2021

WAKSMAN

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ABOUT US

The Waksman Institute of Microbiology is an interdisciplinary research institute devoted to excellence in basic research, located on Busch Campus of Rutgers, The State University of New Jersey. Focus areas include developmental biology, cell biology, biochemistry, structural biology, genetics, and genomics.

The Institute employs faculty teams that concentrate on certain organisms amenable to genetic analysis such as bacteria and fungi (E. coli and yeast), animal systems (e.g., Drosophila and C. elegans), and plants (Arabidopsis, tobacco, and maize). Although the Institute focuses on basic questions in microbial, animal, and plant research, it continues to engage in extensive technology transfer of its basic discoveries.

To support the educational mission of Rutgers, Waksman faculty members hold appointments in academic departments throughout the University. Our researchers train undergraduate students, graduate students, and postdoctoral fellows, as well as engage high school students in research through an outreach program.

Giving

The Waksman Institute is supported by Rutgers University, its endowment and research grants, and gifts from private foundations and individuals. Gifts provide valuable resources to enhance research initiatives and increase student opportunities. Donors may choose to contribute either to the Institute's operating budget, or to a specific initiative. For more information, contact Robert Rossi, Executive Director for Administration and Finance: 848-445-3937, rossi@waksman.rutgers.edu.

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Rutgers Research and Educational Foundation

The Rutgers Research and Educational Foundation (RREF) was established by the Rutgers University Board of Trustees to receive the income from the streptomycin and neomycin inventions as well as other inventions by Waksman Institute faculty and staff.

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CONTENTS

REPORT OF THE INTERIM DIRECTOR

Kenneth Irvine

For all of us in the Waksman Institute, as for all of you, this has been a year like no other. After having most of our research suspended at the beginning of the Covid-19 pandemic, Waksman Institute researchers returned to their labs in July 2020, as soon as Rutgers and state guidelines allowed. During this strange year, Waksman scientists have been working in labs with staggered schedules, while wearing masks and staying 6 feet apart. Our labs have gradually refilled, and we are now back at full capacity, while continuing to follow Covid safety guidelines. I'd like to thank everyone in the Institute for their continued contributions to advancing our research and education efforts under these challenging circumstances. I'd also like to acknowledge Institute members who have helped in ways large and small to combating the pandemic, including staff members who have supported the efforts of RUCDR/iBX in Sars-CoV-2 testing while they occupied Institute lab space.

In addition to the exciting research taking place within the Institute, a highlight of the past year was a virtual symposium and laboratory dedication in honor of Dr. Evelyn Witkin. Dr. Witkin was a Rutgers faculty member from 1971-1991, and a lab Director in the Waksman Institute. She performed high-impact research in bacterial molecular genetics and was a pioneer who opened pathways for women in the biological sciences. Among other accolades, Dr. Witkin was awarded the Lasker prize in 2015 for her discoveries, which is America's the most prestigious prize in the life sciences. Dr. Witkin is one of only two Lasker prize recipients from Rutgers (Selman Waksman is the other). Despite Dr. Witkin's scientific accomplishments, no celebration of her career had ever been held at Rutgers. To rectify this, we held a virtual symposium in her honor in April 2021, which was attended by hundreds of scientists around the world. The symposium coincided with Dr. Witkin's 100th birthday and we were delighted that she was also able to attend. In addition to stellar talks from leading scientists, the symposium featured gracious introductory remarks from President Holloway, Chancellor Molloy, and Senior Vice President for Research Scotto. In conjunction with the symposium, we dedicated one of the new research laboratories in the Institute as the "The Evelyn Witkin Laboratory," to serve as a permanent reminder of this remarkable Rutgers scientist.

Despite this year's challenges, as you look through this report I trust you will be impressed, as I am, by the outstanding accomplishments of our faculty. They have continued to push the frontiers of knowledge across the breadth of the life sciences, while training the next generation of scholars and researchers. They have been ably assisted by our excellent administrative and core facilities staff, working under the direction of Executive Director of Finance and Administration Bob Rossi.

Waksman Mission

The primary mission of the Waksman Institute is to function as an interdisciplinary center of excellence in foundational life sciences research. We achieve this by supporting investigators addressing a broad range of fundamental questions in biology, as well as the development of novel biotechnologies, to push the frontiers of scientific discovery. We also facilitate interdisciplinary research at Rutgers through connections to diverse units, by operating core facilities that enable scientific research, and by supporting seminars and symposia. We also aim to be a leader in research education through the teaching and training of post-doctoral, graduate and undergraduate students and fellows, and through our high school outreach programs.

Overview of the Waksman Institute

The Institute was established in part through royalties that Rutgers received from patents on antibiotics discovered in the laboratory of its first director, Selman Waksman. The most significant of these was streptomycin, the first antibiotic effective against tuberculosis, which was discovered by Waksman's student Albert Shatz. While the initial focus of the Institute was microbiology and antibiotic discovery, the Institute's research mission has evolved and broadened

over 70 years, while remaining true to Dr. Waksman's exhortation that the Institute should be "dedicated to the free pursuit of scientific knowledge for the benefit of all mankind."

The Waksman Institute currently supports investigations into a broad range of fundamental questions in biology, as well as the development of novel biotechnologies, to push the frontiers of scientific discovery. Using microbial, plant, and animal models, Waksman scientists conduct research on diverse topics including morphogenesis, gene regulation, signal transduction, cancer, fertility, metabolism, sustainable energy, congenital and neurologic disorders, together with antibiotic discovery. A key aspect of research in the Institute is its interdisciplinary nature, including faculty from multiple departments and schools, and incorporating approaches from physical and computational sciences into life sciences research. The Institute has a long history of excellence, including Rutger's only Nobel prize winner (Selman Waksman), only Lasker prize winners (Selman Waksman and Evelyn Witkin), and recipients of a number of other major awards and prizes, including Wolf Prize winners, Howard Hughes Medical Institute Investigators, and inductees into the National Academy of Sciences.

Waksman faculty all hold joint appointments within academic departments at Rutgers, and the faculty play active roles in supporting the educational mission of Rutgers University through the teaching and training of post-doctoral, graduate and undergraduate students and fellows. The Institute also provides support and space for educational and outreach programs, including the renowned Waksman Student Scholars Program, which has provided a first introduction to research to thousands of high school students across the United States for almost three decades. The Institute also supports and hosts seminars and symposia that bring descriptions of new discoveries from around the world to the Rutgers campus.

The Waksman Institute also maintains life sciences infrastructure to support the research efforts of both Waksman Institute scientists and outside users, including facilities for confocal microscopy, genomics, fermentation, and a field and greenhouse for growing plants. Descriptions of the facilities and services provide by each of these units are included elsewhere in this annual report.

Faculty

In the academic year 2020-2021, the Institute had sixteen resident faculty members, plus one non-resident member and seven emeriti faculty. The Institute also accommodates eight assistant research professors, nine visiting student/scholar researchers, eighteen research associates, eleven postdoctoral researchers, twenty technical assistants, and eighteen graduate students. The Waksman Institute's total resident population is currently 147, including forty-three undergraduate students who did independent research during the last year in Waksman Institute labs.

Among the Waksman-resident faculty, five are in the Department of Molecular Biology and Biochemistry, five are in the Department of Genetics, four are in the Department of Plant Biology and Pathology, two are in the Department of Chemistry and Chemical Biology, and one is in the Department of Biochemistry and Microbiology (Dr. Dismukes has two departmental affiliations). Of the sixteen current resident members, three are Assistant Professors, two are Associate Professors, seven are Professors, and four are Distinguished Professors, one of whom is also a Board of Governors Professor. Notable faculty awards this past year include the election of Kenneth Irvine as a Fellow of the American Association for Advancement of Science, and the awarding of the Chancellor's Award for Pioneering Research to Chuck Dismukes.

Funding

Competitive acquisition of external grants and contracts forms the major part of our research support. We are proud of the success of all of our faculty in securing external funding. During the past fiscal year, Waksman faculty were supported by $5.35 million dollars in external grants.

ADMINISTRATION REPORT

Robert Rossi

The Waksman Institute's research mission is greatly aided by our administrative and core facilities staff, working under the direction of Executive Director of Finance and Administration, Robert Rossi.
The Institute's administrative staff continues to be kept to the minimum essential staff needed to support our core research mission.

The Institute's Business Office staff of four people have primary responsibilities for budgeting, purchasing, and pre and post award administration of all sponsored awards. In addition, the Business Office staff provide support for required financial reporting to the central University administration. For Human Resource operations at the Institute, we rely on one person who handles all HR responsibilities including hiring, appointments, and work visas.

The Information Technology Office, comprised of just three people, provides critical computing support to all faculty and staff at the Institute. The IT Office also makes recommendations regarding computing procurement and IT infrastructure to support the Institute's research goals and long-term growth.

The Waksman Institute's research mission is also supported by our core central services that include glassware and autoclaving, genomics services, greenhouse facility services, and specialty repair of equipment. The staff for these core central services is kept to the minimum necessary for operational support, and part-time employees are utilized as appropriate.

The Business Office, Human Resource Office, Information Technology Office and research support areas report to the Executive Director of Finance and Administration, and this position in turn reports to the Director of the Waksman Institute.

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INFORMATION AND TECHNOLOGY REPORT

Randall Newman

The Waksman Institute employs three full time staff to maintain the computing resources of the Institute as well as provide software and hardware support to all of our faculty, staff, and students. The IT staff are responsible for maintaining the 24/7 availability of these resources with minimal downtime. Our industry standard raised floor data center is located on the fourth floor in the building›s Old Wing. With dedicated cooling and generator backed up emergency power, it hosts 30+ servers, a high-performance computing cluster with nearly 400 logical compute cores, and over 1PB of enterprise class storage with an offsite backup location for disaster recovery. The servers, storage, and other devices communicate using a combination of high-speed 10Gb Ethernet and 8Gb Fibre Channel fabrics. Extensive server virtualization provided by VMware ESXi is used to make most efficient use of available physical hardware and minimize energy costs.

In addition to its on-site resources, the Institute makes use of a number of shared University resources including the Office of Advanced Research Computing (OARC) Amarel cluster, a shared community-owned advanced computing environment. This large community-model Linux cluster is comprised of tens of thousands of Intel Xeon cores, various models and configurations of NVIDIA GPUs, and multiple 1.5 TB RAM large-memory nodes, all sharing a Mellanox InfiniBand fabric and an IBM Spectrum Scale concurrent-access cluster file system and is ideally suited for many of the Institutes computationally intensive research tasks.
By utilizing Rutgers' Internet 2 connection, Waksman users have access to a high speed, high bandwidth direct connection to 400+ universities and 60 affiliate members of the Internet 2 consortium. The Institute provides its users with a number of traditional office software packages, common molecular biology tools, as well as sequence analysis application suites like Lasergene DNAStar and SnapGene.

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BARBER LAB

Circadian Behavioral Genetics in Diverse Environmental Contexts

Dr. Annika Barber
Molecular Biology & Biochemistry

Summary
We can't do everything all at once. To quote Serge Daan, "An animal performing all its activities in optimal proportions but I random temporal sequence would be continuously making the right decisions at the wrong time." A circadian transcription translation feedback loop, termed the molecular clock, in the brain and peripheral tissues coordinates timing of behavior and physiology relative to changes in the environment, most notably the day-night cycle. This "brain clock" acts as a central pacemaker providing time-of-day cues to other brain circuits and peripheral tissues.

But many aspects of life are unpredictable, so circadian signals must be integrated with sensory cues about the flies' internal and external environment to inform behavioral choices. The Barber lab uses the fruit fly, *Drosophila melanogaster*, as a model system to investigate how neuronal networks regulating conserved behaviors integrate sensory and circadian cues to inform behavioral choices. We use the circadian clock output circuit to understand how co-transmitter signaling by small molecule neurotransmitters and neuromodulatory peptides affects neurophysiology and circadian behaviors such as sleep, locomotor rhythms and feeding. We also seek to understand how environmental context affects circadian signaling at the molecular and circuit levels to alter physiology, behavior and ultimately other aspects of health such as longevity, and survival after traumatic injury. While our work focuses on circadian physiology, our findings elucidate fundamental aspects of how environmental contexts shape neuronal circuits to alter behavior.

Characterization of a signal integration "hub"

The *Drosophila pars intercerebralis* (PI) is an analog of the mammalian hypothalamus and regulates numerous processes including sleep, arousal, locomotor rhythms, feeding, and gene transcription in peripheral tissues. As in the hypothalamus, multiple internal and external sensory pathways converge in the PI, which then releases an array of neuropeptides that influence fly behavior. This project examines how time-of day signals are communicated to the PI by the clock neuron circuit by both fast neurotransmitters and neuropeptide signals, and investigates the role of intra-PI PI signaling in coordinating locomotor and feeding behavior.

We have previously shown that insulin-producing neurons of the PI have rhythmic physiology, despite lacking a molecular clock. Instead, PI neurons receive inputs from upstream brain clock neurons, which drive physiological rhythms. The circadian clock network in *Drosophila* is a well-studied circuit with extensive colocalization of small molecule neurotransmitters and neuropeptides that integrates light and temperature information. We have previously demonstrated clock-to-PI signaling via small molecule neurotransmitters, and are currently extending this work to investigate the role of co-transmission of neurotransmitters and neuropeptides in regulating circadian locomotor and feeding rhythms.

An exciting exploratory arm of this project uses RNA sequencing of *Drosophila* PI neurons to identify new PI peptidergic populations regulating sleep, feeding and circadian locomotor rhythms. Preliminary data from this study demonstrates that despite lacking a molecular clock, insulin producing PI cells maintain rhythmic expression of multiple genes (Figure 1).

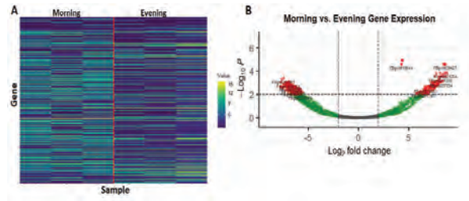

Fig. 1 Diurnal regulation of gene expression in insulin producing cells. (A) Insulin producing cells (IPCs) collected in the morning vs. evening have distinct patterns of transcript expression. Because IPCs do not have their own molecular clock, upstream clock neuron inputs may drive diurnal gene expression. (B) Volcano plot displaying the range of transcripts detected in the differential gene expression from (A). Transcripts indicated in red are significant (padj < 0.05 and Log2fold change > 5)

Defining the role of circadian clocks in gene regulation and health after traumatic injury

Sleep and circadian rhythm disorders are a major feature of traumatic brain injury (TBI); TBI patients not only experience acute insomnia and sleep disorders, but also show delayed rhythms of core body temperature and melatonin secretion for years after injury. Symptoms observed in TBI patients, such as mood disorders and PTSD, are associated with changes in clock gene expression and alterations in circadian timing. At present the field lacks a mechanistic understanding of how molecular clock function is altered by traumatic injury, and whether the presence of molecular clocks in the brain or peripheral tissues improves TBI outcomes. Novel traumatic injury models in the fruit fly that mimic physiological changes observed in humans with TBI allowed us to find that circadian clock function improves survival after traumatic injury in *Drosophila.* We are currently investigating how circadian transcriptional control across tissues is altered by traumatic injury and how chronotherapeutic interventions that strengthen circadian physiology could protect against the long-term sequelae of TBI.

Age x Diet effects on circadian function

Over the course of normal aging, circadian rhythms dampen, which can contribute to age-related decline in health. High-fat and high-sugar diets can also reset clocks in peripheral tissues, leading to circadian desynchrony. Surprisingly, the daily oscillation of clock molecules in the "master clock" in the brain of both flies and humans remains robust, even as circadian behavior and physiology become disorganized with advancing age or dietary changes (Figure 2A). If the clock in the brain is still ticking, why do behavior and physiology become temporally disorganized? Using a fly model, we are examining how circadian outputs from the brain clock become disrupted in normal aging, and in conditions of nutritional stress (Figure 2B).

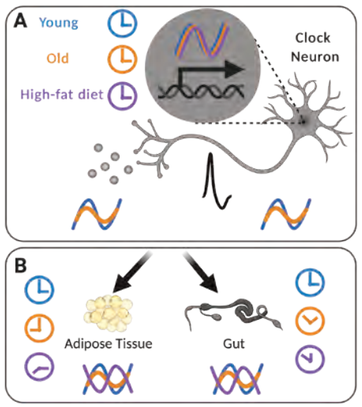

Fig. 2 Degradation of circadian signals by age and dietary challenge. (A) Aging dampens rhythms of neurotransmitter release (left) and physiological properties (right) of brain clock neurons are dampened with age, despite intact transcriptional rhythms. (B). High-fat diet induced loss of circadian phase synchrony is exacerbated in old age, leading to reduced life- and healthspan.

Dietary interventions have the most reproducible effects on lifespan and healthspan across species and affect the molecular circadian clock. Caloric restriction robustly extends lifespan in a clock-dependent manner, while time-restricted feeding paradigms show promise in improving metabolic and muscle tissue function with age, likely via interactions with peripheral circadian clocks. Conversely, consumption of high-fat and high-sugar diets leads to reductions in lifespan and disrupts molecular and behavioral circadian rhythms. The tight links between diet, circadian function, and life/healthspan suggest a diet x clock effect on regulating circadian phase synchrony through unknown mechanisms. Age related changes in circadian function and metabolism are highly conserved in *Drosophila*, allowing us to examine how clock function in peripheral tissues regulates life- and health-span in dietary challenge.

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IRVINE LAB

Developmental Biology

Dr. Kenneth Irvine
Molecular Biology & Biochemistry

Summary
During development, organs grow to a characteristic size and shape. This is essential for normal organ function, and the symptoms of many congenital syndromes stem from defects in organ growth or morphogenesis. Moreover, dysregulation of growth control is associated with tumorigenesis. A detailed understanding of organ growth and morphogenesis will also be required to create functional organs from stem cells, which is a key goal of regenerative medicine. Yet how characteristic and reproducible organ size and shape are achieved remains poorly understood.

Key molecular insights into how growth is controlled have come from the identification and characterization in model systems of intercellular signaling pathways that are required for the normal control of organ growth. Many of these pathways are highly conserved among different phyla. We are engaged in projects whose long-term goals are to define relationships between patterning, growth and morphogenesis in developing and regenerating organs and to determine how patterning inputs are integrated with other factors, including mechanical stress. Much of our research takes advantage of the powerful genetic, molecular, and cellular techniques available in *Drosophila* melanogaster, which facilitate both gene discovery and the analysis of gene function. We also use cultured mammalian cell models.

One major area of research involves investigations of the Hippo signaling network, which has emerged over the past 15 years as one of the most important growth regulatory pathways in animals. We study its regulation, molecular mechanisms of signal transduction, and its roles in different developmental and physiological contexts. We discovered regulation of Hippo signaling by the Dachsous and Fat cadherins over a decade ago, and have continued to define key steps in this branch of Hippo pathway regulation.

Another focus of our investigations of Hippo signaling has involved determining how mechanical forces experienced by cells influence Hippo signaling, and thereby organ growth. Observations that mechanical stress can influence cell proliferation had been made as early as the 1960s, but the molecular mechanisms responsible were unknown. We identified the first biomechanical pathway that could link cytoskeletal tension to Hippo signaling by discovering that the localization and activity of the *Drosophila* Ajuba LIM protein (Jub), and the Warts kinase, are modulated by cytoskeletal tension, providing a direct link between myosin activity and organ growth. We have more recently demonstrated that this mechanism contributes to feedback regulation of growth in compressed cells, and that it contributes to density-dependent regulation of cell proliferation in developing *Drosophila* wings and in cultured mammalian cells. The role of density-dependent mechanical stress in modulating Hippo signaling provides a mechanism through which this pathway can contribute to the regulation of organ size.

We have also investigated molecular mechanisms by which cells can respond to mechanical stress. Jub localization is regulated through a tension-dependent association with α-catenin, and we obtained evidence that this occurs through a tension-induced conformational change in α-catenin that enables Jub binding. We also confirmed that increased Jub recruitment to α-catenin is associated with increased Yorkie activity and wing growth, even in the absence of increased cytoskeletal tension. Additional studies have identified novel roles for Jub in modulating tension and cellular organization, which are shared with the cytohesin Step, and the cytohesin adapter Stepping Stone, and we established that Jub and Stepping Stone together recruit Step to adherens junctions under tension. This work identified a role for Jub in mediating a feedback loop that modulates the distribution of tension and cellular organization in epithelia.

We have also characterized links between mechanical forces and Hippo signaling in mammalian cells, and discovered both conservation of the Jub biomechanical pathway and a role for this pathway in cell density-dependent regulation of mammalian Hippo signaling, including contact-inhibition of cell proliferation. Our studies have provided a molecular understanding of how tissue mechanics can influence Hippo signaling, while also emphasizing that there are

multiple mechanisms by which mechanical forces regulate this pathway.

Our studies of biomechanical regulation of Hippo signaling in mammalian cells led to our recent discovery of a role for the LIM-domain protein TRIP6 in maintaining tension at adherens junctions. TRIP6 and the Jub homologue LIMD1 had each been identified as being required for tension-dependent inhibition of the Hippo pathway LATS kinases and their recruitment to adherens junctions, but the relationship between TRIP6 and LIMD1 was unknown. Using siRNA-mediated gene knockdown we found that TRIP6 is required for LIMD1 localization to adherens junctions, whereas LIMD1 is not required for TRIP6 localization. TRIP6, but not LIMD1, is also required for recruitment of Vinculin and VASP to adherens junctions. Knockdown of TRIP6 or Vinculin, but not of LIMD1, also influences the localization of myosin and F-actin. In TRIP6 knockdown cells actin stress fibers are lost apically but increased basally, and there is a corresponding increase in recruitment of Vinculin and VASP to basal focal adhesions. Our observations identified a role for TRIP6 in organizing F-actin and maintaining tension at adherens junctions that could account for its influence on LIMD1 and LATS. They also suggested that focal adhesions and adherens junctions compete for key proteins needed to maintain attachments to contractile F-actin.

We have also investigated how tissue patterning and mechanics influence organ shape. As one simple model, we have combined genetic analysis, live imaging, and computation image analysis to investigate cellular and molecular mechanism that govern wing shape in *Drosophila*. One unexpected outcome of these studies was the discovery that orientation of cell divisions are not required for normal wing shape. Current studies are investigating how Dachsous-Fat signaling influences the shape of *Drosophila* organs.

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MCKIM LAB

Molecular Genetics of Meiotic Recombination and Chromosome Segregation

Dr. Kim McKim
Genetics

Summary
Aneuploidy, or an abnormal chromosome number, is a leading cause of spontaneous abortions and infertility in women and also causes diseases such as Down, Turner or Klinefelter syndromes. It is caused by errors in meiosis, the process that deposits the correct number of chromosomes into each sperm and oocyte. Using *Drosophila melanogaster* females as a model, we are studying the mechanisms that promote accurate chromosome segregation in oocytes; to understand how oocytes receive the correct number of chromosomes and the mechanisms of errors that lead to aneuploidy. Due to their unique biology, there are probably segregation mechanisms that are unique to oocytes. We are interested in the protein complexes and mechanisms of meiosis and the features of the oocytes that makes them susceptible to chromosome segregation errors.

Meiosis begins with pairs of chromosomes undergoing recombination events. These events not only exchange genetic information and generate diversity in the population, they provide a temporary link between each pair of homologous chromosomes. This linkage allows the chromosomes to orient on a bipolar meiotic spindle such that they segregate from each other during the meiotic division, a process known as bi-orientation. Specifically, prior to separating, each pair of chromosomes bi-orients on a bipolar meiotic spindle such that when the cell divides, the chromosomes move in opposite directions and the chromosome complement is reduced in half. The movement of chromosomes during meiosis is driven by attachments made between the microtubules and the chromosomes. Fertilization then restores diploidy and the next generation begins.

Chromosome-directed oocyte spindle assembly depends HP1 and the Chromosomal Passenger Complex

Oocytes in many organisms assemble bipolar spindles in the absence of centrosomes, and instead, the microtubules assemble around the chromosomes by an unknown mechanism. In *Drosophila* oocytes, spindle assembly, which includes kinetochore assembly and recruitment of spindle assembly factors such as kinesins, requires the chromosomal passenger complex (CPC), which consists of INCENP, Borealin, Survivin and Aurora B kinase. To determine what recruits the CPC to the chromosomes and its role in spindle assembly, we developed a strategy to manipulate the function and localization of INCENP, which is critical for recruiting the Aurora B kinase to certain targets. Localizing INCENP to the centromeres and microtubules was not sufficient to promote bipolar spindle assembly. Furthermore, known pathways that recruit the CPC to centromeres, involving Bub1 or Haspin, are not required for spindle assembly. Instead, we found that Borealin, when fused to INCENP, was sufficient to assemble a fully functional bipolar spindle (Figure 1).

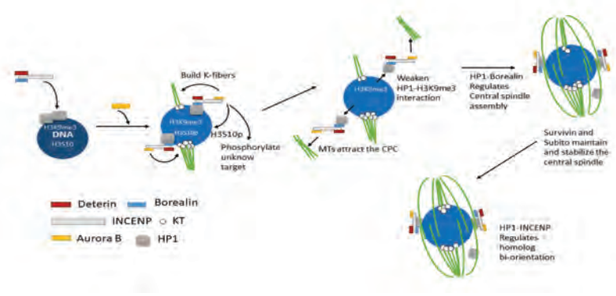

Figure 1: After Nuclear Envelope breakdown, a complex of INCENP, Borealin and Deterin / Survivin is recruited to the chromosomes. Localization studies suggest that CPC recruitment is enriched in heterochromatic regions containing H3K9me3 and HP1. Aurora B is recruited, which results in kinetochore assembly, limited microtubule recruitment in the form of K-fibers, and phosphorylation of other targets including H3S10 and possibly HP1. Aurora B activity also results in Borealin-dependent ejection of HP1 and the CPC from the chromosomes to the microtubules. Once on the microtubules, the Kinesin 6 Subito causes enrichment of the CPC and HP1 in the central spindle. HP1 could be involved in a complex pattern of interactions that bring important spindle proteins together. For example, Aurora B could be brought together with potential phosphorylation substrate Subito, which has a conserved HP1 binding site that is required for its meiotic functions.

Furthermore, an interaction between Borealin and HP1 is crucial for the initial recruitment of the CPC to the chromosomes and is sufficient to build kinetochores and recruit spindle microtubules. We also found that HP1 relocates from the chromosomes to the spindle microtubules along with the CPC, suggesting a mechanism for how the CPC moves from the chromosomes to the microtubules. Recruitment of Aurora B to the chromosomes results in phosphorylation of S10 of Histone 3, which then causes the CPC-HP1 complex to move from the chromosomes to the microtubules. The CPC then becomes concentrated within the *central spindle* (the microtubules bundled into anti-parallel arrays in the center of the spindle) through the activity of the kinesin motor protein Subito. Within the central spindle, rather than at the centromeres, the CPC-HP1 complex is required for homologous chromosome bi-orientation. Thus, HP1 plays a pivotal role in oocyte meiosis, mediating CPC localization to the chromosomes, spindle assembly, transfer to the microtubules, and homolog bi-orientation within the central spindle.

Multiple pools of PP2A regulate spindle assembly, kinetochore attachments, and cohesion in *Drosophila* oocytes

The chromosomal structure that mediates interactions with the microtubules is the kinetochore. Our published and unpublished data support a model that two types of microtubule attachment to the kinetochores are used for the process of bi-orientation: lateral attachments and end-on attachments (Figure 2). Lateral attachments are when the kinetochore interacts along the side of a microtubule bundle. End-on attachments are when the kinetochore interacts with the end of a microtubule bundle. We propose that lateral attachments are required to position chromosomes correctly prior to their separation and movement towards the spindle poles. Lateral attachments are transient and can be corrected if a

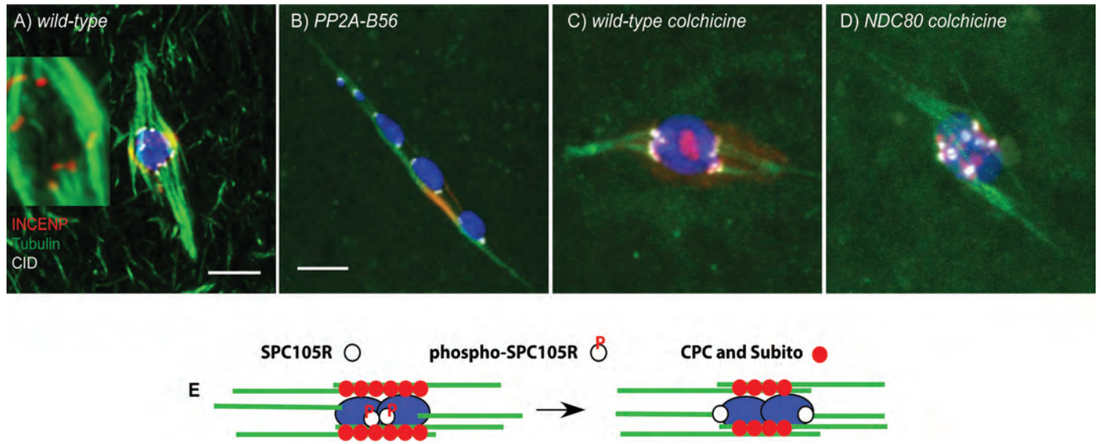

Figure 2: A) A "Lightning" super resolution image showing end-on attachments. Microtubules end at the centromeres (inset). B) Lateral attachments persist in PP2A RNAi oocytes and are visible as centromeres positioned along the walls of microtubules. C, D) A short (15-min) colchicine treatment removes most of the microtubules except those with attachments, revealing end-on attachments in wild-type and lateral attachments in Ndc80 RNAi oocytes, which lack the kinetochore proteins required for end-on attachments. E) Model that lateral attachments are associated with phosphorylated SPC105R, which may occur when SPC105R is located in a region of high Aurora B activity within the central spindle. This region contains a high density of overlapping microtubules organized by the kinesin Subito. The Aurora B kinase localized to this region may phosphorylate SPC105R.

chromosome establishes an incorrect position. End-on attachments are stronger but more permanent. In this model, end-on attachments are only established once each pair of chromosomes have bioriented and established their correct positions for segregation.

The transition between lateral and end-on attachments appears to depend on the kinetochore SPC105R, also known as KNL1 in other organisms. This protein recruits other kinetochore proteins while also interacting with the microtubules. We are studying SPC105R and its partners to investigate how lateral attachments occur, how bi-orientation is achieved, and how the transition from lateral attachments to end-on attachments is regulated.

The transition to between lateral and end-on attachments may be regulated by kinetochore phosphorylation. To investigate the mechanisms that regulate kinetochore phosphorylation, we examined Protein Phosphatase 2A (PP2A) in oocyte meiosis. We found that one form of PP2A, the B56 isoform, is required for establishing end-on microtubule attachments. Based on these data, we hypothesize that phosphorylation of SPC105R, possibly by Aurora B, regulates microtubule attachments. We propose that the transition from lateral to end-on attachments is associated with changes in phosphorylation of SPC105R (Figure 2). When microtubules are interacting laterally with the kinetochore, SPC105R is phosphorylated. For end-on attachments to occur, SPC105R is dephosphorylated by PP2A-B56.

Future directions: the central spindle microtubules interact with the kinetochores to promote bi-orientation

PP2A is required to remove phosphorylation events that regulate microtubule attachment status. It is not know, however, if these phosphorylation events depend on Aurora B kinase of the CPC. Furthermore, although the target of the CPC is probably kinetochore proteins, most of the CPC is located within the central spindle, the microtubules bundled into anti-parallel arrays (Figure 1,2). Aurora B kinase localizes to the central spindle and is required for error correction and homologous chromosome bi-orientation. Prior to bi-orienting, the kinetochores are also located within the central spindle. We are investigating if phosphorylation of kinetochore proteins and maintenance of lateral attachments depends on CPC located on the microtubules. We propose that upon establishing correct bi-orientation, the homologous chromosomes move outwards towards the poles and leave the central spindle region. This would result in loss of phosphorylated SPC105R and end-on attachments. We are testing this model by targeting the CPC to specific structures, such as centromeres or microtubules, and determining the effect on SPC105R phosphorylation and lateral attachments. The central spindle is emerging as a structure that can sense tension, promote error-correction and separate pairs of homologous chromosomes. The activities mediated by Aurora B kinase, SPC105R and lateral attachments, allow the central spindle to direct reductional chromosome segregation at anaphase I.

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RONGO LAB

Stress, Mitochondrial Dynamics, and the Central Nervous System

Dr. Christopher Rongo
Genetics

Summary
Our nervous system is the primary organ by which we sense, interpret, remember, and respond to the outside world and to our own internal physiology. This elaborate system of neurons functions as a communication network, with vast arrays of chemical and electrical synapses between individual neuronal cells. The nervous system also interfaces with other tissues of the body, either directly (e.g., neuromuscular junctions at skeletal muscles) or indirectly (e.g., the release of hormones, biogenic amine neurotransmitters, and neuropeptides into the blood stream), to regulate physiology and behavior, as well as maintain overall body homeostasis. Unlike many bodily tissues, the nervous system is largely incapable of replacing damaged cells once development is complete, making it susceptible to traumatic injury and age-associated decline. The high energy demands of electrochemical signaling, combined with the inability to store energy in the form of glycogen reserves, makes neurons highly dependent on oxygen, oxidative phosphorylation, and mitochondria. The nervous system has evolved multiple mechanisms to maximize mitochondrial function and prevent damage from acute oxygen starvation. Indeed, the underlying etiology of many neurological disorders and diseases, including ischemic stroke, Parkinson's Disease, and Alzheimer's Disease, are due to defects in one or more of these key neurophysiological processes. A more complete understanding of these processes will facilitate better diagnosis and treatment of multiple neurological disorders.

We focus on understanding three areas of neurophysiology. First, we are interested in understanding how the transport and dynamics of mitochondria are mediated along axons and dendrites, as well as at synapses. Second, we are interested in understanding how neurons, synapses, and neuronal mitochondria respond to hypoxic stress (e.g., ischemic stroke). Finally, we are interested in understanding the function of the Ubiquitin Proteasome System (UPS) and its role in cellular aging, including the function of the UPS in neurons, as well as how neurons can regulate the UPS and proteostasis in distal tissues.

We use *C. elegans* to study these areas of neurophysiology because the nematode has a simple nervous system, which is easily visualized through its transparent body, allowing us to observe mitochondria and other structures within neurons in an intact and behaving animal. My lab has used the rich genetic and genomic tools of this organism, and both forward and reverse genetic approaches, to identify multiple genes that function in mitochondrial, hypoxic stress, and UPS biology. The genes we have identified have human equivalents that seem to be playing similar or identical roles in the human brain, suggesting that our findings are likely to be applicable to human health.

The Response Of Neurons To Low Oxygen Levels (Hypoxia And Anoxia).

Environment can impact nervous system function, and neurons can respond to accommodate a changing environment. Specifically, oxygen influences behavior in many organisms, and low oxygen levels (hypoxia) can have devastating consequences for neuron survival due to excitotoxicity from overactivated neurotransmitter receptors and impaired mitochondrial function. In multicellular organisms, cells respond to hypoxia through the Hypoxia Response Pathway. Normal levels of oxygen are sensed by a prolyl hydroxylase (PHD) enzyme, which uses that oxygen to covalently modify key proline residues on the transcription factor HIF alpha. This modification results in the ubiquitination and degradation of HIF alpha. Under hypoxia, PHD enzymes are inactive, resulting in the stabilization of HIF alpha. HIF alpha dimerizes with HIF beta, enters the nucleus, and regulates gene expression so as to minimize the impact of hypoxia on underlying development and physiology.

We have shown that hypoxia blocks the membrane recycling of glutamate-gated ion channels to synapses, thereby depressing glutamatergic signaling. Surprisingly, *C. elegans* HIF alpha, encoded by the *hif-1* gene, does not mediate this effect. Instead, a specific isoform of the prolyl hydroxylase (encoded by the *egl-9* gene in *C. elegans*) recruits

LIN-10, a known PDZ scaffolding protein, to endosomes, where together the two proteins promote glutamate receptor recycling. This is a novel way by which animals can sense and respond behaviorally to oxygen levels, and it suggests that the protective mechanisms are more diverse than originally appreciated.

A complete understanding of the hypoxia response pathway (i.e., EGL-9 and HIF-1) is important for understanding ischemic stroke. In addition, this pathway has become a target of interest for new chemotherapeutics, as HIF-1 is activated and plays a key role in cancer progression and metastasis. Therefore, we broadened our studies of this pathway by conducting RNA-seq and ChIP-seq experiments to identify both HIF-1-dependent and HIF-1-independent targets of hypoxia-induced gene regulation. We also identified over 400 unique metabolites that are regulated by this pathway and correlated with the changes in gene expression. Through this analysis, we found that HIF-1 promotes gluconeogenesis and the generation of the reducing molecule NADPH through the direct transcriptional regulation of PEP carboxykinase. Our findings highlight the importance of upregulating antioxidant responses during hypoxic stress.

Regulators Of Mitochondrial Transport and Dynamics In Neurons.

In addition to being the "powerhouse of the cell," mitochondria play critical roles in mediating calcium buffering, apoptosis, and necrosis. They are also a major source of reactive oxygen species (ROS), which can have both a signaling role and be damaging to cells. Mitochondria are actively transported within neurons to synapses, and damaged mitochondria – a potential threat to the cell – are transported back to the cell body for removal by mitophagy. Mitochondria are also dynamic, undergoing fusion and fission. Fusion is thought to be a mechanism for boosting mitochondrial output and protecting mitochondrial health, whereas fission is thought to be the first step on the way to mitophagy and the removal of damaged mitochondria. Defects in mitochondrial dynamics have a clear role in Parkinson's Disease. Defects in mitochondrial transport have a clear role in Alzheimer's Disease. Thus, an understanding of mitochondrial dynamics and transport is important for our understanding of neurological disorders with mitochondrial etiology, as well as our understanding of aging and age-associated diseases.

Mitochondrial dynamics as a field has largely been studied in single celled yeast; thus, little is known about the machinery that conducts mitochondrial fission and fusion in specialized tissues like neurons. We are studying mitochondrial dynamics in *C. elegans* neurons using a mitochondrially-localized GFP reporter, which makes it easy to visualize individual mitochondria in axons and dendrites of live animals. Using this tool, we performed a forward genetic screen for mutants with defects in mitochondrial transport, dynamics, or mitophagy. We are currently cloning and characterize the underlying genes so as to have a complete understanding of the factors that mediate and regulate mitochondrial biology in neurons.

We also generated a *C. elegans* transgenics strain that expresses MitoKeima, new reporter for mitochondria undergoing mitophagy. MitoKeima has a differential, pH-dependent fluorescence excitation spectra that allows one to discriminate healthy mitochondria in the neutral pH of the cytosol from damaged mitochondria in the low pH environments of autophagosomes, autolysosomes, and lysosomes. Mitophagy can be triggered by mitochondrial stress or even by starvation. Using this and other mitochondrial reporters, we are now examining how mitochondrial dysfunction contributes to genetic models of Alzheimer's Disease and Parkinson's Disease.

Dopamine Signaling Activates The UPS In Distal Epithelial Tissues.

The Ubiquitin Proteasome System (UPS) is a key mechanism by which cells maintain protein homeostasis (proteostasis) by removing misfolded and oxidized proteins. This system comprises many ubiquitin ligases, which tag individual proteins for degradation by the 26S Proteasome. As cells age, UPS activity becomes impaired, resulting in the accumulation of damaged proteins and age-associated physiological decline. By understanding how UPS activity is regulated in neurons and in non-neuronal tissue by neurons, we should be able to provide new therapeutic targets for diseases that involve protein aggregates and disrupted proteostasis.

We previously generated a GFP-based reporter system for UPS activity in *C. elegans*, allowing us to query UPS activity in specific tissues and at specific points along development. We found that epithelial cells undergo a dramatic increase in UPS activity as animals mature. We also found that the humoral neurohormone/biogenic amine neurotransmitters dopamine and serotonin promote UPS activity in epithelia. Mutants for biogenic amine synthesis show

decreased poly-ubiquitination and turnover of our GFP-based UPS substrate. Using RNA-seq and mass spectrometry, we found that biogenic amines promote eicosanoid production from poly-unsaturated fats (PUFAs) by regulating expression of cytochrome P450 monooxygenases. Mutants for one of these P450s share the same UPS phenotype observed in biogenic amine mutants. The production of n-6 eicosanoids is required for UPS substrate turnover, whereas accumulation of n-6 eicosanoids accelerates turnover. Our results suggest that sensory neurons secrete biogenic amines like dopamine and serotonin to modulate lipid signaling, which in turn activates stress response pathways to maintain UPS proteostasis.

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SINGSON LAB

Reproductive Biology, Cell-Cell Interactions

Dr. Andrew Singson
Genetics

Summary
Reproductive success requires that two haploid cells – sperm and egg – unite to form a diploid zygote. Both sperm and egg cells must be differentiated into forms that are highly specialized for their specific roles in fertilization. After fertilization has occurred, the zygote must begin development. From extensive study, the events required for reproductive success are known in some detail. However, the molecular underpinnings of these events generally remain elusive.

Our primary research interests are to understand the molecular mechanisms of sperm-egg interactions and gamete activation. The genetic and molecular dissection of these events will also provide insights relevant to other important cell-cell interactions during the life and development of multicellular organisms. Further, our studies are highly significant with regards to understanding germ cell/stem cell biology, reproductive aging, the mechanisms of molecular evolution and sexual selection.

C. elegans offers a unique opportunity to define sperm and egg components required for fertilization and gamete activation

The nematode *Caenorhabditis elegans* is a well-established model system for the study of many biological processes. My lab has been helping to pioneer the use of *C. elegans* for addressing the mechanisms of sperm-egg interactions. The amoeboid sperm of *C. elegans* despite lacking an acrosome and flagellum, carry out the same basic functions common to all spermatozoa. Many of the genetic and molecular tools developed for *C. elegans* are not available or are very difficult to utilize in other organisms traditionally used for studying fertilization. The most significant advantage of *C. elegans* is the ability to isolate and maintain mutants that affect sperm or eggs and no other cells. We have focused our studies on several classes of sterile mutants. These mutants define genes required for sperm activation, sperm function during fertilization, egg function during fertilization and egg activation.

Sperm function

We characterized the first *C. elegans* gene (*spe-9*) that encoded a protein required for sperm function at fertilization. All other genes with a similar mutant phenotype are now know as "*spe-9* class" mutants. The SPE-9 protein functions as a sperm surface ligand required for sperm to egg signaling during fertilization.

We continue to identify and characterize genes required for sperm function at fertilization taking advantage of the most up to date genetic and molecular tools. Most recently we have been using a new genome engineered balancer chromosomes in our fertility mutant screens. This allows us to screen specific segments of the genome and easily maintain mutants.

We have recently identified candidates for the spe-9 class genes spe-13, spe-36, spe-45, and spe-51 with next generation whole genome sequencing. SPE-45 is a single pass transmembrane molecule with a single immunoglobulin domain (IG) that has a conserved function from worms to humans. SPE-36 and SPE-51 appear to be the first secreted sperm molecules required for fertilization(Figure 1). SPE-51 also has an IG domain and has features that suggest it could be a long sought-after sperm-egg fusogen. SPE-36 encodes an epidermal growth factor (EGF) motif. Our analysis of these genes could serve as a paradigm for mammalian sperm-secreted or reproductive tract-secreted proteins that coat the sperm surface and influence their survival, motility, and/or the ability to fertilize the egg.

In addition to ongoing genetic screens for new sperm function mutants, we will continue to study our current collection of mutants. The molecular characterization of the corresponding genes should help us formulate models on how their encoded proteins function during wild-type fertilization.

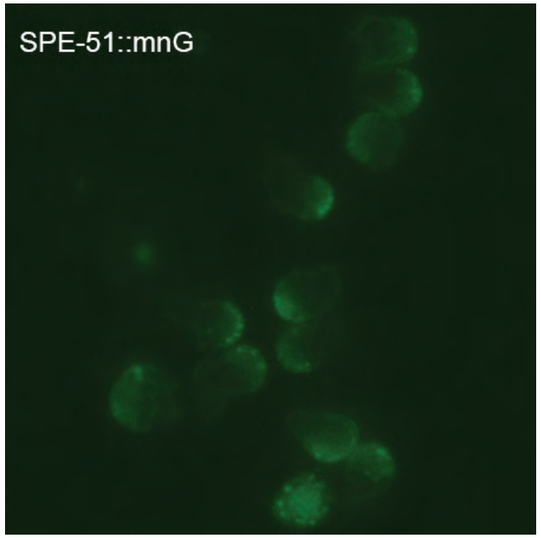

Figure 1. Although the SPE-51 protein is secreted by sperm, it remains associated with the sperm surface and is required for successful fertilization. SPE-51:NeonGreen can be detected on the surface of activated C. elegans sperm.

As we have been defining the molecular components of fertilization, we have seen emerging parallels with other cellular systems. We have recently proposed the concept of a **fertilization synapse**. This framework takes into account the molecular and cellular complexity required for reproductive success.

Sperm activation

Post meiotic sperm differentiation (spermiogenesis) is required for a haploid spermatid to build cellular structures required for motility and interactions with the egg. We recently cloned two new genes (spe-24/zipt-7.1 and spe-43) that are required for C. elegans spermiogensis. The spe-24/zipt-7.1 encoded protein is a zinc transporter and demonstrates zinc as an important second messenger for sperm activation in vivo. The spe-43 gene is a novel transmembrane protein that is alternately spliced. Further characterization of this gene will help us better understand how sperm become competent to move towards and fertilize the egg. The spe-21 gene encodes a DHHC-CRD zinc finger membrane protein. We are characterizing the role of this protein during spermatogenesis and sperm activation. It likely regulates through lipidation the activity and localization of other sperm proteins.

Egg functions

Since starting the lab, an important direction was to make the first effort to identify components required by the oocyte for fertilization using complementary forward and reverse genetic approaches. Despite the substantial time and effort required to initiate these studies, we have been able to identify the first egg components required for fertilization in *C. elegans*. The *egg-1* and *egg-2* genes encode LDL-receptor-repeat containing proteins that are localized to the oocyte plasma membrane. Loss of either *egg-1* or *egg-2* function leads to a significant reduction in fertility. Loss of both genes leads to complete sterility and the production of oocytes that can never be fertilized by wild-type sperm. The *egg-1* and *egg-2* genes are a result of a gene duplication in the *C. elegans* lineage. This gene duplication may provide *C. elegans* with an extra copy/variant of an egg sperm receptor that could enhance fertility and/or or provide more robust gamete interactions across a wider range of conditions. We have developed an innovative new genetic screening strategy that will help us identify more genes like *egg-1/2*.

The oocyte-to-embryo transition

The last class of mutants that we study defines genes required in the egg to trigger development after fertilization. The *egg-3, egg-4* and *egg-5* genes encode inactive protein tyrosine phosphatases or “antiphosphatase” required for egg activation after sperm entry. Recently, through forward genetic screens, we have identified at temperature sensitive allele of the *egg-3* gene. This will provide a genetic tool to not only better understand the regulation of the oocyte-to-embryo transition but will also help us identify additional components of the *egg-3* pathway.

In addition to *egg-1* through *egg-5*, we have a unique collection of egg genes/mutants that are being characterized. A subset of these mutants may alter germ line stem cell behavior. We have been examining a new gene *egg-6* in early events in the one cell embryo just after fertilization.

Reproductive Life Span

We have completed a study examining the reproductive span of male *C. elegans*. We found that male worms have completely lost fertility after only about one third to one half of their lifespan. We find that the loss of the male’s ability to mate is a major factor in this surprisingly short reproductive span. We are following up with comparative reproductive span studies with other nematode species that have different mating strategies.

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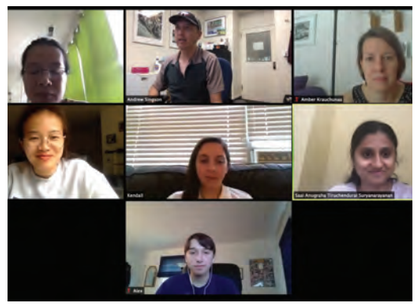

STEWARD LAB

Epitranscriptomics, Modification of mRNA in Drosophila, Neuronal Development

Dr. Ruth Steward
Molecular Biology & Biochemistry

Summary
RNA modifications provide a critical layer of epitranscriptomic gene regulation in most organisms. We study the generation and functional impact of the essential RNA modification 5-hydroxymethylcystosine (5hmrC) in Drosophila.

The 5hmrC modification is introduced to mRNA in vertebrates and Drosophila by the Tet (Ten-Eleven-Translocation) enzyme. Tet proteins have well-documented functions in development, maintaining vertebrate stem cells, are associated with carcinogenesis and neurological disorders. Tet proteins were first identified as DNA-modifying enzymes that function as 5-methylcytosine (5mC) hydroxylases, catalyzing the transition of 5mC to 5hmC on DNA in vertebrates. That Tet proteins also function as RNA-modifying enzymes has been established only recently.

We created an endogenously expressed GFP-tagged Tet gene and found that the protein is seen in embryos from blastoderm stage onwards, most strongly in neuronal cells, and in third instar larvae the gene is strongly expressed in the brain and in most nerve cells. We are studying the neuronal phenotype of Tet mutants. In Tet^{null},the complete loss-of-function mutation, axonal pathfinding is disrupted in the embryonic CNS, and the beta-lobe axons grow across the midline in the mushroom bodies of larval and adult brains. These results underline a requirement for Tet in axon outgrowth or guidance. Further, we observed morphological defects in mature neurons in both the peripheral and central nervous system. These observations are particularly noteworthy considering our results showing that 5hmrC plays a role in translational efficacy. Regulation of translation is known to play an important role in the patterning of both dendritic fields and axons through effects on branching.

Previously, in collaboration with Dr. Fuks' laboratory at the Free University of Brussels, we mapped 5hmrC transcriptome-wide in S2 Drosophila tissue culture cells and could show that Tet modifies ~ 1500 specific transcripts. Our working hypothesis is that Tet, mediated by its DNA-binding domain, localizes at actively transcribed target genes and controls the modification of their nascent transcripts. The 5hmrC mark is then recognized by reader protein(s) that direct the association of the bound mRNA with ribosomes ultimately controlling translational levels. To test this hypothesis, we performed ChIP-Seq experiments. Bioinformatic analysis identified 2242 protein binding peaks, and 1032 of the peaks map to promoter sites and the majority of these Tet peaks co-localize with chromatin modification marks associated with the transcription start site of actively transcribed genes. Gene ontology analyses indicates that Tet-binding genes are preferentially involved in axon outgrowth.

Next, we performed hmeRIP (immunoprecitpitation using commercially available anti-5hmrC antibody) on RNA isolated from wild type (wt) embryos as well as wt and Tet^{null} larval heads in order to map 5hmrC transcriptome-wide. In both preparations, the distribution of modified RNAs was similar to what we had previously observed in RNAs isolated from S2 cells. In S2 cells we had identified ~3000 peaks in ~1500 transcripts, while in embryos we identified about 1815 peaks on 1404 mRNAs, and in larval heads 3711 peaks on 1776 transcripts, results that are highly consistent with each other. Peaks in 507 transcripts were significantly (four fold) reduced in Tet^{null} larval heads compared to wild type. The GO analysis showed that the distribution of peaks is similar to that observed in the ChIP-Seq analysis, transcripts modified by Tet are preferentially involved in axon outgrowth. These results are most encouraging; they indicate an impressive correspondence between our phenotypic analysis and our genomic and transcriptomic approaches.

To determine if there is a link between 5hmrC marks and mRNA levels, we analyzed the input RNA-seq from the 5hmeRIP experiments. When we compared Tet-regulated mRNAs with the targets identified by hmeRIP-seq, a very small percentage (5.5%) of the Tet-regulated mRNAs contained 5hmrC peak. This result indicates, consistent with our model, that the level of the vast majority of Tet-dependent 5hmrC modified RNAs do not change in Tet^{null} brains.

Previously we reported that 5hmrC modified mRNAs are preferentially found on ribosomes, suggesting a correlation between the 5hmrC mark and mRNA translation levels. We addressed this possibility by examining ribosome occupancy across the transcriptome by sequencing ribosome-protected RNA fragments using ribosome profiling (Ribo-seq) analysis. By integrative analysis of Ribo-seq and RNA-seq data in wild type and Tet^{null} larval brain preparations, we found that of 1776 wild type 5hmrC modified mRNAs, 46% (829) show diminished levels of ribosome occupancy in Tet^{null} samples. Further, of the 507 transcripts with reduced 5hmrC marks in Tet^{null} larval heads, 73% (374) also showed reduced translation.

Based on the results listed above we identified Tet target genes implicated in axon guidance that showed Tet protein binding to the promoter region, whose level of the 5hmrC mark on the mRNA was reduced in Tet^{null}, and that also showed a reduction in ribosome occupancy. Two such Tet targets are Robo2 and Slit. Robo2 is the receptor of Slit, which is expressed in the ipsilateral and commissural axons of the CNS and controls axon guidance and midline repulsion. When we carefully analyzed the Tet embryonic axon phenotype, we found a midline crossing phenotype similar to that observed in slit and Robo2 embryos and, further, in Tet mutant larval brains the levels of both proteins are reduced.

Thus our results support the model we proposed above; Tet binds to target genes mediated by its DNA-binding domain and modifies nascent mRNAs resulting in the positive control of translation of the modified mRNAs. This process occurs preferentially in nerve cells and can control the outgrowth of axons in the embryo and at late larval stages.

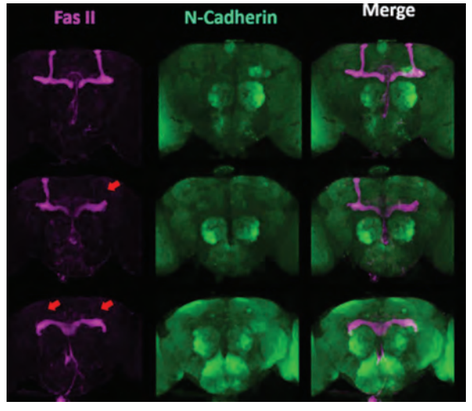

Figure legend: Loss of mushroom body α lobe(s) in Tet^{4XXC} mutants. Fasciclin II stains the mushroom bodies (red) N-cadherin is used as a cell marker (green). Top, wildtype brain, bottom, two different phenotypes observed in Tet^{4XXC} brains.

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EBRIGHT LAB

Transcription: Structure, Mechanism, Regulation, and Antibacterial Drug Discovery

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Summary
Transcription--synthesis of an RNA copy of genetic information in DNA--is the first step in gene expression and is the step at which most regulation of gene expression occurs. Richard H. Ebright's lab seeks to understand structures, mechanisms, and regulation of bacterial transcription complexes and to identify, characterize, and develop small-molecule inhibitors of bacterial transcription for application as antituberculosis agents and broad-spectrum antibacterial agents.

Structures of Transcription Complexes

Transcription initiation in bacteria requires RNA polymerase (RNAP) and the transcription initiation factor σ. The bacterial transcription initiation complex contains six polypeptides (five in RNAP, one in σ) and promoter DNA, and has a molecular mass of 0.5 MDa.

Understanding bacterial transcription initiation will require understanding the structures of polypeptides in bacterial transcription initiation complexes and the arrangements of these polypeptides relative to each other and relative to promoter DNA.

We are using x-ray crystallography to determine high-resolution structures of transcription initiation complexes, fluorescence resonance energy transfer (FRET) to define distances between pairs of site-specifically incorporated fluorescent probes, photocrosslinking to define polypeptides near site-specifically incorporated photocrosslinking probes, and protein footprinting and residue scanning to define residues involved in contacts. In support of these activities, we are developing procedures to incorporate fluorescent probes and photocrosslinkers at specific sites within large multisubunit nucleoprotein complexes, and we are developing automated docking algorithms to integrate structural, biophysical, biochemical, and genetic data in order to construct models for structures of complexes.

Mechanism of Transcription

Transcription complexes are molecular machines that carry out complex, multistep reactions in transcription initiation and elongation:

(1) RNA polymerase (RNAP) binds to promoter DNA, to yield an RNAP-promoter closed complex.

(2) RNAP unwinds ~14 base pairs of promoter DNA surrounding the transcription start site, rendering accessible the genetic information in the template strand of DNA, and yielding an RNAP-promoter open complex.

(3) RNAP begins synthesis of RNA as an RNAP-promoter initial transcribing complex. During initial transcription, RNAP uses a "scrunching" mechanism, in which RNAP remains stationary on promoter DNA and unwinds and pulls downstream DNA into itself and past its active center in each nucleotide-addition cycle, resulting in generation of a stressed intermediate.

(4) After RNAP synthesizes an RNA product ~10-15 nucleotides in length, RNAP breaks its interactions with promoter DNA, breaks at least some of its interactions with sigma, escapes the promoter, and begins transcription elongation as a transcription elongation complex. Energy stored in the stressed intermediate generated by scrunching during initial transcription is used to drive breakage of interactions with promoter DNA and interactions with sigma during promoter escape.

During transcription elongation, RNAP uses a "stepping" mechanism, in which RNAP translocates relative to DNA in each nucleotide-addition step. Each nucleotide-addition cycle during initial transcription and transcription elongation can be subdivided into four sub-steps: (1) translocation of the RNAP active center relative to DNA (by scrunching in initial transcription; by stepping in transcription elongation); (2) binding of the incoming nucleotide; (3) formation of the phosphodiester bond; and (4) release of pyrophosphate.

Crystal structures have been reported for transcription elongation complexes without incoming nucleotides and for transcription elongation complexes with incoming nucleotides. Based on these crystal structures, it has been proposed that each nucleotide-addition cycle is coupled to an RNAP active-center conformational cycle, involving closing of the RNAP active center upon binding of the incoming nucleotide, followed by opening of the RNAP active center upon formation of the phosphodiester bond. According to this proposal, the closing and opening of the RNAP active center is mediated by the folding and the unfolding of an RNAP active-center structural element, the "trigger loop."

To understand transcription initiation, transcription elongation, and transcriptional regulation, it will be necessary to leverage the available crystallographic structural information, in order to define the structural transitions in RNAP and nucleic acid in each reaction, to define the kinetics of each reaction, and to define mechanisms of regulation of each reaction.

We are using FRET and photocrosslinking methods to define distances and contacts within trapped intermediates in transcription initiation and transcription elongation. In addition, we are using FRET with stopped-flow rapid mixing, and photocrosslinking with quenched-flow rapid mixing and laser flash photolysis, to monitor kinetics of structural transitions. Finally, and most importantly, we are using single-molecule FRET, single-molecule DNA nanomanipulation, and combined single-molecule FRET and single-molecule DNA nanomanipulation, to carry out single-molecule, millisecond-to-second timescale analysis of structural transitions.

Regulation of Transcription: Regulation of Transcription Initiation

The activities of bacterial transcription initiation complexes are regulated in response to environmental, cell-type, and developmental signals. In most cases, regulation is mediated by factors that bind to specific DNA sites in or near a promoter and inhibit (repressors) or stimulate (activators) one or more of the steps on the transcription initiation pathway.

To provide the first complete structural and mechanistic descriptions of activation, we study two of the simplest examples of activation in bacteria: (1) activation of the *lac* promoter by catabolite activator protein (CAP) and (2) activation of the *gal* promoter by CAP. These model systems each involve only a single activator molecule and a single activator DNA site and, as such, are more tractable than typical examples of activation in bacteria and substantially more tractable than typical examples of activation in eukaryotes (which can involve tens of activator molecules and activator DNA sites).

We have established that activation at *lac* involves an interaction between CAP and the RNA polymerase (RNAP) alpha-subunit C-terminal domain that facilitates closed-complex formation. Activation at gal involves this same interaction and also interactions between CAP and the RNAP alpha-subunit N-terminal domain, and between CAP and sigma, that facilitate isomerization of closed complex to open complex.

Together with collaborators, we are using electron microscopy, x-ray crystallography, and NMR to determine the structures of the interfaces between CAP and its targets on RNAP. In addition, we are using FRET, photocrosslinking, and single-molecule FRET and single-molecule DNA nanomanipulation methods to define when each CAP-RNAP interaction is made as RNAP enters the promoter and when each interaction is broken as RNAP leaves the promoter.

Regulation of Transcription: Regulation of Transcription Elongation, Pausing, and Termination.

Recently we have extended our studies of transcriptional regulation to encompass regulation at the level of transcription antipausing, transcription and antitermination.

The transcription antitermination factor Q, which is produced by lambdoid bacteriophage during lytic infection, is one of two classic textbook examples of regulators of gene expression that function at the level of transcription pausing

and transcription termination (e.g., *Molecular Biology of the Gene*). (The other classic textbook example is the structurally and mechanistically unrelated regulator N, which is produced by bacteriophage lambda and functions in an earlier phase of lambdoid bacteriophage infection.)

Q proteins function by binding to RNA polymerase-DNA-RNA transcription elongation complexes (TECs) and rendering TECs unable to recognize and respond to transcription pausing and transcription termination signals. Q proteins are targeted to specific genes through a multi-step binding process entailing formation of a "Q-loading complex" comprising a Q protein bound to a Q binding element and a sigma-containing TEC paused at an adjacent sigma-dependent pause element, followed by transformation into a "Q-loaded complex" comprising a Q protein and a translocating, pausing-deficient, termination-deficient TEC.

Q proteins from different lambdoid bacteriophages comprise three different protein families (the Qλ family, the Q21 family, and the Q82 family), with no detectable sequence similarity to each other and no detectable sequence similarity to other characterized proteins. Q proteins from different protein families are thought to be analogs (with identical functions but unrelated structures and origins), rather than homologs (with identical, interchangeable functions and related structures and origins).

Q proteins have been the subject of extensive biochemical and genetic analysis spanning five decades. However, an understanding of the structural and mechanistic basis of transcription antitermination by Q proteins has remained elusive in the absence of three-dimensional structural information for Q-dependent antitermination complexes.

We are systematically determining high-resolution single-particle cryo-EM structures of Qlambda-, Q21-, and Q82-dependent transcription antitermination complexes.

Results for Q21 reveal that Q21 forms a torus--a "nozzle"--that extends and narrows the RNA-exit channel of RNA polymerase, that the nascent RNA is threaded through the Q nozzle, and that the threading of the nascent RNA through the Q nozzle precludes the formation of pause and terminator RNA hairpins.

Narrowing and extending the RNA-exit channel of RNA polymerase by attaching a nozzle and threading RNA through the nozzle is a remarkably straightforward mechanism for antitermination and almost surely will be a generalizable mechanism.

Attaching a nozzle and threading RNA through the nozzle has the additional remarkable consequence of generating a topological connection--an unbreakable linkage--between the antitermination factor and the RNA emerging from RNA polymerase. This enables exceptionally stable association and exceptionally processive antitermination activity and has implications for engineering highly efficient, tightly regulated, gene expression for synthetic biology applications.

Regulation of Transcription: Transcription-Translation Coupling.

Most recently we have extended our studies of transcriptional regulation to encompass transcription-translation coupling.

In two of the three domains of life--the bacteria and the archaea--transcription and translation occur in the same cellular compartment, occur at the same time, and are coordinated processes, in which the rate of transcription by the RNA polymerase (RNAP) molecule synthesizing an mRNA is coordinated with the rate of translation by the first ribosome ("lead ribosome") translating the mRNA.

We recently have reported cryo-EM structures that define the structural basis of transcription-translation coupling in the bacterium *E. coli* . The results show that two bacterial transcription factors, NusG and NusA, serve as transcription-translation-coupling factors that physically bridge RNAP and the ribosome. NusG functions as a flexible connector--a "tow chain"--that potentially enables the RNAP "locomotive" to pull the ribosome "locomotive." NusA functions as a flexible connector--a "coupling pantograph"--that potentially both enables RNAP to pull the ribosome and enables RNAP to be pushed by the ribosome.

In current work, we are determining cryo-EM structures that define the structural basis of transcription-translation coupling by RfaH, a specialized homolog of NusG that mediates coupling transcription-translation coupling at a subset of genes that have a specific DNA site required for RfaH to load onto RNAP.

In further current work, we are determining cryo-EM structures that define intermediates in the establishment of transcription-translation coupling by NusG and RfaH, intermediates in the break-down of transcription-translation coupling by NusG and RfaH, and effects of transcription-translation coupling by NusG and RfaH on formation and function of pause and termination hairpins.

In further current work, we are determining cryo-EM structures that define the structural basis of transcription-translation coupling in archaea, which possess a cellular RNAP that is closely related in subunit composition and structure to eukaryotic RNAP II, but that is only distantly related to bacterial RNAP.

Inhibitors of Transcription; Antibacterial Drug Discovery

Bacterial RNAP is a proven target for broad-spectrum antibacterial therapy. The suitability of bacterial RNAP as a target for broad-spectrum antibacterial therapy follows from the fact that bacterial RNAP is an essential enzyme (permitting efficacy), the fact that bacterial RNAP-subunit sequences are highly conserved (providing a basis for broad-spectrum activity), and the fact that bacterial RNAP-subunit sequences are not highly conserved in human RNAPI, RNAPII, and RNAPIII (providing a basis for therapeutic selectivity).

The rifamycin antibacterial agents--rifampin, rifapentine, rifabutin, and rifamixin--bind to and inhibit bacterial RNAP. The rifamycins bind to a site on bacterial RNAP adjacent to the RNAP active center and prevent extension of RNA chains beyond a length of 2–3 nucleotides. The rifamycins are in current clinical use in treatment of Gram-positive and Gram-negative bacterial infections. The rifamycins are of particular importance in treatment of tuberculosis; the rifamycins are first-line antituberculosis agents and are among the only antituberculosis agents able to clear infection and prevent relapse. The clinical utility of the rifamycin antibacterial agents is threatened by the existence of bacterial strains resistant to rifamycins. Resistance to rifamycins typically involves substitution of residues in or adjacent to the rifamycin-binding site on bacterial RNAP--i.e., substitutions that directly interfere with rifamycin binding.

In view of the public health threat posed by drug-resistant and multidrug-resistant bacterial infections, there is an urgent need for new classes of broad-spectrum antibacterial agents that (1) target bacterial RNAP (and thus have the same biochemical effects as rifamycins), but that (2) target sites within bacterial RNAP that do not overlap the rifamycin-binding site (and thus do not show cross-resistance with rifamycins).

We have identified new drug targets within the structure of bacterial RNAP. Each of these new targets can serve as a potential binding site for compounds that inhibit bacterial RNAP and thereby kill bacteria. Each of these new targets is present in most or all bacterial species, and thus compounds that bind to these new targets are active against a broad spectrum of bacterial species. Each of these new targets is different from targets of current antibiotics, and thus compounds that bind to these new targets are not cross-resistant with current antibiotics. For each of these new targets, we have identified at least one lead compound that binds to the target, and we have synthesized analogs of the lead compound comprising optimized lead compounds. Several of the lead compounds and optimized lead compounds are extremely promising: they exhibit potent activity against a broad spectrum of bacterial pathogens (including *Staphylococcus aureus* MSSA, *Staphylococcus aureus* MRSA, *Enterococcus faecalis*, *Enterococcus faecium*, *Clostridium difficile*, *Mycobacterium tuberculosis*, *Bacillus anthracis*, *Francisella tularensis*, *Burkholderia mallei,* and *Burkholderia pseudomallei*) and exhibit no cross-resistance with current antibiotics.

In support of this work, we are identifying new small-molecule inhibitors of bacterial RNAP by analysis of microbial and plant natural products, by high-throughput screening, and by virtual screening. We are also using genetic, biochemical, biophysical, and crystallographic approaches to define the mechanism of action of each known, and each newly identified, small-molecule inhibitor of bacterial RNAP, and we are using microbiological approaches to define antibacterial efficacies, resistance spectra, and spontaneous resistance frequencies of known and new small-molecule inhibitors of bacterial RNAP.

We seek to address the following objectives: to develop new classes of antituberculosis agents and broad-spectrum antibacterial agents, to develop antibacterial agents effective against pathogens resistant to current antibiotics, to develop antibacterial agents effective against pathogens of high relevance to public health, and to develop antibacterial agents effective against pathogens of high relevance to biodefense.

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NICKELS LAB

Regulation of Gene Expression in Bacteria

Dr. Bryce Nickels
Genetics

Summary
Proper control of transcription, the first step in gene expression, is essential for organismal development, cellular response to environmental signals, and the prevention of disease states. We aim to understand mechanisms of transcription and its regulation. Transcription in all cells is carried out by members of a family of conserved multi subunit RNA polymerases (RNAP). Within this family, Escherichia coli RNAP serves as an exceptional model system for mechanistic studies of transcription and as a paradigm for understanding gene expression in bacteria. For our studies of transcription, we use a range of approaches including molecular biology, genetics, biochemistry and highthroughputsequencing (HTS) methods that enable us to probe RNAP function at an unprecedented scale.

Mechanism and impact of gene expression control by "nanoRNAs".

It had been widely accepted that, in living cells, the initiation of RNA synthesis by RNAP occurs solely via use of nucleoside triphosphate (NTP) substrates, "*de novo* initiation." Our studies have challenged this conventional paradigm by establishing that under certain cellular conditions a significant fraction of transcription initiation does not occur *de novo*, but rather relies upon use of 2- to ~4-nt RNAs, "nanoRNAs," that serve as primers for RNAP. Furthermore, we have established that the impact of nanoRNA-mediated priming on gene expression and cell physiology in *E. coli* is highly significant. Nevertheless, having only recently discovered that nanoRNA-mediated priming occurs *in vivo*, the full extent to which nanoRNA-mediated priming impacts gene expression and cell physiology across diverse organisms remains a major area of interest and represents a frontier of our current knowledge.

Use of non-canonical initiating nucleotides (NCINs) facilitates "ab initio capping" of nascent RNA.

The chemical nature of the 5' end of RNA is a key determinant of RNA stability, processing, localization, and translation efficiency. Recently it has been shown that some bacterial RNA species carry a 5'-end structure reminiscent of the 5' 7-methylguanylate "cap" in eukaryotic RNA. In particular, RNA species containing a 5'-end nicotinamide adenine dinucleotide (NAD^+) or 3'-desphospho-coenzyme A (dpCoA) have been identified in both Gram-negative and Gram-positive bacteria. It has been proposed that NAD^+, reduced NAD^+ (NADH), and dpCoA caps are added to RNA after transcription initiation, in a manner analogous to the addition of 7-methylguanylate caps. We have shown instead that NAD^+, NADH, and dpCoA are incorporated into RNA during transcription initiation, by serving as noncanonical initiating nucleotides (NCINs) for *de novo* transcription initiation by bacterial RNA polymerase (RNAP). In addition, we have identified key promoter sequence determinants for NCIN-mediated initiation, shown that NCIN-mediated initiation occurs *in vivo*, and shown that NCIN-mediated initiation has functional consequences by increasing RNA stability *in vivo*. We have further shown that eukaryotic nuclear and mitochondrial RNAPs can perform NCIN-mediated initiation indicating NCIN-mediated "*ab initio* capping" likely occurs in all organisms.

Together with our work on nanoRNA-mediated priming, our studies of NCIN-mediated initiation add to an emerging picture that NTPs are not the only substrates for transcription initiation *in vivo*. In current work, we are determining the full extent to which NCIN-mediated initiation impacts gene expression in bacterial cells and investigating the possibility that NCIN-mediated initiation provides a direct regulatory connection between metabolism and gene expression.

Development and application of high-throughput sequencing-based approaches for analysis of transcription.

A distinguishing feature of our lab has been our development of methods to monitor transcription across extensive sequence space both in vitro and in vivo. We have developed methods to measure yields and 5' end sequences for RNA products generated in vitro or in vivo for each of ~4^{10} (~1,000,000) barcoded promoter sequences ("massively parallel

transcriptomics." In published work, we used massively parallel transcriptomics to obtain a complete understanding of the determinants of transcription start site and a complete understanding of how promoter sequence modulates NCIN mediated initiation with NAD. We have also developed methods to identify positions of RNAP-DNA crosslinking with single-amino-acid, single-nucleotide resolution for complexes generated in vitro or in vivo on each of ~4^{10} (~1,000,000) barcoded promoter sequences ("massively parallel protein DNA photo crosslinking"). Together, these methods enable us to systematically analyze the contributions of DNA sequence, transcription factors, and reaction conditions to RNAP activity in each step of transcription.

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SEVERINOV LAB

Mechanisms of Transcription in Microorganisms

Dr. Konstantin Severinov
Molecular Biology & Biochemistry

Summary

Our laboratory studies bacteria, their interactions with phages, plasmids and transposons, and with each other. The following research projects were actively pursued during the last year.

Studies of CRISPR-Cas bacterial adaptive immunity

CRISPR-Cas (Clustered Regularly Interspersed Palindromic Repeats/CRISPR *a*ssociated *s*equences) loci provide bacteria with adaptive immunity to phages and plasmids. We study diverse CRISPR-Cas systems from *Escherichia coli*, *Thermus thermophilus,* human pathogen *Clostridium difficile,* and thermophilic archaea. To better understand the fundamental aspects of CRISPR-Cas function, evolution, and ecology, we developed highly efficient experimental model systems for *in vivo* studies of CRISPR-Cas interference and CRISPR adaptation. In *T. thermophilus*, we were able for the first time to set an experimental system to study spacer acquisition by the Type III CRISPR-Cas system of this thermophilic bacterium, the most enigmatic an complex CRISPR-Cas type. In *E. coli*, we were identified spacer intermediates (prespacers), excised from foreign DNA and on their way to become CRISPR array spacers and showed that these prespacers associate with both CRISPR adaptation and interference machineries. This result is of fundamental importance as it shows that the two arms of CRISPR defense – the spacer acquisition machinery and the interference machinery that destroys foreign DNA – are functionally and physically linked.

We use the diversity of CRISPR spacers to monitor adaptations of bacterial populations to viruses and performed extensive analysis of short-term and long-term dynamics of CRISPR spacer repertoire in *C. difficile* communities in human gut and archaeal communities in acidic hot springs in Japan.

Structure-activity analyses of peptide antibiotics

Ribosomally-synthesized post-translationally modified peptides RIPPs) form a broad and diverse class of molecules with highly unusual structures and potentially useful properties, such as antibiotic activity. We use powerful bioinformatics pipelines to predict new RIPPs. We next determine their structures, characterize enzymes involved in their synthesis, and determine the modes of their antibacterial action. Structure-activity analysis of new RIPPs leads to development of molecules that are not found in nature but possess superior properties and may be used to treat bacterial infections. Studies of enzymes that provide immunity to microcins allow us to better understand the mechanisms of antibiotic resistance and look for compounds that overcome the resistance. Our powerful bioinformatics pipelines had retrieved several novel diverse putative microcin biosynthetic clusters and these predictions are being validated.

Structure-functional analysis of novel transcription enzymes and phage-encoded transcription regulators

We bioinformatically predict and functionally and structurally characterize bacteriophage-encoded transcription enzymes (DNA-dependent RNA polymerases) that are very distantly if at all related to cellular enzymes. A high-resolution structure of a non-canonical RNA polymerase from Crass phage, the most abundant virus in the human gut, was determined in collaboration with Petr Leiman from UTM. Surprisingly, the structure of this enzyme is different from universally conserved cellular transcription enzymes structures but is closely related to the structures of RNA-dependent RNA polymerases involved in RNA interference (RNAi) in eukaryotes. This exciting finding suggests that the RNAi mechanisms may be partially based on enzymes "borrowed" from phages. We also determined the structure of another RNA polymerase, encoded by a jumbo phage AR9, and gained insights into the very unusual details of promoter recognition by this enzyme. We also continue our studies of how phages control the transcription of their own genes by modifying host transcription apparatus. In collaboration with Rob Lavigne group from University of Leuven

in Belgium we uncovered a novel way of controlling host and viral transcription by proteolytic digestion of host RNA polymerase triggered by acetylation.

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YADAVALLI LAB

Bacterial stress response, antimicrobial resistance and biology of small proteins

Srujana Samhita Yadavalli
Genetics

Summary

Antimicrobial resistance is an alarming problem of our present and future. Stress response networks that are meant to protect bacteria against challenges in their environment, are increasingly being co-opted to promote antimicrobial resistance. Understanding the biochemical and regulatory pathways that underlie this resistance is of utmost importance to tackle the growing threat of untreatable multidrug resistant bacterial infections. Our research is broadly focused on two distinct themes of bacterial stress response regulation, which have been under-appreciated in the past – (i) small protein regulators and (ii) epitranscriptomic regulators. The long-term goal of our research is to expand our understanding of these emerging classes of gene expression regulators, by characterizing their regulatory functions and interactions with the stress response networks. To this end, we use a wide-range of tools from classical genetics, biochemistry to high-throughput sequencing, proteomics, single-cell gene expression analysis by fluorescence microscopy. These investigations will not only provide a basis for how antimicrobial resistance mechanisms evolve but will also uncover potential targets better suited for drug development and combat the spread of such resistances.

Small protein regulators – an entire class of proteins (<50 amino acids) was completely missed due to initial length cut-offs in conventional genome annotation. These small proteins encoded by short, non-canonical open reading frames have been discovered in all three kingdoms of life. Despite the advances in small protein discovery, there has been little progress in terms of finding the functions of these new-found proteins. Only a handful of the >150 small proteins in *Escherichia coli* have known functions, and it is becoming increasingly apparent that these proteins participate in diverse cellular processes and play key regulatory roles. The PhoQ/PhoP two-component system is an important pathway for survival in response to signals such as low Mg^{2+}, acidic pH, osmotic upshift and the presence of cationic antimicrobial peptides, and regulates virulence in *E. coli*, *Salmonella*, *Yersinia* and related bacteria. The PhoQ sensor kinase is regulated by small protein MgrB via negative feedback inhibition. In our most recent research, we elucidated the mechanism of the interactions between MgrB and PhoQ by utilizing a combination of *in vivo* and *in vitro* approaches, such as bacterial two-hybrids, protein domain swaps, reporter gene assays (Figure 1). Traditional biochemical methods to study proteins are biased towards proteins much larger than 50 amino acids. Therefore, our lab is developing tools specifically tailored to study the functions of small proteins, by identifying their functional targets and physiological roles in the cell. Additionally, we are interested in studying the differences in small proteome composition between commensal *E. coli* and related pathogens

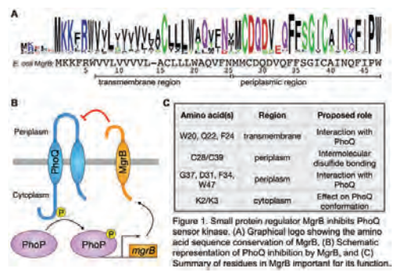

Figure 1. Small protein regulator MgrB inhibits PhoQ sensor kinase. (A) Graphical logo showing the amino acid sequence conservation of MgrB. (B) Schematic representation of PhoQ inhibition by MgrB, and (C) Summary of residues in MgrB important for its function.

Epitranscriptomic regulators – these are proteins at the interface of epitranscriptome and stress response. An epitranscriptome consists of all the RNA modifications in a cell, a significant subset of which are tRNA modifications, whose primary function is in maintaining the efficiency and fidelity of protein synthesis; however, their significance is not always apparent. Recent findings show that many tRNA modifications are directly determined by cellular or

environmental factors such as nutrient availability, pH, growth phase and oxidative stress. It is now becoming evident that the enzymes involved in the biosynthesis of tRNA modifications, either directly or indirectly may play pleiotropic regulatory roles within the cell by acting as links between the epitranscriptome, metabolism and stress responses. Previously, we showed that treating *E. coli* with sublethal concentrations of an antimicrobial peptide (C18G) causes cells to filament in a PhoQ/PhoP-dependent manner. Our work demonstrated that this block in cell division is not due to cell wall/membrane damage induced by the antimicrobial peptide, but instead the result of a high stimulus through this two-component system. Filamentation is mediated by an enzyme, QueE, which participates in the biosynthesis of a tRNA modification called queuosine. QueE is upregulated upon strong activation of PhoQ, which directly binds and inhibits the division complex in *E. coli* (Figure 2). The control of septation by QueE may protect cells from antimicrobial peptide stress via stimulation of the PhoQ/PhoP signaling system. Currently, we are investigating the mechanistic details of cell division inhibition by this tRNA modification enzyme, QueE and determining if its role in stress response confers a fitness advantage.

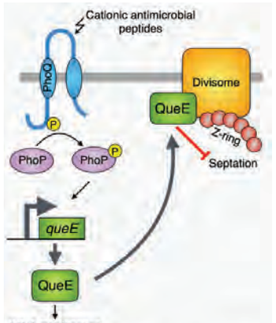

Figure 2. *E. coli* PhoQ/PhoP two-component system modulates cell division via QueE upon strong stimulation by antimicrobial peptides.

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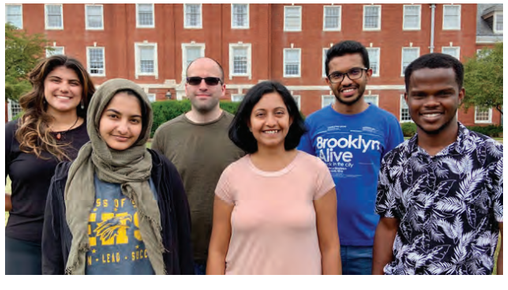

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DISMUKES LAB

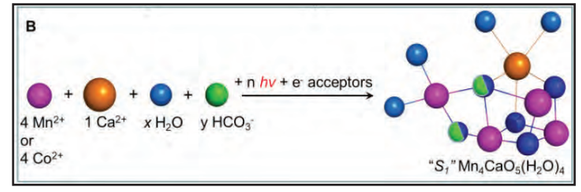
Biological and Chemical Approaches to Renewable Energy Research

Dr. G. Charles Dismukes
Chemistry & Chemical Biology
Microbiology & Biochemistry

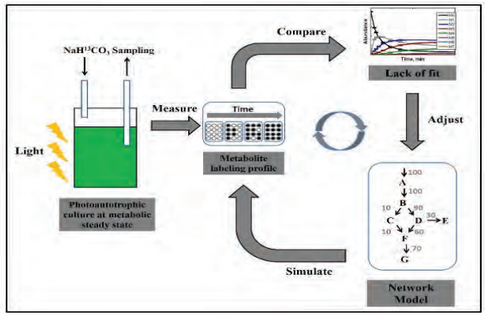
Summary

The Dismukes research group conducts fundamental and applied research in the areas of renewable energy production via biological and chemical approaches. The biological approach focuses on investigations of the light reactions and central carbon metabolism in photosynthetic microorganisms and their symbionts. The chemical approach involves synthesis of bioinspired electrocatalysts and their integration with electrolyzers and artificial photosynthetic systems. The disciplinary approaches used are genetic engineering, materials synthesis by design, electrochemistry and catalysis. The goal is to produce sustainable processes for renewable fuels and biomass production. Our laboratories are located in the Waksman Institute of Microbiology and the New Chemistry Laboratory at Rutgers University. In the 2020-July 2021 period the group was comprised of 28 researchers and interns (listed below).

1) ***Why Did Nature Choose Manganese Exclusively to Make Oxygen on Earth?*** **Waksman.** All contemporary oxygenic phototrophs split water using a single invariant cluster comprised of Mn_4CaO_5 (the WOC) as the catalyst within Photosystem II of natural photosynthesis. This cluster is unstable outside of PSII and can be reconstituted, both in vivo and in vitro, using the elemental aqueous ions and light, called photoassembly. Here we demonstrate the first functional substitution of manganese in any oxygenic reaction center by in vitro photoassembly. Following complete removal of the inorganic cofactors from cyanobacterial PSII microcrystals (PSIIX), photoassembly with free cobalt (Co^{2+}), calcium (Ca^{2+}) and water (OH^-) reassembles >90% of PSII centers. This process occurs 3-fold faster using Co^{2+} vs. Mn^{2+}, due to a higher quantum yield for PSIIX-mediated photochemistry (*): $Co^{2+} \rightarrow * \rightarrow Co^{3+}Q_A^-$. This kinetic preference for Co^{2+} over native Mn^{2+} during photoassembly is offset by significantly poorer catalytic activity and photodamage. The resulting reconstituted Co-PSIIX oxidizes water by the standard four-flash photocycle, though produces 4-fold less O_2 per PSII, attributed to faster charge recombination ($Co^{3+}Q_A \leftarrow Co^{4+}Q_A^-$) in the catalytic cycle. More severe photoinactivation of reconstituted Co-PSIIX occurs (3-fold faster than reconstituted Mn-PSIIX), attributed to oxidative damage to PSII during the catalytic cycle. The physico-chemical basis for these differences is discussed. Manganese offers two advantages for oxygenic phototrophs that explains its exclusive retention throughout Darwinian evolution: significantly slower charge recombination ($Mn^{3+}Q_A \leftarrow Mn^{4+}Q_A^-$) permits more water oxidation at low and fluctuating solar irradiation (net energy conversion), and much greater tolerance to photooxidative damage at high light intensities (Mn^{4+} less damaging than Co^{4+}). Supported by DOE-BES. Collaboration: Arizona State U.



2) Photoautotrophic Carbon Fluxomics. **Waksman.** Metabolic pathways for model organisms can be found in textbooks. However, these are widely modified across the ToL and novel pathways for making carbon products abound in nature that remain to be discovered. Improving the abysmal solar to biomass efficiency of plants and algae through metabolic engineering is an additional goal. Our approach is to use mass spectrometry techniques to decipher pathways for carbon fixation. This is illustrated by flux balance analysis and isotopically nonstationary metabolic flux analysis (INST-MFA, figure) to quantitatively understand carbon flux distributions and pathway used by phototrophs during photosyn-



thesis. It enables discovery of kinetic bottlenecks that limit efficiency, new roles for existing metabolic pathways and completely new pathways not previously known. NSF-MCB and GCEP. Collaborations: RU Plant Biology; USTC China.

3) *Bridging the gap between Kok-type and kinetic models of photosynthetic electron transport within PSII.* Waksman. Historically, two modeling approaches have been developed independently to describe photosynthetic electron transport (PET) from water to plastoquinone within Photosystem II (PSII): Markov models account for losses from finite redox transition probabilities but predict no reaction kinetics, and ordinary differential equation (ODE) models account for kinetics but not for redox inefficiencies. We have developed an ODE mathematical framework to calculate Markov inefficiencies of transition probabilities as defined in Joliot-Kok-type catalytic cycles. We adapted a previously published ODE model for PET within PSII that accounts for 238 individual steps to enable calculation of the four photochemical inefficiency parameters (miss, double hit, inactivation, backward transition) and the four redox accumulation states (S-states) that are predicted by the most advanced of the Joliot-Kok-type models (VZAD). Using *only* reaction kinetic parameters without other assumptions, the *RODE*-calculated time-averaged (eg., equilibrium) inefficiency parameters and equilibrium S-state populations agree with those calculated by time-independent Joliot-Kok models. *RODE* also predicts their time-dependent values during transient photochemical steps for all 96 microstates involving PSII redox cofactors. We illustrate applications to two cyanobacteria, *Arthrospira maxima* and *Synechococcus sp.* 7002, where experimental data exists for the inefficiency parameters and the S-state populations, and historical data for plant chloroplasts as benchmarks. *Significant findings: RODE* predicts the microstates responsible for period-4 and period-2 oscillations of O_2 and fluorescence yields and the four inefficiency parameters; the latter parameters are not constant for each S state nor in time, in contrast to predictions from Joliot-Kok models;some of the recombination pathways that contribute to the backward transition parameter are identified and found to contribute when their rates exceed the oxidation rate of the terminal acceptor pool (PQH_2); prior reports based on the assumptions of Joliot-Kok parameters may require reinterpretation. Supported by DOE-BES-PS.

4) *Regulation of linear electron flow (LEF) in the Z-scheme from water oxidation to CO_2 reduction.* Waksman.
We are developing a method to measure CO_2 carboxylation in living phototrophs that avoids the pitfalls of infrared spectroscopy. It is a variant of the chlorophyll fluorescence induction method that uses fast repetition rate method (FRRF). The figure shows Chl emission (Fv/Fm) from PSII within algal cells produced by 180 trains of flashes (a total of 9000 pulses) given over 3 minutes. Three phases can be observed that were identified as originating from changes in the yield of PSII charge separation as downstream electron acceptor pools are successively filled, first the PQ pool, then the $NADP^+$ pool. Finally, nearly full recovery of PSII charge separation occurs when CO_2 is carboxylated by RuBisCO present in excess, thus consuming the transient pool of NADPH produced by LEF. The method is fast, sensitive and can be applied in the field with suitable instrumentation. We are applying this method to characterize adaptation to changing CO_2 levels in the environment by various plants and microalgae that do carboxylation by different metabolic pathways (C_3, C_4, CAM or use dissolved inorganic carbon). Supported by DOE-BES-PS; Collaborations: RU Plant Biology and TU Delft.

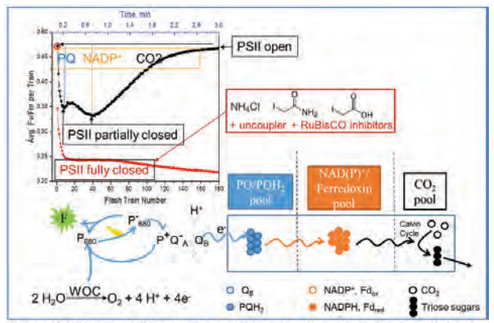
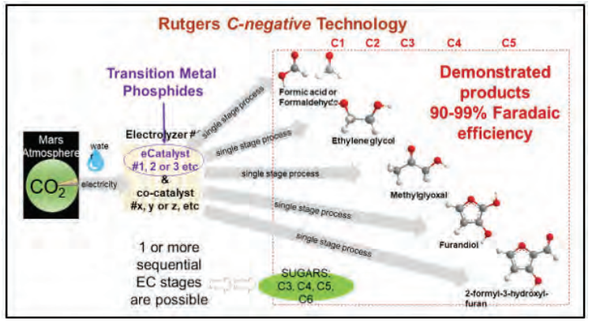


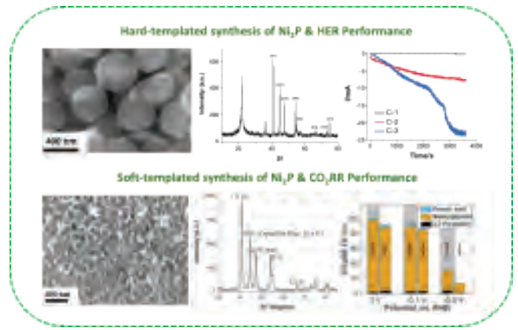
Figure 12. Changes in Chl fluorescence Fv/Fm under illumination measured by FRRF.

5) *Bioinspired Electrocatalysts for Water Splitting and CO_2 Reduction*. CCB. Human civilization is on a crash course to consume our natural resources and poison our environment unless we learn how to use renewable feedstocks like water and CO_2 to make chemicals and food. In this project we apply the principles learned from enzymes to synthesize better heterogeneous catalysts for the generation of H_2 and O_2 from water (like Photosystem II) and CO_2 conversion (like acetogens and methanogens). Bioinformatics and data science tools are used to determine the chemically relevant attributes for catalysis by the CO_2 reducing enzymes. The synthesized catalysts are made from earth abundant elements and in the best cases can exceed the activity of commercial catalysts used today. Using this

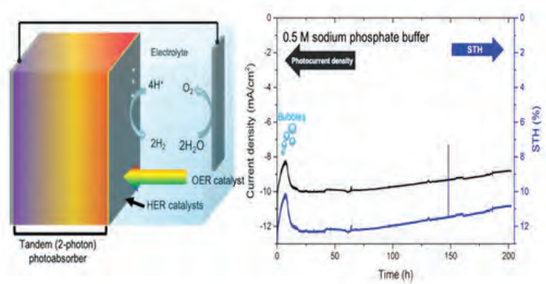
strategy, we have produced noble-metal-free catalysts for water oxidation ($LiCo_2O_4$), water reduction to hydrogen (Ni_5P_4), and selective CO_2 reduction to C_1 to C_5 products (Nickel Phosphides). The image depicts our electrolytic process for converting water and CO_2 into C_1 to C_5 products. Supported by RenewCO_2-SBIR, NASA-CO_2 Challenge, Rutgers Goldman Prize; Pray Family Fund. Collaborations: UPenn and CU Boulder.



***6) Synthesis of Transition Metal Phosphides with Defined Structural Phase, Facet and Morphology for Electrocatalysis.* CCB.** In this project, we use a templating approach to synthesize nickel phosphide catalysts for hydrogen evolution and CO_2 reduction reactions. We aim to develop robust protocols to synthesize morphologically controlled and crystalline nanocatalysts that can electrochemically produce sustainable chemicals. So far, we have used silica as a hard template and cetyltrimethylammonium bromide as the soft template which act as frameworks to synthesize binary transition metal phosphide compounds to test as catalysts. XRD and SEM characterizations have revealed phase purity and unique morphology of the synthesized nanomaterials. These catalysts are currently being evaluated for HER and CO_2RR. HER results show high current densities can be obtained (~25mA/cm^2 and ~400mA/cm^2 for hard and soft template Ni_2P respectively. CO_2RR results show soft-templated Ni_2P catalyst can convert CO_2 to C_1, C_3 and C_4 products with high Faradaic efficiencies. DFT computational studies of HER are being conducted with the Rappe group at UPenn. Support: DOE NREL-LDRP and subcontract to RenewCO_2 LLC.



7) *Best-in-class Platinum Group Metal-free Catalyst Integrated Tandem Junction PEC Water Splitting Devices.* CCB. Solar energy conversion to fuels requires initially the splitting of water into its elements, H_2 and O_2. Our goal is to build a tandem solar fuel cell to split water using sunlight using earth abundant materials that are globally scalable. This entails development of a dual absorber photocell for red photons and near infrared photons coupled to Rutgers patented OER and HER catalysts, respectively. Thus far, we have achieved an overall efficiency for sunlight into hydrogen of 12% with more than 200 h of stability. Support DOE-EERE-HydroGEN. Collaborator: NREL.



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DONG LAB

Cell Polarity and Asymmetric Division in Plants

Dr. Juan Dong
Plant Biology

Summary

Cell polarity, in both animals and plants, is of paramount importance for developmental and physiological processes. The establishment and maintenance of cell polarity is required for asymmetric cell division (ACD) and indispensable for multi-cellular organisms to generate cellular diversity. Through ACD, a single mother cell can produce daughter cells with distinctive identities in developmental differentiation. Our research focuses on the mechanisms by which cell polarity is initiated and maintained in the stomatal lineage cells in Arabidopsis and the mechanisms by which differential cell fates are specified in plant ACD. The identification of the plant-specific protein BASL (Breaking of Asymmetry in the Stomatal Lineage) provided strong evidence that plant cells have the capability to polarize non-transmembrane proteins and utilize such polarized protein distribution to regulate asymmetric cell division (Figure 1).

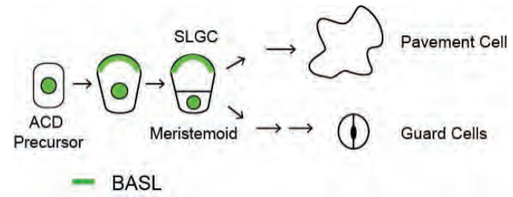

Figure 1. BASL localization and stomatal asymmetric cell fate

By using BASL as an anchor for screens to isolate genetic and physical interactors and by using the features of the protein itself as a probe for cell's ability to correctly establish polar cortical localization, our lab is in the process of building a model for plant cell polarization and its regulation in ACD. We are also interested in understanding the molecular mechanisms underlying how cell polarity orients cell division plant and how differential daughter cell fates are specified by the cortical polarity complex. Our work focuses on the identification of additional polarized proteins and of mutants that display specific subsets of polarity defects. At a larger extent, some of our work also include the characterization of novel regulators in stomatal development in Arabidopsis. Through collaborative effort, we also expand our research interest towards understanding the mechanical and physiological features of functional stomatal guard cells in plants.

Membrane trafficking for polarity initiation and establishment in plant cells

The molecular mechanisms for protein polarization in plants have been extensively studied in two systems: 1) vesicular trafficking-based polarization of PIN auxin efflux carriers (membrane embedded proteins) and 2) cytoskeleton-dependent and –independent positive feedback loop-based ROP polarization (small Rho-like GTPases from plants). Polar trafficking of BASL, a non-membrane novel protein, has not been successfully connected to both pathways and might represent an unknown mechanism. Fluorescence Recovery After Photobleaching (FRAP) was performed on GFP-BASL and the recovery curves suggested that BASL dynamics is more comparable to the membrane-embedded PIN proteins, hinting the possible regulation of membrane trafficking in BASL polarization. We recently identified a family of plant specific proteins associated to the endomembrane system to regulate BASL polarization (Wang and Li, submitted). This direction is currently pursued in the lab.

BASL scaffolds the BSL phosphatases to enable the progression of stomatal asymmetric cell division

Our previous work showed that BASL is phosphorylated and activated by MAPK 3 and 6 (MPK3/6) and becomes po-

larized to the cell cortex, where it recruits the MAPKK Kinase YODA and MPK3/6 to inhibit stomatal differentiation in one of the two daughter cells. Recent work showed that, prior to a stomatal ACD, the polarity complex employs POLAR to recruit the GSK3-like kinase BIN2 that releases the suppression of YODA on stomatal differentiation, therefore stomatal ACD is promoted. Therefore, the stomatal polarity complex by scaffolding different signaling molecules could promote the division potential before an ACD and suppress the division potential after an ACD. However, how the transition of these two seemingly opposing procedures can be achieved by the same polarity complex remained a major challenge towards understanding stomatal ACD. Here, by using immunoprecipitation combined with mass spectrometry (IP-MS), we identify a family of protein Ser/Thr BSL phosphatases, as BASL partners (Guo, 2021 Nat Plants). Genetic analysis places BSL upstream of the YDA MAP kinase cascade and downstream of the plasma membrane receptors. In addition, the founding member BSL1 colocalizes with BASL in a polarized manner at the cell periphery. Interestingly, the recruitment of the BSL phosphatases in the polarity module confers a negative role to BIN2 complex but a positive role to the YDA MAPK module (Fig. 2). Thus, our study reveals a crucial function of the BSL phosphatases in bridging the two opposing protein functional modules to control the balance of cell-division potential and cell-fate determination in plant ACDs.

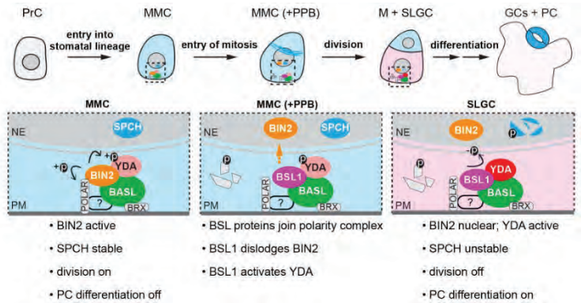

Figure 2. Working model for BSL phosphatases to function in stomatal ACD.
The BSL proteins function as the spatiotemporal molecular switch enabling the coordination of cell division and cell-fate differentiation in stomatal ACD. BSL1 join the polarity complex in the ACD mother cells that are committed to cell division. Association of BSL1 with the polarity complex dislodges BIN2 from the cell membrane to prevent subsequent rounds of cell division and activates the YDA MAPK signaling cascade to promote cell-fate differentiation. BIN2, GSK3-like kinase; YODA, MAPK Kinase Kinase; SPCH, bHLH transcription factor; BASL and POLAR, polarly localized scaffolding proteins.

Protein Phosphatase 2A promotes stomatal development by stabilizing SPEECHLESS in Arabidopsis

Stomatal guard cells control gas exchange that allows plant photosynthesis but limits water loss from plants to the environment. In Arabidopsis, stomatal development is mainly controlled by a signaling pathway comprised of peptide ligands, membrane receptors, a mitogen-activated protein kinase (MAPK) cascade, and a set of transcriptional factors. The initiation of the stomatal lineage requires the activity of the bHLH transcriptional factor SPEECHLESS (SPCH) with its partners. Multiple kinases were found to regulate SPCH protein stability and function through phosphorylation, yet no antagonistic protein phosphatase activities have been identified. Here, we establish the conserved PP2A phosphatases as positive regulators of Arabidopsis stomatal development. We show that mutations in genes encoding PP2A subunits result in lowered stomatal production in Arabidopsis (Bian et al., 2020 PNAS). Genetic analyses place the PP2A function downstream of the MAPK cascade, but upstream of SPCH. Pharmacological treatments support a role for PP2A in promoting SPCH protein stability. We further show that SPCH directly binds to the PP2A-A subunits in vitro. In plants, non-phosphorylatable SPCH proteins are less affected by PP2A activity levels. Thus, our research identifies PP2A as the missing phosphatases that function antagonistically with the known kinases to maintain the phosphorylation balance of the master regulator SPCH in stomatal development (Fig. 3).

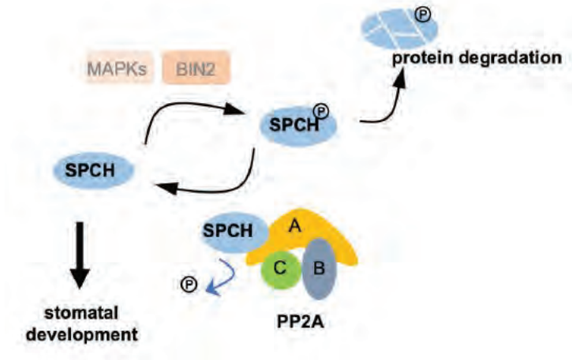

Figure 3. Working model for PP2A phosphatases in stomatal development.
PP2A promotes stomatal development by stabilizing the SPCH protein. PP2A might function in opposition to the identified kinases (MAPKs and BIN2) to balance the phosphorylation status of SPCH in the initiation of stomatal.

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GALLAVOTTI LAB

Molecular Mechanisms of Plant Development

Dr. Andrea Gallavotti
Plant Biology

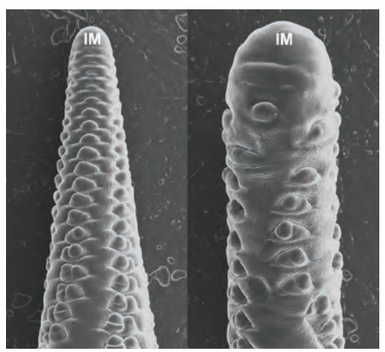
Summary
In stark contrast to animal development, plant development mainly occurs after embryogenesis. This ability is provided by small groups of stem cells called meristems that are continuously formed and maintained throughout development. Meristems are responsible for the formation of all lateral organs, such as leaves, branches and flowers, and as such, are primary determinants of plant architecture and of the morphological variation we observe in different plant species. Meristems are also responsible for the ability of plants to constantly adapt growth to changes in the surrounding environment. Understanding the molecular mechanisms of meristem formation and function can therefore answer basic questions on the regulation of organogenesis and cell fate specification that eventually drive maize productivity in different environments.

In our laboratory we study the formation, activity and maintenance of meristems. In particular, we focus on a class of meristems, called axillary meristems, that are responsible for the formation of branches and flowers in plants. We use maize as a model system for our research because of the vast genetic and genomic resources available, and because of its agricultural importance.

Identification of cis-regulatory modules in plant genomes

Transcription factors (TFs) are proteins that recognize short DNA sequence motifs in regulatory regions of their target genes and thus control the gene expression changes responsible for plant developmental programs and environmental responses. To expand our currently limited view of the functional non-coding space in maize and other plant species, we are using DAP-seq, a cost-effective *in vitro* technique to map genome-wide TF binding events. We observe that many TFs often bind within close proximity to one another forming putative cis-regulatory modules (CRMs; also commonly referred to as enhancers; Figure 1). These CRMs frequently overlap with regions of accessible chromatin and can be located both proximally and distally at regions far away from genes. Such proximal and distal CRMs were for example observed in several plant architecture-related maize genes.

The overal goal is to obtain a highly integrated view of how multiple TFs contribute to the control of certain transcriptional programs. This is important because a significant percentage of trait-associated variants in crop species lie within non-coding regions and likely affect TF binding. Our goal is to explore how cis-regulatory variation contributes to phenotypic diversity in maize and other species, including the model plant Arabidopsis, by coupling identification of CRMs with precise CRISPR-based editing of TF binding sites. This work is currently supported by a collaborative grant from the National Science Foundation in the Tools, Resources and Technology Advances track (TRTech-PGR).



In *Bif3* mutants overexpressing *ZmWUS1* the size of ear inflorescence meristems (IM) is dramatically increased. Scale bars, 500μm.

The role of the phytohormone auxin in maize development

The plant hormone auxin directs a multitude of developmental responses. How auxin can trigger many different developmental responses is still a major unanswered question. Recent work has highlighted how the combinatorial activity of different components of the auxin signaling pathway may be responsible for the specificity of auxin response. Auxin is perceived by the nuclear auxin receptor TIR1/AFB, part of an E3 ligase that rapidly degrades Aux/IAA co-receptor proteins and disrupts their recruitment of TOPLESS (TPL) corepressor proteins that silence transcription. The auxin-dependent degradation of Aux/IAAs frees interacting activating ARF transcription factors from TPL repression, allowing them to activate downstream genes. Aux/IAAs and ARFs belong to large families of transcriptional regulators whose combinatorial interaction is believed to trigger specific developmental responses.

As part of a collaborative research project sponsored by the National Science Foundation Plant Genome Research Program we are investigating the specificity of auxin function in developmental pathways and discovering new genes involved in auxin biology and meristem development. We used DAP-seq to analyze the DNA binding behavior of the maize ARF family and to identify the direct targets of their regulation. Using this approach, we created the largest dataset of ARF targets in any plant species. Furthermore, we discovered that ARFs belonging to different phylogenetic groups have different binding behavior in terms of sequence recognition and genomic distribution.

Using traditional genetic screens, we have identified several genes that affect auxin function and meristem development. Among these is *ZmWUS1*, a key regulator of meristem size that determines the number of rows of seeds in maize ears (Figure 1).

Transcriptional repression in maize shoot development

Transcriptional repression is a fundamental tool in a cell's repertoire of molecular mechanisms for the dynamic regulation of gene expression. In most eukaryotes, such repression typically involves corepressor proteins that do not bind DNA directly, but instead interact with DNA-binding transcription factors (TFs) that act in specific developmental and signaling pathways and suppress transcriptional output.

The maize co-repressor REL2, a functional homolog of the TPL protein mentioned above, was originally isolated in a forward genetic screen for inflorescence defects. Mutations in the *REL2* gene give rise to pleiotropic defects throughout development, thus providing an excellent tool to study how plants use transcriptional repression mechanisms in numerous developmental processes. Notably, *rel2* mutants increase the size of meristems.

We identified a large number of TFs that contain specific repressor motifs that allow the interaction with REL2-type corepressors and repress the transcription of their target genes. According to the repressor motif embedded in their sequences, these transcriptional regulators interact with REL2 using distinct mechanisms. Among the TFs interacting with REL2 is ZmWUS1. We are currently investigating REL2 function in regulating the size of inflorescence meristems to determine whether this occurs via ZmWUS1 interaction or independently of it. This research is sponsored by a new collaborative grant from the Division of Integrative Organismal Systems of the National Science Foundation.

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MALIGA LAB

Plastid Molecular Genetics

Dr. Pal Maliga
Plant Biology

Summary
Plastids are semi-autonomous organelles with a relatively small (120-180 kb), highly polyploid genome present in 1,000 to 10,000 copies per cell. The best-known plastids, chloroplasts, convert sunlight into chemical energy. Plastid engineering, in contrast to nuclear engineering, offers higher protein yields, the opportunity to express several genes controlling complex traits, and a natural tool to prevent transgene flow via pollen. We have developed protocols for transformation of the tobacco (*Nicotiana tabacum*) plastid genome, for efficient post-transformation excision of the marker genes, and high-level expression of recombinant proteins. During the past year we made significant progress in the following areas.

Extending plastid transformation from *Arabidopsis thaliana* to *Brassica napus*, an oilseed crop

Plastid transformation has been inefficient in *Arabidopsis thaliana* due to a natural tolerance of Arabidopsis to spectinomycin, the selective agent employed to enrich transformed plastid genomes. Tolerance to spectinomycin has been linked to a duplication of the ACCase biosynthetic pathway in chloroplasts. We have shown that plastid transformation is 100-fold more efficient in Arabidopsis lines defective in the plastid-targeted *ACC2* nuclear gene (Yu et al. Plant Physiol. 175: 186-193, 2017). This information has been obtained in the the Col-0 ecotype that is recalcitrant to plant regeneration. Last year we reported *ACC2* defective lines in the RLD and Ws ecotypes, which readily regenerate plants from cultured cells (Yu et al., Plant Physiol. 181: 394-398, 2020). We hypothesized that plastid transformation competent *Brassica napus* can be obtained by the deletion of all nuclear encoded, chloroplast targeted ACCase copies using CRISPR-Cas9. *B. napus* (2n= 38, AACC) is a recent interspecific hybrid of *B. rapa* (2n=20, AA) and *B. oleracea* (2n=18, CC) and is expected to have at least two *ACC2* copies, one from each parent. Our preliminary data suggested five unique *ACC2* copies in cv. Westar. However, the genetic map has only two *ACC2* copies, one that is *B. rapa*-like and one that is *B. oleracea*-like. We designed sgRNAs that could simultaneously knock out all nuclear *ACC2* copies and expressed Cas9 from an egg cell-specific promoter. Four T0 transgenic lines were obtained by Agrobacterium-mediated hypocotyl transformation. Amplicon sequencing confirmed deletions in 10 T1 progeny, in seven of which no wild-type *ACC2* gene remained. We shall now evaluate the feasibility of obtaining transplastomic *Brassica napus* in the *ACC2* knockout background.

Re-engineering Agrobacterium for T-DNA delivery to chloroplasts

The current bottleneck of plastid transformation in Arabidopsis is the difficulty of obtaining fertile plants from transplastomic tissue culture cells. Tissue culture limitations in Arabidopsis nuclear gene transformation were overcome by using Agrobacterium to directly transform the female gametocyte, and identification of nuclear transgenic events by germinating the resulting seedlings on a selective medium. Our goal is to re-engineer Agrobacterium for T-DNA delivery to chloroplasts to directly transform the plastids in the female gametocyte. T-DNA export from *Agrobacterium* to plant cells occurs by the type 4 protein secretion machinery. Recently, we obtained proof of concept that proteins can be directly exported from *Agrobacterium* to chloroplasts. The protein of our choice was the phiC31 phage site-specific integrase (Int), because visitation of the recombinase to chloroplasts created a permanent footprint (Matsuoka, A and Maliga, P. Prospects for Reengineering Agrobacterium for T-DNA delivery to Chloroplasts. Plant Physiol. 186: 215-220, 2021). We are now working on re-targeting the proteins involved in T-DNA transfer. VirD2 has been reengineered by removing all nuclear localization signals (NLSs) and providing chloroplast targeting sequences. Import of the engineered VirD2 (plastid VirD2 or Pt-VirD2) will be shown in a split GFP assay, in which Pt-VirD2-11 delivery, containing the small GFP-11 peptide, is indicated by fluorescence complementation with a split (non-fluorescent) GFP1-10 molecule in chloroplasts (Figure 1A). As the first step, we set up a split GFP complementation assay in *E. coli.* We designed a family of seven Pt-VirD2-11 genes by removing the VirD2 nuclear localization signals, providing a chloroplast-targeting transit peptide (TP) at the N-Terminus and inserting the small GFP-11 peptide at the N- and C-terminal region of VirD2. We also expressed a GFP1-10 protein in *E. coli* which does not fluoresce when illuminated with UV light. However, when both the GFP-1-10 and Pt-VirD2-11 proteins are co-expressed in the same *E. coli* cell, we could readily detect GFP fluorescence under UV light (Figure 1B). Next we shall be working on GFP complementation of Pt-VirD2-11 and GFP1-10 in chloroplasts. Proof of concept will be obtained in tobacco; the results will be applied to Arabidopsis floral dip transformation. Side-stepping the tissue culture process by floral dip transformation of plastids in the female gametocyte will lead to widespread applications of Arabidopsis plastid genome engineering.

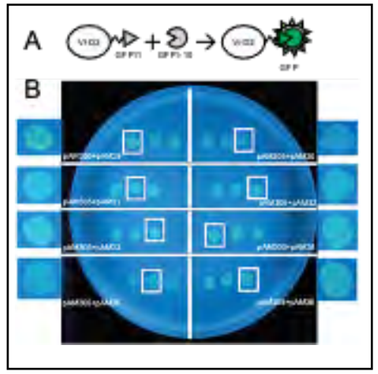

Figure 1. Split GFP complementation in *E. coli.* (**A**) Schematic of the split GFP system. (**B**) Fluorescence in *E. coli* confirms complementation of Pt-VirD2-11 and GFP1-10 (pAM305). Positive control pAM29, sulfit-reductase-GFP11; negative control pAM30, Pt-VirD2 without GFP11.

Engaging undergraduate students in research

Engaging undergraduate students in research is part of the broader impact of research activity supported by the National Science Foundation. We integrate research and education by training undergraduates to facilitate full participation of women and underrepresented minorities in STEM fields. We also host students from Farmingdale State College, a Primarily Undergraduate Institution, to expose the Farmingdale students to the research University environment. The students come through our collaborator, Associate Professor Kerry A. Lutz, who is Co-PI on the NSF Grants. Participating students during 2020-2021 from Rutgers were Mugdha Parulekar, Alifya Quresh and Julia Ferranti. From Farmingdale State College Ana Candina spent the summer of 2021 at Rutgers.

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ZANDER LAB

Transcription Factor-driven Epigenome Dynamics in Plants

Dr. Mark Zander
Plant Biology

Summary
Increased crop productivity will be essential to address the imminent food demands predicted in human population models. This is increasingly difficult to accomplish because global climate change is projected to expand the geographic range of numerous pathogens, as well as shift the geographic ranges of crop suitability. To achieve this goal, we need to increase crop productivity and resilience via improved tolerance to abiotic and biotic stresses, and to utilize marginal lands for crop production. Especially the deciphering of molecular mechanisms underlying plant responses to increased temperatures are thus critically needed to breed more stress-tolerant crop plants ultimately ensuring our food security in the future.

A key regulatory process of stress pathways is the interplay between master transcriptions factors (TFs) and the epigenome (all chemical modifications of DNA and histone proteins) that lay the transcriptional foundation for a robust stress response. Despite their agronomic importance, spatiotemporal information about the genome-wide actions of plant master TFs and their interactions with the epigenome are scarce due to two major problems. First, the missing scalability of genomic profiling tools such as ChIP-seq (chromatin immunoprecipitation analysis coupled with next-generation sequencing) and second the dilution and obscurity of biological information in existing datasets due to bulk measurements which is a fundamental problem of biological research. My laboratory uses the model plant species *Arabidopsis thaliana* as well as industrial hemp to tackle these problems to better understand transcription factor-driven epigenome dynamics in response to environmental changes.

Visualization of elevated temperature-induced epigenome dynamics at cell type resolution

The interpretation of TF activity and epigenome dynamics is difficult. Unlike the very dynamic range of number of mRNA molecules per gene and per cell after induction, the dynamic range of TF binding and epigenomic changes is way smaller, thus far more prone to get diluted and become invisible in bulk measurements. We investigate TF-governed regulatory networks in elevated temperature responses at organ and cell type level to overcome these inherent challenges. Our focus lies on the main plant organs (root, hypocotyls, petioles, leaves) to generate an organ-level atlas of elevated temperature-induced transcriptional and epigenomic reprogramming. In addition, we also profile the major cell types of the aboveground plant tissues (epidermis, vascular tissue, palisade and spongy mesophyll cell, endodermis, and cortex) into my analyses. Nuclei from the various cell types will be purified with INTACT (isolation of nuclei tagged in specific cell types) and subsequently subjected to epigenome profiling and expression analyses. Taken together, these experiments will yield a very comprehensive understanding of TF-initiated transcriptional networks at unprecedented resolution.

Comprehensive analysis of the interplay between transcription factors and the epigenome

The functional relationship between transcription factors and the epigenome is highly complex depending on epigenome feature, type of TF and targeting mechanism of the respective chromatin modifying enzyme complexes. DNA methylation at cis-regulatory elements in humans and in Arabidopsis, for example, can prevent the binding of TFs. To investigate the interplay between TFs and the epigenome in more detail, we conduct TF ChIP-seq in Arabidopsis mutants that are deficient in known chromatin regulators. Unlike mammalian systems where chromatin regulator mutants are often not viable, chromatin regulator mutants in Arabidopsis are mostly viable thus providing an ideal toolkit to study the impact of an altered chromatin landscape on TF binding. Time course experiments will be conducted to capture dynamic TF binding together with mRNA expression. This approach allows us to visualize how TF binding dynamics by certain chromatin states including the resulting transcriptional consequences.

Investigating the role of the epigenome in stress responses of hemp

The role of the epigenome in regulating stress responses of plants is also still enigmatic. What is the contribution of the chromatin in integrating environmental stimuli? Are stimulus-induced changes of the chromatin architecture just reflections of regulatory events initiated by sequence-specific DNA binding proteins or is there a hidden layer of information (e.g. epigenetic memory) embedded in certain chromatin states? To shed light into this complexity, we apply a variety of genetic, genomic, and proteomic tools to investigate epigenome dynamics in industrial hemp. Natural variation in species harbors enormous information about how gene regulatory networks were shaped in course of adaptation of "ecotypes" to a diversity of growth environments/habitats but also because of domestication. Thus, we also analyze the natural genetic and epigenetic variation in various hemp accessions to exploit to discover the molecular determinants underlying the phenotypic complexity of stress response pathways.

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CORE FACILITIES

Cell and Cell Products Fermentation Facility

OVERVIEW

The Cell and Cell Products Fermentation Facility (CCPFF) is a nonprofit state of the art facility designed to help the Waksman Institute researchers, university faculty as well as other scientific/research/biotechnology industry develop new fermentation technologies and provide bench top and pilot scale fermentation projects for research and biologics production. We offer flexibility and versatility in supplying biomolecules including; proteins, enzymes, antibiotics, growth factors, natural flavors and cosmetic substrates to industry engineering; microbiological and molecular groups. We are equipped to handle most requests with the exceptions: pathogenic or opportunistic organisms and mammalian and insect cell lines. We can conduct both scale-up and production to maximize research potential for both upstream and downstream processing.

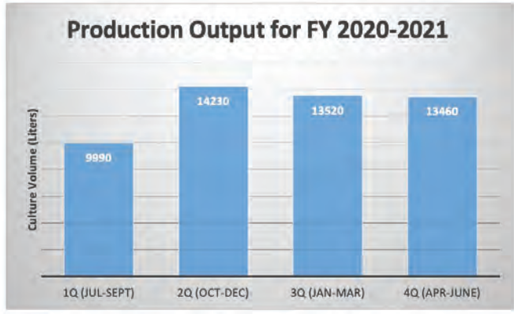
For upstream processes, the facility is currently equipped with 2 units of 1000L Bio Flo Eppendorf reactors and 3 units of 125L 610 Eppendorf reactors for scale-up studies and pilot scale work. For smaller scale projects, we have 3 units of 40L 510 Eppendorf reactors with working volumes between 12-32L Liters for research and optimization studies. We have multiple options for downstream applications including clarification by macro/ultra/nanofiltration or centrifugation. The facility has a continuous centrifuge for large scale separation, Beckman Avanti refrigerated floor centrifuge for smaller batches with multiple rotors for 10mL conical tubes up to 6, 1L centrifuge bottles and two smaller centrifuges for sample preparation. The facility has an assortment of hollow fiber filters for clarifying, concentrating, and diafiltering solutions of different volumes. Further downstream processing includes cell disruption using a GEA Niro Pony NS2006L. The facility has three refrigerated Innova Eppendorf shakers for seeding tanks and small projects and provides analytical services for testing proteins and metabolites using protein gel analysis and a YSI 2900D Chemistry analyzer for glucose, glycerol, methanol as well as other chemistries

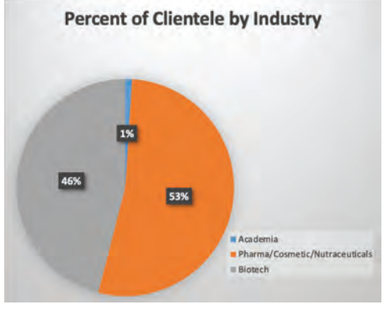
FISCAL YEAR 2020-2021

Over the past year, the scientists at the fermentation facility have completed projects for numerous biotech, pharmaceutical and cosmetic industries as well as a couple of university and university affiliates. For this fiscal year and

despite the pandemic, the total production output exceeded last fiscal year's output and is now over 52,000 liters (65 % increase from last year) of microbe cultures of E. coli, P. pastoris, Streptomyces spp. and various strains of yeast and fungus. The revenue generated from all these works have continuously provided valuable support to the research goals of the Institute as a whole and will continue to do so in the years to come.

Dr. Arvin Lagda oversees and directs the overall operation of the facility, while the day to day projects is handled by a team of fermentation experts led by Ms. Amanda Rodriguez (Production manager) and supported by Dr. Sergey Druzhinin as Laboratory Scientist and Mr. Andrew Cloud as Laboratory Technician. The highlight for this fiscal year is the incorporation of Industry Standards Quality Management Systems based on current Good Manufacturing Practice (cGMP) and the Joint International Pharmaceutical Excipients Council (IPEC) & the Pharmaceutical Quality Group guidelines into our work flow systems. This is an ambitious but doable task that will ensure product quality, safety and work efficiency.





FUTURE GOALS

Our goal over the next few years is to increase and diversify our research and production capabilities which will be tailored fit to the needs of our Waksman Institute researchers. This includes incorporation of technologies necessary for the in-house pilot scale production and purification of commercial proteins/enzymes and research grade plasmid DNA for research use. We also want to incorporate a broader range of clientele as well as create a broader outreach to the Rutgers community by collaborating with other core and research facilities. Further, we plan to contribute further in the scholarship goals of the Rutgers University by extensive collaboration with other academic departments through teaching/course integration, lectures, facility tours and internship training programs for students.

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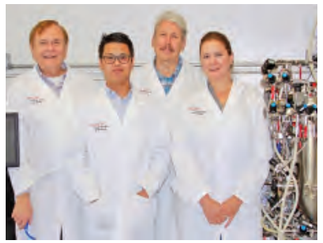
Mr. Andrew Cloud, Laboratory Technician
Mr. Nathan Hill, Laboratory Technician



Waksman Genomics Core Facility

Waksman Genomics Core and Instrumentation Laboratory (FY 2020-2021)

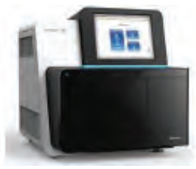
Waksman Genomics Core and Instrumentation Laboratory (WGCF) provides access to high-throughput next generation sequencing and molecular biology instruments to the Waksman Institute's researchers as well as Rutgers research community. Since 2008, the core offers single read, paired-end, and multiplex sequencing using various Next Generation Sequencers but as of March 2020, the facility evolved from providing sequencing services to a shared Instrumentation laboratory with emphasis on molecular biology tools. One of the main goals of the Facility is to provide support to the research mission and vision of the Waksman Institute by providing access to faculty and researchers with up-to-date molecular biology and standard laboratory instruments.

The facility's workhorses include the NextSeq500, Illumina's desktop sequencing instrument that provides roughly 120 Gb data from its 2x150 bp configuration. NextSeq 500's push-button operation provides a thirty-hour turnaround time for an array of popular sequencing applications such as single human genome, 20 transcriptome or up to 16 exomes in a single run. Whereas MiSeq with relatively long read-length and low throughput, is best suitable for small genome sequencing and targeted sequencing.

In addition to the sequencing equipment, WGCF also offers access to Real-Time PCR on Thermo Fisher's StepOnePlus system, DNA shearing services using Covaris, as well as nucleic acid qualification services using fluorometer Qubit, NanoDrop , Agilent bioanalyzer, Typhoon Imager and Scintillation Counter. Access is also available for several units of high speed refrigerated centrifuges and a couple of Ultracentrifuges.

New to this year is the acquisition of a Tecan Spark Plate Reader, Bio-Rad GelDoc XR and Bio-Rad CFX Opus 384 well Real Time PCR. All of which are available for the benefit of Waksman Institute faculty and researchers.

Our mission is to keep the core facility as comprehensive and up to date as possible in order to increase research productivity all across the Waksman Institute of Microbiology.



Illumina NextSeq500 for NGS

Illumina MiSeq for NGS

Fragment Analyzer

StepOne Plus Real Time PCR

Bio Rad CFX

Tecan Spark

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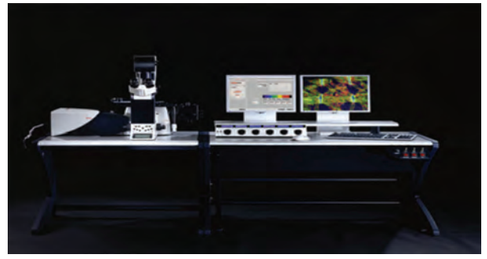
SUPPORT SERVICES

Waksman Confocal Imaging Facility

The Waksman Confocal Microscope Core Facility has two Leica TCS confocal microscopes, the SP5 II and the SP8, as well as two workstations. Our scanning confocal microscopes are capable of high resolution imaging of labeled cell components in three-dimensional space by optical sectioning. Imaging can be done with most standard fluorophores in live or fixed samples.

Both Leica TCS confocal models have inverted microscopes, spectral detection allowing for dynamic adjustment of detected emission wavelengths, sequential scanning to further reduce emission signal overlap, easy to use programs for creating Z-stacks and 3-D images, deconvolution software, multiple programs for post-imaging, and hybrid detectors for large dynamic range, increased signal sensitivity and decreased background noise, as well as standard PMT detectors, Z-stack compensation, and photon counting. The SP5 II is also capable of resonant scanning which significantly reduces imaging times**,** and it has notch filters, FRET, FRAP**,** deconvolution, and a motorized stage allowing multi-position time lapse, mark and find, and tile scanning. Powerful and easy to use, Lightning Deconvolution on the SP8 significantly improves image resolution during acquisition by utilizing adaptive technology.



The Waksman Core Facility has approximately 50 trained users, primarily Waksman researchers, from ten laboratories and is used an average of 62 hours per week (a slight reduction from previous years due to the pandemic). The Confocal Manager provides training, troubleshooting, and consultation on the use of our confocal microscopes. The future aim of the facility is to continue to provide exceptional imaging capabilities to Waksman researchers.

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Waksman Greenhouse

Our state of the art Greenhouse facility, completed in 2006, features 4,200 square feet of growing space. The facility is divided into two identical, independently-controlled bays topped by a roof structure with a height of 14 feet at the truss to better enable the proper growth of corn plants. These two bays feature heating, cooling, shade curtains, growth lights, and roof vents, and are connected by an adjoining headhouse, which includes an outdoor weather station (providing sensor inputs for precipitation detection, solar readings, temperature, humidity, wind speed, and wind direction).

This newer construction replaced the original Waksman greenhouse constructed in 1986, which encompassed 3,600 square feet of growing space divided into six rooms and a roof structure only 7.5 feet at the truss. Insect netting, a cost-effective and environmental-friendly alternative to the use of pesticides, is implemented as a physical barrier to exclude insect pests from the facility in order to reduce the necessity for insecticides.

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CHARLES AND JOHANNA BUSCH FELLOWS

Predoctoral Research

Hiep Tran
Steward Lab

Research Summary

Study Tet protein in regulating RNA hydroxymethylation and brain development

Chemically modified ribonucleotides in rRNA, including mRNA, have been known for decades. Recently, the mapping these modifications by next generation sequencing as well as the discovery of enzymes that deposit ("writer"), eliminate ("eraser"), and bind ("reader") to the modifications revealed the biological functions of the modifications. We have previously shown that *Drosophila* Tet is responsible for hydroxymethylating ribocytosine (5hmrC) in mRNA and that 5hmrC is enriched in *Drosophila* brain mRNAs. Loss of Tet at early stage resulted in severe defect in *Drosophila* brain demonstrating that Tet is required for development of the nervous system.

Tet protein has two domains: a zinc finger CXXC-type DNA-binding domain and a 2OG-Fe(II) dioxygenase domain. By a gene replacement method using CRISPR/Cas9 and homologous directed repair (HDR), I have generated mutant lines in which the absolutely conserved C598 in the DNA-binding domain was changed to A (Tet^{AXXC} line), and a second line, in which H1886 to Y and D1888 to A mutations in the dioxygenase domain were induced (Tet^{YRA} line). Tet^{AXXC} shows defects in the axonal development of the mushroom body, the *Drosophila* brain structure essential for learning and memory while, the Tet^{YRA} exhibits a very mild phenotype, indicating that the two protein domains have specific functions. Recent result showed that a high portion of genes are upregulated in Tet^{AXXC} which are not seen in Tet^{YRA}. In addition, mutations on human TET3 gene have been found in individuals and families, which are affected by intellectual disability, suggesting that Tet is crucial for proper human brain development. To further study Tet protein, I am addressing two questions. How Tet regulates axon projection in developing brain? And which genes are regulated by Tet domains to fulfill its neurological function?

I hypothesize that Tet regulates brain development via controlling axon projection to the right compartments of the brain. Loss of Tet will lead to neurological defects in which resulted in behavioral phenotype such as learning and memory. I also hypothesize that genes, which are upregulated in TetAXXC, are responsible for axonal and neurological defects have been found in Tet mutant brain. Besides, to study the mechanism of how Tet modifies RNA, I am coordinating with people in the lab to test if Tet binds to RNA and identify Tet complex with modified RNA.

Hoa Vu
Nickels Lab

Research Summary

Multiplexed unnatural-amino acid mediated protein-DNA photo-cross-linking define RNA polymerase (RNAP)-DNA interactions in transcription initiation and elongation in living cells

In transcription initiation, RNA polymerase (RNAP) binds to promoter DNA, unwinds a turn of promoter DNA to yield an RNAP-promoter open complex (RPo) containing an unwound "transcription bubble," and selects a transcription start site (TSS). In initial transcription, RNAP remains bound to promoter DNA as an initial transcribing complex (ITC) and synthesizes an RNA product of a threshold length of ~11-15 nt. In promoter escape, which occurs upon synthesis of a threshold-length RNA product, RNAP breaks free of the promoter to yield a transcription elongation complex (TEC) that synthesizes the rest of the RNA product.

Structural studies performed *in vitro* have provided snapshots of the protein-nucleic acid interactions that occur in RPo, in the ITC, and in the TEC for a handful of representative sequences. However, it is unclear whether the structural snapshots identified in these studies provide mechanistic insight into transcription from all sequences or whether these studies provide mechanistic insight into transcription that occurs in living cells, from sequences located on the chromosome.

My research focuses on the development of a multiplexed protein-DNA photo-crosslinking method (Bpa scanning method) to identify changes in RNAP-DNA interactions that occur in transcription and define, for each step of transcription, the sequencedependent variations in RNAP-DNA interactions that modulate RNAP activity.

The method entails formation of transcription complexes *in vivo* using a collection of 88 RNAP derivatives on three subunits (beta, beta' and sigma) that containing a photo-crosslinker p-benzoyl-L-phenylalanine (Bpa) at specific positions. These derivatives formed complexes with 9 different promoters (LacCONS and lambda Pr' derivatives) in transcription initiation and early elongation stages *in vivo*. The interactions of these derivatives and the promoters were investigated by high throughput sequencing of the crosslinking materials. The results showed that the Bpa scanning method was able to define the interactions of RNAP and the DNA promoter at the nucleotide and amino acid resolution during transcription initiation and early elongation in *E. coli*. In principle, these methods can be adapted to interrogate protein-nucleic acid interactions that occur in other biological processes in *E. coli* or in eukaryotes.

Ishita Jain
Severinov Lab

Research Summary

CRISPR–Cas adaptive immune systems protect prokaryotes from phages and other foreign genetic material. These systems comprise of CRISPR (Clustered regularly interspaced short palindromic repeats) arrays of identical repeats separated by unique spacers and *cas* (CRISPR-associated) genes. My research is focused on understanding the interference and adaptation processes in Class 2 systems which rely on single-subunit effectors (type II, V, and VI).

As a predoctoral fellow this year, my aim was to understand if there is *in vivo* trans ssDNA cleavage due to *Francisella novicida* Cas12a (FnCas12a) activity as was shown previously *in vitro*. I used the FnCas12a system heterologously expressed in *E. coli* cells to perform *in vivo* experiments.

I first confirmed the presence of interference (dsDNA *cis* cleavage) by FnCas12a using a transformation efficiency assay of target plasmid. Next, I tested a putative Cas12a ssDNase activity on genomic DNA and its effect on cell growth. I observed plasmid loss caused by Cas12a *cis* activity (cleavage of dstarget DNA). Cell growth results suggested that there was no significant damage of genomic DNA by target activated FnCas12a (ssDNA *trans* cleavage) probably due to protection by single-stranded DNA binding proteins. Then using M13 phage, whose genome is present in both ssDNA and dsDNA forms during the infection cycle, I tested Cas12a cleavage of phage DNA. I measured growth and phage propagation kinetics, to see if M13 phage targeting led to any changes in either growth or plaque counts. There were reduced plaque counts of M13 containing protospacer and PAM due to FnCas12a *cis* cleavage activity. I did not detect any PAM-independent activation of Cas12a ssDNase *trans* activity for M13 containing the protospacer without the functional PAM. Lastly, I wanted to separate FnCas12a *cis* and *trans* cleavage activity (if any). The results suggested that the ssDNA form of wild type M13 is not cleaved *in trans* due to FnCas12a activity. It could be due to inefficient Cas12a *trans* activity or/and protection of M13 ssDNA by single-stranded DNA binding proteins.

The results from this project will contribute towards the main aim of my PhD research – to explore Class 2 CRISPR-Cas systems by comparative analysis of interference and adaptation mechanisms. Overall, my PhD project is focused on investigating target recognition and spacer integration requirements in the RNA-guided RNA-targeting Cas13a system and the DNA-targeting Cas12a and Cas12b systems. Acquiring this data will allow meaningful mechanistic comparisons of these systems and will inform on best ways to use them for practical applications.

Srividya Venkatramanan
Irvine Lab

Research Summary

Investigating the role of LIM proteins in the regulation of Hippo signaling and the cytoskeleton

The Hippo signaling network controls organ growth and cell fate in a wide range of animals, and when dysregulated, can contribute to oncogenesis. Hippo signaling mediates its effects through regulation of the transcriptional co-activator proteins YAP1 and TAZ (Yorkie in *Drosophila*). YAP1 and TAZ (collectively, YAP proteins) are inhibited by Hippo signaling through phosphorylation by the LATS kinases LATS1 and LATS2 (Warts in *Drosophila*). TRIP6 and LIMD1 have each been identified as being required for tension-dependent inhibition of the Hippo pathway LATS kinases and their recruitment to adherens junctions, but the relationship between TRIP6 and LIMD1 was unknown. Using siRNA-mediated gene knockdown we observed that TRIP6 is required for LIMD1 localization to adherens junctions, whereas LIMD1 is not required for TRIP6 localization. TRIP6, but not LIMD1, is also required for recruitment of Vinculin and VASP to adherens junctions. Knockdown of TRIP6 or Vinculin, but not of LIMD1, also influences the localization of phosphorylated myosin light chain and F-actin. In TRIP6 knockdown cells actin stress fibers are lost apically but increased basally, and there is a corresponding increase in recruitment of Vinculin and VASP to basal focal adhesions. Our results thus identify a role for TRIP6 in organizing F-actin and maintaining tension at adherens junctions that could account for its influence on LIMD1 and LATS. They also suggest that focal adhesions and adherens junctions compete for key proteins needed to maintain attachments to contractile F-actin.

Yamei Zuo
Singson Lab

Research Summary

Fertilization is a critical process for the majority of species. Successful fertilization requires a healthy egg and sperm to recognize each other, bind, fuse and form a zygote (Bhakta et al. 2019). However, the molecules and underlying mechanisms for fertilization are still unclear in all species.

In our lab, we use C. elegans to study the molecules that are required for a successful fertilization process. The *spe-13(hc137)* mutation was first discovered over 30 years ago through an EMS mutagenesis screen for C. elegans spermatogenesis-defective mutants on chromosome I by L'Hernault et al. The gene was cloned over ten years ago by Singson lab and *spe-13(hc137)* is a splicing defect mutant with a single nucleotide change in the beginning of the 3rd intron. *Spe-13* was also predicted to be a small transmembrane protein that genetically interacts with *spe-9* gene.

The spe-13 mutant males and hermaphrodites exhibit healthy and normal gonad morphology yet fail to produce healthy embryos and a normal number of progenies. The sperm number and sperm morphology were also shown to be comparable with the wild type. All phenotypic analysis of *spe-13(hc137)* mutant indicates that the gene has a distinguished role in gamete interactions at fertilization. Even though the *spe-13* mutants have severe fertilization defects, *spe-13(hc137)* could still produce an average of 5 progenies per hermaphrodite (vs ~300 to wild type). To determine the phenotype of the *spe-13* null allele for its function, I tested the its mRNA expression in spe-13(hc137) to check if *spe-13(hc137)* can be considered as a null allele. The rtPCR results showed that there was still a comparable amount of *spe-13* mRNA expression in *spe-13(hc137)* to wild type. Furthermore, the results also showed that the *spe-13(hc137)* mutant would produce two types of mRNA due to the splicing defect at the end of the 3rd exon. Therefore, *spe-13(hc137)* cannot be considered as a *spe-13* null allele and we're in the process of ordering a CRISPR knock out null allele strain. With the spe-13 null allele, we would be able to determine the "true" phenotype of losing the *spe-13* gene, which would either be completely sterile or still be a leaky phenotype like *spe-13(hc137)*. To check the localization of SPE-13 protein and its interactions with other sperm or egg proteins, we are planning on creating a CRISPR knock in SPE-13-GFP allele. Right now, we're trying to amplify the DNA to get ready to for injection to create the knock in allele. By gathering all the additional information, we will be able to get into a more detailed picture of the roles *spe-13* plays in fertilization.

Samuel Adeniyi Adeleye
Yadavalli Lab

Research Summary

I study an enzyme, QueE, that plays a role in the Queuosine(Q) biosynthesis and as a cell division inhibitor. Queuosine (Q), a hyper-modified guanosine, is a universally conserved tRNA modification in specific tRNAs' anticodon loop (G34UN). Prokaryotes synthesize Q *de novo* from guanosine triphosphate (GTP), while eukaryotes can only acquire Q from microbial or dietary sources through the salvage pathway. The role of QueE in Q-synthesis is well characterized. However, it was only in the last decade that the new function as an inhibitor of cell division was reported. When *E. coli* cells are exposed to sub-inhibitory levels of cationic antimicrobial peptides, this signal is sensed by a two-component signaling system, broadly conserved among gammaproteobacteria, including *E. coli,* Salmonella*,* and related species. Upon strong activation of the PhoQ/PhoP system by the antimicrobial peptide, cell division is inhibited via upregulation of QueE, which binds to the division complex leading to filamentation. However, the mechanistic details of how QueE inhibits cell division and whether this secondary function is independent of QueE's role in the tRNA modification pathway are unclear. I proposed that QueE is a moonlighting protein whose roles in cell division and Q-biosynthesis are functionally discrete. Using alanine scanning and APB-gel tRNA northern blot analyses, I have identified specific residues that affect either one, both, or neither of the functions, supporting my hypothesis that QueE's roles in tRNA modification and antimicrobial activity peptide stress response are independent of each other. I am currently investigating the binding partners of QueE within the *E. coli* divisome using a combination of biochemical and genetic tools, including affinity tag pull-downs, in vivo cross-linking in tandem with mass spectrometry, bacterial two-hybrid assays, and phenotypic screens. This study will deepen our understanding of how RNA-modification enzymes can potentially act as regulatory links between translation and stress response.

Jason Gregory
Gallavotti Lab

Research Summary

Transcriptional corepressors of the TOPLESS (TPL) family function as master regulators of plant development, encompassing all nine phytohormone pathways. In maize, the TPL family is comprised of four members, RAMOSA1 ENHANCER LOCUS2 (REL2), REL2-LIKE1 (RELK1), REL2-LIKE2 (RELK2), and REL2-LIKE3 (RELK3). Prior characterization of rel2 mutants revealed pleiotropic vegetative and reproductive phenotypes such as defective axillary meristem initiation and inflorescence meristem (IM) maintenance in single recessive mutants (Liu et al, Plant Phys 179:348-363, 2019). Recently, the mutant short upright (su) was identified in an unbiased EMS mutagenesis screen for rel2 enhancers. Bulked Segregant Whole Genome Sequence analysis was performed and identified RELK1 as the causal gene in two independent alleles. Double rel2;relk1 mutant plants appear shorter with very upright tassel branches and enhanced ear fasciation. Expression analysis in rel2 mutant inflorescences indicates that RELK1 is strongly upregulated, suggesting a buffering mechanism between the two family members in agreement with the genetic enhancement. Additionally, preliminary data from CRISPR knock-out lines of both RELK2 and RELK3, two closely duplicated genes co-orthologous to Arabidopsis TPL, revealed a branchless phenotype in rel2;relk mutant tassels. This analysis suggests that functional diversification occurred in the maize TPL family of transcriptional corepressors. We are currently investigating the role of each family member in maize development, and in particular in the regulation of the size of ear inflorescence meristems as it directly impacts grain yield.

WAKSMAN FACULTY COURSES
Undergraduate and Graduate

Undergraduate
Undergraduate students from departments in the School of Arts and Sciences (SAS) and the School of Environmental and Biological Sciences (SEBS) are trained in a state of the art molecular biology research laboratory. Most of the faculty take the students into their laboratories to perform independent research projects through the summer and academic year. Many of these students go on to receive the Waksman Undergraduate Research Fellowship to support their research efforts.

Graduate
Graduate students from a wide range of programs conduct their Ph.D. or M.S. dissertation research projects with faculty at the Waksman Institute and are eligible for the Busch Graduate Fellowship Program to support their thesis research. Predoctoral candidates can be funded a maximum of four years, while postdoctoral applicants can be funded for one year. Core Ph.D. courses for the Molecular Biosciences Program, along with numerous upper level lecture and seminar courses, are taught at the Waksman Institute each semester.

Faculty-Taught Graduate and Undergraduate Courses 2020-2021

Advanced Plant Genetics
Chemical Biology Of Transcription
Chemical Bonding
Core Seminars In Plant Biology
Genetic Analysis I
Genetic Analysis II
Genetics And Cell Biology Of Fertilization
Introduction To Research In Genetics
Microbial Biochemistry
Molecular Biology And Biochemistry
Molecular Biosciences
Molecular Genetics
Structural Biology, Structural Biophysics
Thesis Writing And Communication In Genetics

WAKSMAN STUDENT SCHOLARS PROGRAM
High School Outreach

Summary
With the emergence of the cyberinfrastruture in molecular biology over the past years, there has been a "revolution" in modern research that parallels the physics revolution that occurred at the turn of the 20th century. Molecular biology, evolution, genomics, and bioinformatics are rapidly growing disciplines that are changing the way we live and our understanding of how the world functions. To compete successfully in the global economy, the United States needs to be at the forefront of technology and science. This will require a citizenry that is technologically literate and capable of contributing to, and making use of, this cyberinfrastructure. Additionally, a fundamental understanding of the basic underlying principles of modern biology will be required to make informed choices about scientific issues.

For 28 years, faculty and scientists at the Waksman Institute have collaborated with high school teachers and their school administrators in an effort to address these issues. Our strategy has been to engage high school students and their teachers in authentic scientific research, in an effort to bridge the gap between how scientific research is conducted versus how science is taught.

The Waksman Student Scholars Program (WSSP) is designed to connect high schools with the research community at Rutgers by encouraging teachers and students to engage in a genuine research project in molecular biology and bioinformatics. Its primary goal is to develop a research climate in the schools by establishing, supporting, and sustaining on-going interactions among research scientists and teams of high school students and teachers.

The 2020-2021 WSSP consisted of two interrelated parts: a Summer Institute (SI) and an Academic Year Program (AYP). Due to the pandemic most of these activities were conducted using an on-line, virtual, format. In July 2020, 53 students from 31 different schools participated in a one-week virtual SI. The SI program used a "flipped classroom" structure. Initially, students independently viewed videos or read notes that provided background information on molecular biology and bioinformatics and steps of the WSSP research project, then took quizzes to help them gauge their understanding of the material. Students then participated in four daily discussion seminars that went more in depth into the research project and addressed any questions they had. Students were provided with data generated by the WSSP staff and used Internet resources to process and analyze their data.

After the SI, teachers and students returned to their schools and recruited additional students who contributed to the research project during the academic year. Some teachers incorporated the project into existing research courses or advanced placement biology courses at their schools. Others conducted the project as after school clubs. These courses and clubs provided additional students beyond those who attended the SI with opportunities to conduct and contribute to the research project. During the fall of 2020, teachers from most of the WSSP schools conducted the project virtually using online resources provided by project faculty.

In addition to the activities based at the Waksman Institute, the WSSP also supported the program at sites beyond the central New Jersey region. Dr. Forrest Spencer at John Hopkins University, in Baltimore MD helped run the AYP for two high schools in MD. Working with the Lawrence Livermore National Laboratory, Livermore, CA, students from three schools in CA and two schools in HI participated in the program.

Due to the pandemic many of the WSSP clubs were cancelled and the laboratory activities for many of the classes were not held. As a result, the number of students participating in the WSSP project dropped from 1215 during the 2019-2020 AYP to 917 from 41 different high schools in the 2020-2021 AYP. 14 schools conducted the in-person laboratory experiments this last year. The WSSP provided the reagents and supplies for these schools to conduct the experiments. Schools that did not conduct in person laboratory activities were provided with novel DNA sequence

data that was generated by the WSSP staff for each participating student to analyze on-line.

The Research Question

The 2020-2021 research project focused on identifying the genes and proteins of the duckweed, *Landoltia punctata.* Duckweeds are fresh-water aquatic plants that is used in bioremediation and can be potentially used for biofuel. Several research laboratories at Rutgers are currently investigating these plants.

To conduct the project, a plasmid cDNA library from *Landoltia punctata* was prepared by the WSSP staff. If the schools conducted the in-person laboratory activities, students grew bacterial cultures of individual clones from the cDNA library, performed minipreps of the plasmid DNA, cut the DNA with restriction endonucleases, performed polymerase chain reactions, analyzed DNA electrophoretically, had inserts from their clones sequenced, and analyzed these sequences with the aid of a computer program called the DNA Sequence Analysis Program (DSAP) that was developed by the project director and faculty. Students who did not conduct the laboratory experiments were each provided with two sequences that were generated by the WSSP staff. A total of 1122 DNA sequences were analyzed by the students in the 2020-2021 SIs and AYPs. 619 of these sequences have been or will be submitted for publication on the National Center for Biotechnology Information (NCBI) DNA sequence database citing the students' names as contributing authors.

WISE

The WSSP project involves both students and teachers conducting research at their high schools during the academic year. Since involvement in the yearlong program requires the participation from a teacher who has attended a WSSP SI and the support of the schools, many students from schools that are not involved in the program cannot conduct the research project. To accommodate these students, we offered summer programs called **Waksman Institute Summer Experience (WISE)** in which students perform the same research project as conducted in the WSSP. Due to the pandemic, these programs were all held on-line, in the same manner as the WSSP SI. One advantage to conducting a virtual WISE program (vWISE) is that we were not limited to the number of students who could attend the Program by the number of students who could work safely in the laboratory. As a result, we were able to increase the number of participants from 36 during previous in-person WISE programs to 67 in June 2020, 64 in August 2020, and 93 in January 2021. Students from states across the US along with several other nations were able to participate in these programs. The summer vWISE programs were conducted over a one-week period with 4 discussion sessions each day. The January vWISE program was conducted in two nightly sessions each week over a nine-week period. Students were each provided two DNA sequences and the vWISE students completed the analysis of 408 of these sequences and 364 were published on the NCBI database.

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PRESENTATIONS & MEETING ABSTRACTS

Waksman Institute Hosted Seminars
David Jaciuch, PhD, Centre for Cognition in Small Brains, University of Sheffield: Selective Tau protein expression in different clock circuits of the Drosophila brain disrupts different aspects of activity/sleep-rhythms

Robert Stedfeld. PhD, Sr. Scientist, Benlai Chai, PhD, Bioinformatics Scientist, Nada Abdel, MSc, Sr. Business Development Manager: Rapid, Sensitive and More Accurate Metagenomic Solutions: From Targeted Amplicon Panels to Direct Sequencing

Monica Dus, PhD, University of Michagan, Ann Arbor: Persistent Epigenetic reprogramming of Taste by Diet

Richard Ebright, PhD, Waksman Institute and Department of Chemistry and Chemical Biology: Structural basis of Transcription-translation Coupling

Monica Dus, PhD, University of Michagan, Ann Arbor: Persistent Epigenetic Reprogramming of Taste by Diet

Avital Rodal, PhD, Brandeis University: Routing and Remodeling Membranes at the Synapse

Witkin Symposium: Multiple Speakers: Dr. Evelyn Witkin, 100th Birthday and Research Accomplishments

Dr. Li-Qing Chen, Assistant Professor, University of Illinois at Urbana-Champaign: Local Glucose availability determines seed germination under ABA treatment

Waksman Institute Sleep Seminar: Nicholas Stavropoulos, Assistant Professor, NYU Neuroscience Institute and Department of Neuroscience and Physiology: What can our evolutionary cousins teach us about the regulation of sleep?

Waksman Student Scholars Programs
Virtual Waksman Student Scholars Program Summer Institute (vWSSP-SI), July 6-July 10, 2020, 53 students.

Virtual Waksman Institute Summer Experience (vWISE June-20), June 22-June 26, 2020, 67 students.

Virtual Waksman Institute Summer Experience (vWISE August-20), July 27-July 31, 2020, 64 students.

Virtual Waksman Institute Summer Experience (vWISE January-21), January 25-March 24, 2021, 93 students

Barber:
University of Pennsylvania Chronobiology and Sleep Institute Clock Meeting, Dec. 2020

University of Michigan Kavli Neuroscience Scholars Invited Speaker, Jan. 2021

Fong SY, Kolesnik A, Fetchko M, Gallagher P, Barber AF, Sehgal A. (2021) Drosophila clock cells use multiple mechanisms to transmit time-of-day signals in the brain. Poster presented at Genetics Society of America Drosophila virtual meeting

Dong:
Seminar (Virtual) at the College of Life Science, China Agricultural University, Beijing, China
Title: Polarity factors and regulators in plant asymmetric cell division.

Seminar (Virtual) at the Biology Department, University of Massachusetts, Amherst, MA
Title: Polarity factors and regulators in plant asymmetric cell division.

Seminar (Virtual) at the Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee, Knoxville
Title: Polarity factors and regulators in plant asymmetric cell division.

The 2020 Plant Biology Worldwide Summit (Virtual), American Society of Plant Biologists
Title: A polarized molecular switch for plant asymmetric cell division.

Seminar at the Symposium for Plant Molecular Biology, Peking University
Title: A polarized molecular switch for plant asymmetric cell division.

Seminar at the Department of Biology, McGill University, Montreal, Canada
Title: Polarity factors and regulators in plant asymmetric cell division.

Seminar at the Biology Department, University of Massachusetts, Amherst, MA
Title: Polarity factors and regulators in plant asymmetric cell division.

Dismukes:
Anika F Jalil, Development of Falling Thin Film Liquid Phase Electrolyzer for CO2 Reduction, May 2020.

Kyra Yap, Transition Metal Phosphides for the Electrochemical Reduction of CO2, May 2020.

Kyle Mani, Bridging the gap between probabilistic (Markov) and kinetic (ODE) models of photosynthetic electron transport within Photosystem II, May 2021

Ebright:
"Therapeutics for drug-resistant bacteria: myxopyronins," CARB-X Global Antimicrobial Partnership Advisory Board Meeting, Boston University, Boston Massachusetts, 2020 (remote).

"RNA polymerase: the molecular machine of transcription." International Union of Microbiological Societies Congress, Daejon, South Korea, 2020 (plenary address; remote).

"RNA polymerase: the molecular machine of transcription." International Union of Microbiological Societies Congress, Daejon, South Korea, 2020 (plenary address; remote).

"Therapeutics for drug-resistant bacteria: arylmyxopyronins." Centers of Excellence in Translational Research Review Meeting, National Institute of Allergy and Infectious Diseases, Rockville Maryland, 2021 (remote).

"Antibacterial drug discovery targeting bacterial RNA polymerase: myxopyronin (Myx)," Department of Chemistry, Clarkson University, Potsdam New York, 2021 (remote).

"Structural basis of transcription-translation coupling." Institute for Quantitative Biomedicine, Rutgers University, Piscataway, New Jersey, 2021 (remote).

"Structural basis of transcription-translation coupling." Tianfu Medical Microbiology Forum, Chengdu Medical College, Chengdu, China, 2021 (keynote address; remote).
"Structural basis of transcription-translation coupling." Stanford-SLAC Cryo-EM Center, Stanford University, Palo Alto, California, 2021 (remote).

"Structural basis of transcription-translation coupling." Institute for Structural and Molecular Biology Annual Meeting, University College, London, United Kingdom, 2021 (keynote; remote).

Wang, C., Molodtsov, V., Winkerman, J., Firlar, E., Kaelber, J., Blaha, G., Su, M., Nickels, B., and Ebright, R.H. (2020) Structural basis of bacterial transcription-translation coupling. RNA polymerase: the molecular machine of transcription. International Union of Microbiological Societies Congress, Daejon, South Korea, 2020, November 16 19, 2020.

Mandal, S., Feng, Y. Ebright, Y., Li, S., Wilde, R., Mandal, S., Jiang, Y., Chatterjee, S., Srivastava, A., Degen, D., Talaue, M., Connell, N., Zimmerman, M., Dartois, V., Mustaev, A., Lucumi, E., Diamond, S., Cooper, C., Mdluli, K., Fotouhi, N., Ma, Z., Kaneko, T., and Ebright, R.H. (2015) Novel small-molecule inhibitors of Mycobacterium tuberculosis RNA polymerase: Nα-aroyl-N-aryl-phenylalaninamides (AAPs). British Society for Antimicrobial Chemotherapy Annual Meeting. Helmholtz Institute for Pharmaceutical Research, Saarland, Germany, February 2-3, 2021 (virtual).

Fermentation:
Lagda, AC. "New Jersey Core Facilities Showcase" Engage 2020, Princeton University, Princeton, New Jersey. November 04, 2020

Lagda, AC. "ABRF Core Administration Virtual Town Hall-How to Leverage Core Marketplace and RRIFDs to Raise Awareness of Your Core Facility" April 21, 2021.

Rodriguez, A., Druzhinin, S., Cloud, A. and Lagda, AC. "Current Good Manufacturing (cGMP) Practices according to 21 CFR 117" conducted by the Food Innovation Center, Rutgers University. April 26-27, 2021.

Gallavotti:
Gallavotti, A. Genetic and genomic dissection of maize inflorescence architecture. Northeast Regional Meeting of the Society for Developmental Biology, April 9-10, 2021.

Gallavotti, A. Genetic and genomic dissection of maize inflorescence architecture. University of California Riverside. December 4, 2020.

Gallavotti, A. Genetic and genomic dissection of maize inflorescence architecture. Cornell University, College of Agriculture and Life Sciences, November 13, 2020.

Dong, Z., Xiao, Y., Chau, J., Galli, M., Gallavotti, A., Whipple, C., Chuck, G. The tasselsheath4 gene establishes developmental fields within floral phytomers via microRNA mediated mutual repression. Maize Genetics Conference Abstract 63:T12. March 8-12, 2021 (online conference).

Chen, Z., Li, W., Gaines, C., Buck, A., Galli, M., Gallavotti, A. Structural variation at the maize WUSCHEL1 locus alters stem cell organization in inflorescences. Maize Genetics Conference Abstract 63:T14. March 8-12, 2021 (online conference).

Griffin, B., Montes-Serey. C., Galli, M., Gallavotti, A., Walley, J. REL2 acetylation in plant pathogen interactions. Maize Genetics Conference Abstract 63:P145. March 8-12, 2021 (online conference).

Gregory, J., Liu, X., Gallavotti, A. Functional dissection of the REL2 corepressor family. Maize Genetics Conference Abstract 63:P181. March 8-12, 2021 (online conference).

Galli, M., Chen, Z., Li, M., Marand, A., Earp, T., Lu, Z., Krogan, N., Schmitz, R.J., Huang, S.C., Gallavotti, A. Mining cis-regulatory modules in the maize genome. Maize Genetics Conference Abstract 63:P190. March 8-12, 2021 (online conference).

Irvine:
May 11, 2021 Seminar (online), "Biomechanical regulation of organ growth", at Cal Tech, Pasadena CA

April 21, 2021 Seminar (online), "Biomechanical regulation of organ growth", at CINJ, New Brunswick, NJ

Feb 24, 2021 Seminar (online), "Biomechanical regulation of organ growth", at Oregon State University, Oregon

Feb 26, 2021 Seminar (online), "Biomechanical regulation of organ growth", at UMass Boston, MA

June 22-26 2020 Workshop (online) on Molecular Biology & Development of Drosophila

Maliga:
Matsuoka, A. and Maliga, P. "Prospects for reengineering Agrobacterium for T-DNA delivery to chloroplasts." Joint MA-ASPB / University of Maryland Plant Virtual Symposium, May 27-28, 2021.

Matsuoka, A. and Maliga, P. "Prospects for reengineering Agrobacterium for T-DNA delivery to chloroplasts." PB21 Plant Biology Worldwide Summit (online), July 19-23, 2021.

Rongo:
Rongo, C. Title: Uncovering Novel Mechanisms of Hypoxic Stress Response Using C. elegans. Presented at the Department of Biological Sciences, Seton Hall University, Apr. 15, 2021.

Singson:
The Genetics Society of America, The Allied Genetics Conference
Presented by Amber Krauchunas: SPE-36 is an EGF-motif containing secreted sperm protein required for fertilization in C. elegans.

Rutgers University School of Arts and Sciences Honors Program Colloquium "A fertility clinic for worms"

Yadavalli:
An epitranscriptomic enzyme moonlights in bacterial stress response. Evelyn M. Witkin 100th Birthday Symposium, Rutgers University, April 2021.

Small proteins and epitranscriptomic factors: Emerging regulators of bacterial gene expression. Division of Life Sciences – New Faculty Symposium, Rutgers University, April 2021.

Photo cross-linking mass spectrometry-based mapping of small protein interactions. Small Proteins, Big Questions: Virtual Conference on Prokaryotic Small Proteins, January 2021.

PATENTS & PUBLICATIONS

Patents

Dismukes:
Lewis/Bronsted acid/base co-catalysts for direct electrochemical CO2 reduction to hydrocarbons on nickel phosphides, Dismukes, G., A. Laursen, and K. Calvinho, USPTO, Filed May 2021.

Nickel phosphide catalysts for direct electrochemical CO2 reduction to hydrocarbons. Issued 2020, Dismukes, G.C., A. Laursen, M. Greenblatt and K. Calvinho, US Patent & Trademark Office 10,676,833.

Publications:

Barber:
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Barber AF, Fong SY, Kolesnik A, Fetchko M, Sehgal A. (2021) Drosophila clock cells use multiple mechanisms to transmit time-of-day signals in the brain. Proc Nat Acad Sci USA 118: e2019826118

Dismukes:
Bridging the gap between Kok-type and kinetic models of photosynthetic electron transport within Photosystem II. Mani, K., A. Zournas, and G.C. Dismukes, Photosynth Res, 2021. https://doi.org/10.1007/s11120-021-00868-6

CO2 electro-reduction on Cu3P: Role of Cu(I) oxidation state and surface facet structure in C1-formate production and H2 selectivity. Laursen, A.B., et al., Electrochimica acta, 2021. DOI: 10.1016/j.electacta.2021.138889

Symbiosis extended: exchange of photosynthetic O2 and CO2 mutually power metabolism of lichen symbionts. ten Veldhuis, M.-C., G. Ananyev and G.C. Dismukes, Photosynth Res 143, 287–299 (2020). https://doi.org/10.1007/s11120-019-00702-0

Water and Vapor Transport in Algal-Fungal Lichen: Modeling constrained by Laboratory Experiments, an application for Flavoparmelia caperata. Potkay, A., M.C. Ten Veldhuis, Y. Fan, C.R.C. Mattos, G. Ananyev and G.C. Dismukes, Plant Cell Environ, 2020. 43(4): p. 945-964. DOI: 10.1111/pce.13690

Realtime kinetics of the light driven steps of photosynthetic water oxidation in living organisms by "stroboscopic" fluorometry. Gates, C., G. Ananyev and G.C. Dismukes, Biochim Biophys Acta Bioenerg, 2020. 1861(8): p. 148212. 10.1016/j.bbabio.2020.148212

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Xue X, Bian C, Guo X, Di R and Dong J#. (2020) The MAPK substrate MASS proteins regulate stomatal development in Arabidopsis. Plos Gen. 16(4):e1008706. doi: 10.1371/journal.pgen.1008706.

Yang K#, Wang L, Le J, Dong J#. (2020) Cell polarity: Regulators and mechanisms in plants. J Integr Plant Biol. 62(1):132-147. doi: 10.1111/jipb.12904.

Ebright:
Mazumder, A., Lin, M., Kapanidis, A., and Ebright, R.H. (2020) Closing and opening of the RNA polymerase trigger loop. Proc. Natl. Acad. Sci. USA 117. 15642–15649.

Wang, C., Molodtsov, V., Firlar, E., Kaelber, J., Blaha, G., Su, M., and Ebright, R.H. (2020) Structural basis of transcription-translation coupling. Science 369, 1359-1365.

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Mazumder, A., Wang, A., Uhm, H., Ebright, R.H., and Kapanidis, A. (2021) RNA polymerase clamp conformational dynamics: long-lived states and modulation by crowding, cations, and nonspecific DNA binding. Nucl. Acids Res. (in press) (https://doi.org/10.1093/nar/gkab074).

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Gallavotti:
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Irvine:
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Venkatramanan, S., Ibar, C., Irvine, K.D. TRIP6 is required for tension at adherens junctions. J. Cell Science 134, jcs.247866

Maliga:
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Maliga P, Tungsuchat-Huang T, Lutz KA, 2021. Transformation of the Plastid Genome in Tobacco: The Model System for Chloroplast Genome Engineering. Methods Mol Biol 2317, 135-53.

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McKim:
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Wang, L. I., T. DeFosse, J. K. Jang, R. A. Battaglia, V. F. Wagner et al., 2021 Borealin directs recruitment of the CPC to oocyte chromosomes and movement to the microtubules. J Cell Biol 220.

Nickels:
Ssl2/TFIIH function in Transcription Start Site Scanning by RNA Polymerase II in Saccharomyces cerevisiae Tingting Zhao T, Vvedenskaya IO, Lai W, Basu S, Pugh BF, Nickels BE, and Kaplan CD.
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The structural and mechanistic basis of RNA extension in reiterative transcription initiation:
RNA slipping without DNA scrunching. Liu Y, Winkelman JT, Yu L, Pukhrambam C, Zhang Yu, Nickels BE, and Ebright RH. (2021) bioRxiv

Promoter-sequence determinants and structural basis of primer dependent transcription initiation in Escherichia coli. Skalenko KS, Li L, Zhang Y, Vvedenskaya IO, Winkelman JT, Cope A, Taylor DM, Shah P, Ebright RH, Kinney JB, Zhang Y and Nickels BE.
Proc. Natl. Acad. Sci. U.S.A. (2021) Jul 6;118(27):e2106388118.

A spatiotemporal molecular switch governs plant asymmetric cell division. Guo X, Park C-H, Wang Z-Y, Nickels BE, and Dong J.
Nature Plants (2021) May;7(5):667-680. doi: 10.1038/s41477-021-00906-0.

Rongo:
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Ovchinnikov, S., Bikmetov, D., Livenskyi, A., Serebryakova, M., Wilcox, B., Mangano, K., Shiriaev, D. I., Osterman, I. A., Sergiev, P. V., Borukhov, S., Vazquez-Laslop, N., Mankin, A. S., Severinov, K., and Dubiley, S. (2020) Mechanism of translation inhibition by type II GNAT toxin AtaT2. Nucleic Acids Res., 48, 8617-8625

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