham, Joshua Thomas, Dan Griffith, Alex Holehouse, Pankaj Karande, Jennifer M. Hurley

The molecular clock regulates many important cellular processes, timed by the interactions of inherently flexible or disordered proteins that comprise a negative feedback loop. However, identifying the binding regions involved in these disordered protein-protein interactions is not tenable using typical biophysical methods. Since disordered proteins interact through short linear binding motifs (SLiMs), we took the primary sequence of a highly disordered protein and divided it into a set of overlapping short peptides that can be synthesized and printed in a microarray format, then challenged with an interacting protein of interest. This high-throughput method termed Linear motif discovery using rational design (LOCATE) identifies candidate SLiMs and tests their specificity through the use of further scrambles, point mutations, and truncations. To validate our method we chose the largely disordered 989 a.a. protein FREQUENCY (FRQ) as the basis of our peptide array and challenged it with its well-known Nanny protein, FRQ-interacting RNA Helicase (FRH), two components that close the negative feedback loop of the clock within

Neurospora crassa. We gained insight into the importance of electrostatics for this dynamic complex, as conserved positive electrostatic “islands” within FRQ’s sequence were consistently bound by the mainly negatively surface-charged FRH. We validated our method by replicating the original FRH-binding domain, but demonstrated that this specific FRH-binding SLiM included additional “hotspot” residues outside the previously genetically-identified region. Targeted in vivo mutations of these “hotspot” residues led to the unexpected discovery that FRQ can close the loop without FRH, while FRH is necessary for clock robustness. LOCATE is therefore new high-throughput method for identifying SLiMs and “hotspots” within disordered clock proteins that allows the dissection of clock protein interactions involved in circadian timekeeping and output regulation.

17. A novel transmission ratio distortion, and a possible trans-species polymorphism affecting MSUD in *Neurospora*.

Durgadas P Kasbekar

Neurospora ascospores become round if the round ascospores (r) gene is silenced by MSUD. MSUD occurs during a sexual cross and silences genes that lack a homologous DNA sequence on the homologous chromosome. Small RNA molecules are made from the unpaired gene, incorporated into a silencing complex, and guide it to eliminate their cognate mRNA. In a cross of a normal (N) strain with one carrying an ectopic r gene (N x ::r), MSUD eliminates mRNA from r, and the round ascospores fraction gives an estimate of MSUD efficiency. N x ::r crosses in the *N. crassa* OR background made more than 99% round ascospores, while control ::r x ::r and N x N crosses made fewer than 1%. But in the *N. crassa* ""BS"" background, and in N. tetrasperma 85, N x ::r crosses produced fewer than 60% and 10% round ascospores. The Rsp-1 mutant of N. tetrasperma strain T-220 is now lost. All ascospores from Rsp-1 x N were round. Rsp-1 likely was a deletion that unpaired and silenced the r allele. The mirroring of MSUD efficiency difference between *N. crassa* OR and BS and N. tetrasperma T-220 and 85, raised the possibility of a trans-species polymorphism. That is, the presence in the two species of MSUD genes more similar to each other than to their alternative alleles in the same species. We examined 106 f1 progeny from OR x BS. MSUD was more efficient in crosses with progeny that inherited the OR chromosome (chr.) 7 than with its BS homologue. Additionally, the OR vs. BS alleles on chr. 1 and 5 did not show 1:1 segregation, it was 36:70 and 38:68. But chr. 2, 6, and 7 did (49:57, 56:50, &47:59). Chr. 3 and 4 and mt DNA were not examined. Extraneous factors then interrupted this study. The BS strains are deposited in the FGSC. Others are welcome to verify this study and take it forward. Ack: D. A. Giri performed the experiments. DPK is an INSA Sen. Sci."

18. Circadian clock control of ribosome heterogeneity and function: the role of RPL-31, a non-essential ribosomal component, in rhythmic translation

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

Recent studies on ribosomes indicate that there is heterogeneity of composition that can be within cells to control mRNA specificity or be between cell types and developmental states. Here we examine ribosome heterogeneity dependent on the circadian clock in *N. crassa*. Intact particle mass spectrometry was performed on ribosomes from WT and clock mutant cells over time, where 358 proteins were detected and 7 cycled in abundance dependent on the circadian clock. We focused on RPL-31, a non-essential ribosomal protein found near the peptide exit channel and known in yeast to interact with the RAC (ribosome associated complex). DJC-2, a homolog of the yeast RAC component Zuotin, a DnaJ type co-chaperone that contacts RPL-31, was also rhythmic in the ribosome samples. RPL-31-HA incorporation in purified ribosomes was confirmed and its abundance depended on the clock, peaking in the subjective night. We generated a ∆rpl-31 strain and show that similar to yeast, this strain is sensitive to cold and paromomycin. To examine the role of RPL-31 in circadian control of translation, RNA sequencing and ribosome footprint sequencing was performed on the knock out over a time-course. ∆rpl-31 abolished the translation efficiency (TE) rhythms for 98% of the mRNAs with rhythms in WT. Because of the role yeast RPL31 plays in translation fidelity, we are currently mining our ribosome footprint sequencing data to determine if there are rhythms in translation fidelity dependent on the clock and RPL-31. Furthermore, we are developing a dual luciferase reporter system to examine stop codon read through, a hallmark of impaired fidelity, over circadian time. Future plans include confirming DJC-2/ZUO rhythms and examination of the impact of these rhythms on translation fidelity and protein folding. Together our work suggests that the clock governs daily changes in ribosome composition that impact rhythms in translation efficiency and may affect translation fidelity and protein folding.

19. A Unified Model for Entrainment by Circadian Clocks: Dynamic Circadian Integrated Response Characteristic (dCiRC)

Zheming An, Benedetto Piccoli, Martha Merrow, Kwangwon Lee

Circadian rhythm is a ubiquitous phenomenon, and it is observed in all biological kingdoms. In nature, their primary characteristic or phenotype is the phase of entrainment. There are two main hypotheses related to how circadian clocks entrain, parametric and non-parametric models. The parametric model focuses on the gradual changes of the clock parameters in response to the changing ambient condition, whereas the non-parametric model focuses on the instantaneous change of the phase of the clock in response to the zeitgeber. There are ample empirical data supporting both models. However, only recently has a unifying model been proposed, the circadian integrated response characteristic (CiRC). In the current study, we developed a system of ordinary differential equations, dynamic CiRC (dCiRC), that describes parameters of circadian rhythms and predicts the phase of entrainment in zeitgeber cycles. dCiRC mathematically extracts the underlying information of velocity changes of the internal clock that reflects the parametric model and the phase shift trajectory that reflects the non-parametric model from phase data under entraining conditions. As a proof of concept, we measured clock parameters of 26 *Neurospora crassa* ecotypes in both cycling and constant conditions using dCiRC. Our data showed that the subjective morning light shortens the period of

the clock while the subjective afternoon light lengthens it. We also found that individual ecotypes have different strategies of integrating light effects to accomplish the optimal phase of entrainment, a model feature that is consistent with our knowledge of how circadian clocks are organized and encoded. The unified model dCiRC will provide new insights into how circadian clocks function under different zeitgeber conditions. We suggest that this type of model may be useful in the advent of chronotherapies.

20. The substituting role of ANXC4 during Ca2+-dependent membrane damage response systems in *Neurospora crassa*

Linda Matz, Marcel Schumann, André Fleißner

Germinating spores of *Neurospora crassa* interact and fuse with each other. This process involves cell wall degradation and plasma membrane merger, which bears the risk of cell lysis. We identified three proteins, PEF1, ANX14 and ANXC4, as part of different membrane repair mechanisms with overlapping functions. Initial studies indicated that PEF1 and ANX14 accumulate at the fusion point upon fusion-induced lysis. Since membrane damage can also be induced by membrane-targeting chemicals, we also tested the subcellular dynamics of all three proteins in response to such compounds, including the anti-fungal drug nystatin, the plant defence compound α-tomatine and newly described antifungal salamander alkaloids. PEF1 and ANX14 are recruited to the plasma membrane in response to α-tomatine and the alkaloids, while only PEF1 is responding to nystatin. ANXC4 is not responding to any of these compounds. Interestingly, however, ANXC4 shows robust membrane recruitment in an ∆anx14 mutant background, suggesting a kind of backup function, when the regular repair machinery is defective. Deletion of either anx14 or anxc4 resulted in increased lysis rates of fusing germling pairs compared to the wild type or the Δpef1 mutant. Lysis further increased by 2-fold in a Δanx14 Δpef1 double mutant, suggesting that PEF1 and ANX14 function independently. There is, however, no increase in the ∆anx14 ∆anxc4 mutant, indicating that ANX14 and ANXC4 function in the same pathway. When grown on α-tomatine, ∆pef1 is more sensitive to the toxin than ∆anx14 or ∆anxc4. Taken together, these observations suggest that the annexins are more important for the repair of mechanically-induced damage, while PEF1 is mainly involved in the response to membrane-targeting drugs. We hypothesize that membrane repair constitutes the first step of resistance against membrane damaging compounds. Future studies aim at revealing and fully characterizing the different membrane repair mechanisms in *N. crassa*."

21. Reverse and Forward genetic screens identify new and old elements impacting the *Neurospora crassa* Circadian Clock.

Felipe Muñoz, Valeria Caballero, Luis F. Larrondo

Eukaryotic circadian oscillators share a common circuit architecture, a negative feedback loop in which a positive element activates the transcription of a negative one that then represses the action of the former, inhibiting its own expression. We sought to identify additional transcriptional regulators modulating the *Neurospora crassa* clock, following a

reverse genetic screen based on luminescent circadian reporters and a collection of transcription factors knockouts, successfully covering close to 60% of them. Besides the canonical core clock components WC-1 and WC-2, none of the tested transcriptional regulators proved to be essential for rhythmicity. Nevertheless, we identified a set of 23 transcription factors that when absent lead to discrete, but significant, changes in circadian period. While the current level of analysis does not provide mechanistic information about how these new players modulate circadian parameters, the results of this screen reveal that an important number of light and clock-regulated transcription factors, involved in a plethora of processes, are capable of modulating the clockworks. In addition, during the screen processes we identified mutants displaying clock-phenotypes, and through a subsequent forward genetic analyses we showed that in at least two cases the phenotypes were not associated with the absence of the gene of interest (knockout), but instead to unlinked mutations. Through genome resequencing, and genetic analyses we have been characterizing these spontaneous mutations responsible of circadian alterations, confirming key roles for known components and also identifying potential new candidates involved in the clockworks. iBIO, FONDECYT 1171151 and HHMI International Research Scholar grant.

22. The role of DFG-5 in targeting *Neurospora crassa* glycoproteins for cell wall incorporation and for extracellular secretion.

Pavan K Patel, Stephen J. Free

The GH76 family of α-1,6-mannanases are important cell wall biosynthetic enzymes in S. cerevisiae, C. albicans, and *N. crassa*. The *N. crassa* dfg-5 gene encodes an α-1,6 mannanase needed for crosslinking glycoproteins into cell wall glucan/chitin matrix, an important step in the biosynthesis of the fungal cell wall. Here, we investigate the role of the DFG-5 enzyme in the processing of the N-linked galactomannan and targeting the glycoproteins for cell wall incorporation. We used site-directed mutagenesis of HIS6-tagged DFG-5 to identify amino acid that are required for DFG-5 activity. We report that aspartate residues at positions 116 and 117 represent the active site and an aspartate at position 76 and a glutamate at position 130 are required for DFG-5 to function in cell wall biogenesis. We also report that co-purification experiments with HIS6-tagged DFG5 protein demonstrated that DFG5 forms stable interactions with a large number of cell wall glycoproteins. HIS6-tagged DFG-5 co-purification experiments in a mutant that is unable to make the N-linked galactomannan demonstrates that the presence of an N-linked galactomannan is required for these glycoprotein interactions. Using a Western blot assay, we found that DFG-5 specifically associates with well-known cell wall glycoproteins, and it does not form associations with secreted glycoproteins. The most novel aspect of our report is that DFG-5 plays a central role in the extracellular targeting of the cell wall and secreted glycoproteins and that the ability of DFG-5 to discriminate between the cell wall and secreted glycoproteins is the key event in targeting the two different types of glycoproteins to their final destinations.

23. Mechanisms of circadian clock control of CPC-3 activity in *Neurospora crassa*

Ebimobowei Preh, Deborah Bell-Pedersen

The circadian clock in *Neurospora crassa* regulates rhythms in the phosphorylation and activity of the conserved translation initiation factor eIF2α, which promotes rhythmic mRNA translation (Karki et al., 2020). Cycling phosphorylated eIF2α levels requires rhythmic activation of the eIF2α kinase CPC-3 (the homolog of yeast and mammalian GCN2). However, the mechanisms controlling rhythmic CPC-3 activation are not fully understood. Studies in Saccharomyces cerevisiae suggested that 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, I examined the interaction of CPC-3::V5 and GCN1::HA with ribosomes. The tagged strains were confirmed to be functional by their ability to promote eIF2α phosphorylation. Ribosomes were purified by sucrose density gradient ultracentrifugation from cultures grown in constant dark (DD) and harvested during the subjective day (DD18), corresponding to the time of peak CPC-3 activity. While CPC-3::V5 and GCN1::HA were found to co-sediment with monosomes and polysomes, the interaction of CPC-3::V5 with ribosomes was arrhythmic in DD. Experiments are currently in progress to determine if the interaction of GCN1::HA with the ribosome is under control of the circadian clock, and if mutations in a conserved CPC-3 ribosome binding domain alter the levels and/or rhythmic accumulation of phosphorylated eIF2α.

24. Elucidating the Fungal immune system and testing the potential role of Nucleotide-binding domain Leucine-rich repeat-like proteins (NLR-like) against bacterial antagonists.

Frances Grace Stark, Ksenia Krasileva

Filamentous fungi are hosts to pathogens such as viruses, bacteria, parasitic fungi, and grazing nematodes. Besides RNAi to protect fungal genomes from mycoviruses, a fungal inducible defense upon recognition of bacteria has yet to be fully described. Genes encoding nucleotide-binding domain Leucine-rich repeat-like (NLR-like) proteins are present in abundance in the genomes of filamentous fungi. NLRs are intracellular receptors known to mediate cross-kingdom, antagonistic communication in plants and metazoans. Although a role for NLR-like proteins has been described for self/non-self recognition systems known as heterokaryon incompatibility (HI), evidence of cross-kingdom surveillance of fungal NLR-like proteins is lacking. In order to investigate if fungal NLR-like proteins participate in an inducible response like plant and animal NLRs, I utilize *Neurospora crassa* and various bacteria with a primary focus on the seventeen putative NLR-like proteins encoded in the *N. crassa* genome. I show that exposure of *N. crassa* to bacteria and bacterial secretions results in an environmental-dependent response, Including, but not limited to: growth defects, possible growth upregulation, macroconidial production, and cell death. This result suggests that *N. crassa* is initiating many

transcriptional changes and possible programmed cell death upon recognition of bacteria that might be constituting a putative immune response with the function of either not contacting antagonistic bacteria (cell death) or relocating (conidiation). In order to investigate these responses, I plan on conducting RNAseq, reverse genetics of NLR-like genes, and Genome Wide Association (GWAS) studies of *N. crassa* environmental isolates. The discovery of genes underlying an immune-like response within the kingdom of fungi will not only lead to a better understanding of basic fungal biology but possibly novel cell death pathways to target destructive fungi or bacterial/fungal relationships.

25. FREQUENCY Phosphosite Mutations Perturb Temperature Compensation of the *Neurospora* Circadian Clock

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

The molecular circadian clock in animals and fungi consists of a transcription-translation feedback loop that is highly regulated post-translationally throughout the circadian day by phosphorylation events. The positive arm of the clock, a heterodimeric complex of transcription factors (White Collar-1 and White Collar-2 in *Neurospora*), activates the transcription of the negative arm of the clock, Frequency (FRQ), which complexes with Casein Kinase I to inactivate the positive arm via phosphorylation, thereby inhibiting their own transcription. Several key features define circadian rhythms, including the capacity to be entrained by external cues, the ability to continue oscillating in the absence of such cues, and incredibly, the maintenance of a consistent period across temperatures (temperature compensation/TC). We have previously demonstrated that a classical *N. crassa* period mutant with overcompensated TC (in which period lengthens with temperature), *prd-3*, is a CKII hypomorph. This led to a model in which CKII phosphorylates FRQ preferentially at higher temperatures, which compensates for the increased levels of FRQ present at such temperatures. Given that FRQ phosphorylation may contribute to the TC mechanism, we further probed which phosphosites on FRQ are important for TC by determining the circadian period of strains bearing serine to alanine mutations on FRQ at 20°C, 25°C, and 30°C, using a transcriptional reporter in which the *frq* promoter drives luciferase expression. We found that no disruption of a single phosphosite on FRQ alone can perturb TC, but that when several key sites are mutated together, the strains are abnormally compensated against temperature. Both under-compensation and over-compensation TC phenotypes were observed, depending on which phosphosites were mutated. Our results further support a role for FRQ phosphorylation in TC and begin to define the precise mechanism that underlies how circadian period is temperature compensated.

26. Interspecies interactions in filamentous ascomycete fungi are mediated by a highly conserved cell-cell communication mechanism

Natascha Stomberg, Hamzeh H. Hammadeh, Antonio Serrano, Ulrike Brandt, André Fleißner

Cell fusion is essential for the development of most eukaryotic organisms, its molecular basis is, however, only poorly understood. An established model organism to study cell-cell-fusion is *Neurospora crassa*. Germinating spores of this fungus grow towards each other and fuse to form a supracellular network. This type of cell-cell fusion is common in many other filamentous ascomycete fungi. Fusion germlings of *N. crassa* employ a specific signaling mechanism, which is often referred to as a “cell dialog”. In this process, the two fusion partners coordinately alternate between signal sending and signal receiving. This unusual cellular behavior involves the alternating recruitment of the MAP kinase MAK-2 and the SO protein to the plasma membrane. To test if this mechanism is conserved in other fungi, we investigated the role of the MAK-2 and SO homologs in the grey mold *Botrytis cinerea*. We observed a similar dynamic membrane recruitment of the two proteins in interacting cell tips, suggesting that the "cell dialog" signaling mechanism is indeed conserved. When *N. crassa* and *B. cinerea* spores are mixed, interactions between the two species frequently occur, which result in mutual attraction and cell-cell contact. However, interspecies fusion was never observed. These findings suggest that the so far unknown signal and receptor that mediate cell-cell communication are also conserved, and that downstream mechanisms are in place that prevent interspecies fusion after cell-cell contact. In addition, we found that the presence of *N. crassa* can reprogram developmental decisions in *B. cinerea*. In the grey mold, cell fusion and pathogenic growth appear to be mutually exclusive. When confronted with *N. crassa*, however, *B. cinerea* undergoes fusion under growth conditions, which usually trigger infectious growth. We hypothesize that the pathogenic development may be suppressed in the presence of the so far unknown fusion signals.

27. Generating an accurate map of temperature responses mediated by hsp promoter elements

Cyndi Tabilo, Veronica Del Rio and Luis F. Larrondo

The Heat Shock Response (HSR) is a highly conserved mechanism that provides protection under stressing high temperatures through the action of Heat Shock Proteins (HSP). In the filamentous fungus *Neurospora crassa*, three hsp genes responsible for this response have been characterized: hsp30, hsp70 and hsp80. The transcriptional expression of these genes is regulated upon subtle changes of temperature; but the degree at which the putative transcriptional regulatory elements present in their promoters modulate this response has not been systematically determined. Herein, we analyzed different sections of each hsp promoter, by assessing the expression of a destabilized luciferase reporter. We analyzed and mapped their response in a temperature gradient

and different treatment times. The results reveal an accurate response depending on the temperature and time exposure with a wide range of response levels, anywhere from 10- to 5000- fold induction. Of the tested promoters only hsp30 displayed a highly inducible and tunable response, highly sensitive to discrete temperature changes. We are currently defining the minimal cis-elements required to assemble cognate synthetic promoters maintaining the properties of hsp30 transcripcional dynamics. These studies provide an unprecedented view into the regulation of the *N. crassa* hsp genes while also enabling the use of hsp30 derived cis-elements as tools for biotechnological or basic research endeavors. iBIO, FONDECYT 1171151 and The Richard Lounsbery Foundation.

28. Accounting for the Collar: Quantification of the Endocytic Collar in Three Fungal Species

Joseph G. Vasselli, Ellen Kainer, and Brian D. Shaw

The fundamental cellular unit of a filamentous fungus is the hypha, which grow in unidirectional or polarized manner. Hyphal growth requires a balance of exocytosis through the Spitzenkörper and endocytosis just distal to the apex in the sub-apical collar. These coordinated processes are essential for maintenance of polarized growth. Understanding this means of growth regulation is both medically and agriculturally essential, as most fungal patho-systems require polarized growth to initiate disease. We hypothesize that the spatio-temporal localization of endocytic activity, as well as the rate of endocytic activity, plays an important role in regulating the growth rate of hyphae. Using XYZT confocal microscopy on fluorescence protein tagged fimbrin in three species, Colletotrichum graminicola, *Neurospora crassa*, and *Aspergillus nidulans*, we quantified a strong positive association between the distance from the apex to the endocytic collar and hyphal growth rate in all three species. Other measured parameters were less strongly associated with growth rate including hyphal diameter and the size of the endocytic collar. FRAP was used to determine endocytic rate as per a previous study in *N. crassa* that concluded that endocytosis function in hyphal growth was to remove excess membrane, and a comparison between the studies will be made.

29. 3-color live-cell imaging reveals dynamic sub-cellular distributions of core clock components

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

Circadian rhythms are endogenous daily oscillations driven by a molecular clock that helps organisms better coordinate their metabolism and behaviors with the environment. *Neurospora crassa* is the most well-studied fungal circadian model. The clock regulates more than 40% of the expression of the *Neurospora* genome and affects its spores' development and liberation. *Neurospora crassa* uses a phosphorylation-driven transcription/translation negative feedback loop (TTFL) as the core clock mechanism, which describes an oscillator composed of positive and negative elements. The White Collar Complex (WCC) is a heterodimeric transcription factor and serves as the positive element. FRQ, stabilized by forming the FRQ-FRH complex which also binds CK1, is the

negative element. Molecular components of the circadian clock have been described over thirty years of genetic and molecular biological studies. However, little is known about their spatiotemporal regulation at the sub-cellular level. We are developing strategies to overcome extant difficulties in applying live-cell imaging to *Neurospora* circadian research, including clock components' low abundance and unpredictable structure. Through 3-color live-cell imaging of WCC (red) and FRQ (green) and a nuclei marker (blue), we can potentially elucidate the dynamics of their subcellular localization and protein-protein interactions in high spatiotemporal resolution."

30. New NuA4 subunits reveal a crucial role of dynamic expression of the negative arm in the *Neurospora* clock

Bin Wang, Xiaoying Zhou, Arminja N. Kettenbach, Hugh D. Mitchell, Jennifer J. Loros, and Jay C. Dunlap

Post-translational modifications (PTMs) on histones have been found to play diverse functions in regulating chromatin events and gene expression. The operation of circadian clocks heavily replies on finely and timely tuned expression of the proteins comprising core oscillators. However, most studies of PTMs' effects on circadian clocks were conducted using static systems in which circadian clocks were terminated due to the essential role of PTMs on gene expression. In the *Neurospora* circadian system, the White Collar Complex (WCC), a heterodimeric transcription factor formed from White Collar-1 (WC-1) and White Collar-2 (WC-2), induces transcription of the circadian pacemaker gene frequency (frq). FRQ, the gene product of *frq*, interacts with FRH (FRQ-interacting helicase) and CK-1 to repress its own transcription by inhibiting WCC. In this study, genetic screening identified a new gene, designated as eaf-8, required for the normal circadian period determination in *Neurospora*. Absence of *eaf-8* leads to a long circadian period, delayed phase, defective overt circadian output under certain temperatures, and compromised temperature compensation. EAF-8 strongly associates with the NuA4 histone acetyltransferase complex, as well as with the transcription elongation factor BYE-1, and loss of eaf-8 reduces H4 acetylation and RNA polymerase (Pol) II occupancy at *frq* and other known circadian genes. Reciprocally, expression of *eaf-8*, *bye-1*, histone hH2Az, and several NuA subunits is controlled by the circadian clock, indicating that the molecular clock regulates the basic chromatin status. Taken together, our data identify a new type of the NuA4 complex formed by EAF-8 and BYE-1 with conventional NuA4 subunits identified in yeast, which is required for timely and dynamic *frq* expression and thereby a normal circadian period.

31. Examining changes to Eukaryotic Genome Organization Upon Altering Epigenetic Mark Levels

Ashley Ward, Andrew D. Klocko

Eukaryotic DNA is comprised of two general forms of chromatin: active euchromatin and silent heterochromatin, each of which possess epigenetic marks involved in regulating gene expression. These chromatin forms are then further organized into active and silent

"compartments" where DNA loops facilitate long-range interactions. The mechanisms underlying this organization are not fully understood and it is unknown whether changing levels of epigenetic modifications, impact long-range interactions. This work explores how variations in epigenetic marks alter genome organization by using the filamentous fungus, *Neurospora crassa*, which has similar DNA compaction to humans, but a smaller genome which is amenable to high-throughput chromosome conformation capture sequencing (Hi-C) methods. Genome organization was characterized in multiple *Neurospora* strains containing deletions of genes encoding proteins found within silencing complexes, specifically the histone deacetylase complex (HCHC). In *Neurospora*, the HCHC removes active acetyl groups from histones thereby silencing chromatin. Deletion of HCHC genes results in increased histone acetylation as well as size dependent changes in DNA methylation within heterochromatic regions. Genome organization of mutant strains lacking the HCHC members CDP-2 and CHAP were assessed. Here I present the findings of these mutant strains where the loss of HCHC members caused genome wide organizational changes and detail additional research of a double mutant strain lacking CDP-2 and the DNA methyltransferase DIM-2 to elucidate if these genome changes are a result in the altered histone acetylation or DNA methylation levels. All told, my research suggests that epigenetic marks play a role in organizing the genome of eukaryotic organisms.

32. The kinase activity of COT-1 is essential for proper polarization and interaction of germinating conidiospores in *Neurospora crassa*

Lucas Well, Ulrike Brandt, Oded Yarden, André Fleißner

Cot-1 (Colonial Temperature sensitive 1) was one of the first temperature sensitive mutants found in an early forward genetics mutant screen of *Neurospora crassa*. When grown at restrictive temperatures, the mutant loses the activity of the serine/threonine protein kinase COT-1, which results in a hyperbranching, dendritic-spine-like phenotype. Besides this severe polarity defect, it also generates more septa and thicker cell walls compared to the wild type. To investigate the kinase COT-1 via live cell imaging we employed a chemical genetics approach, in which the replacement of a single amino acid residue renders the kinase sensitive to an ATP analog. Addition of the chemical inhibitor, inhibits the kinase within minutes. Inhibition of COT-1 in germinating conidiospores results in the formation of multiple germtubes and a swollen spore body. In wild type, germinating conidia interact and fuse into a supracellular network. When COT-1 is inhibited, the germling interaction rate is significantly reduced. Germling interactions involve the dynamic, alternating membrane recruitment of the MAP kinase MAK-2 and the SO protein at the growing cell tips. When COT-1 is inhibited, the proteins permanently accumulate at the growing tips at the same time, indicating that COT-1 activity is required for the proper dynamics of these signaling factors. Introduction of the known suppressor mutation gul-1 into the analog sensitive strain improved the general vitality and the interaction rate. In summary, our data revealed a novel function of COT-1 in cell-cell communication and fusion. Further studies aim to identify the role and function of this kinase in the complex signaling network mediating cell-cell interactions in *N. crassa*.

33. Does *Neurospora crassa* need different MTOCs?

Rosa Ramírez Cota, Rocío Evelyn Macias Díaz, Olga Alicia Callejas-Negrete, Michael Freitag, Reinhard Fischer, Robert W. Roberson, Rosa R. Mouriño-Pérez

γ-Tubulin ring complexes (γ-TuRC) mediate nucleation and anchoring of microtubules (MTs) to microtubule organizing centers (MTOCs). In fungi, the spindle pole body (SPB) is the functional equivalent of the centrosome, which is the main MTOC, and in addition, non-centrosomal MTOCs (ncMTOCs) contribute to MT formation in some fungi. Such ncMTOCs were characterized in *Schizosaccharomyces pombe* and in *Aspergillus nidulans*. In *A. nidulans* they are anchored at septa (sMTOC) and share components of the outer and the inner plaque of the SPB. Here we show that the *Neurospora crassa* SPB is embedded in the nuclear envelope, with the γ-TuRC targeting proteins PCP-1Pcp1/PcpA located at the inner face and APS-2Mto1/ApsB located at the outer face of the SPB. PCP-1 is a specific component of nuclear MTOCs while APS-2 is also present in the septal pore. Although γ-tubulin was only detected at the nucleus, spontaneous MT nucleation took place in the apical and subapical cytoplasm during recovery from benomyl-induced MT depolymerization experiments. MT dynamics were monitored with the MT plus end tracking protein MTB-3 and revealed MT polymerization from septa. Septal MT polymerization was dependent on the septal proteins SPA-10Spa10 and SPA-18Mto2/Spa18 (i.e., the APS-2/SPA-18 protein complex). We conclude that in *N. crassa* the SPB is the only MT nucleator site, but the septal pore aids to MT network arrangement trough the anchorage of the MT plus-ends.