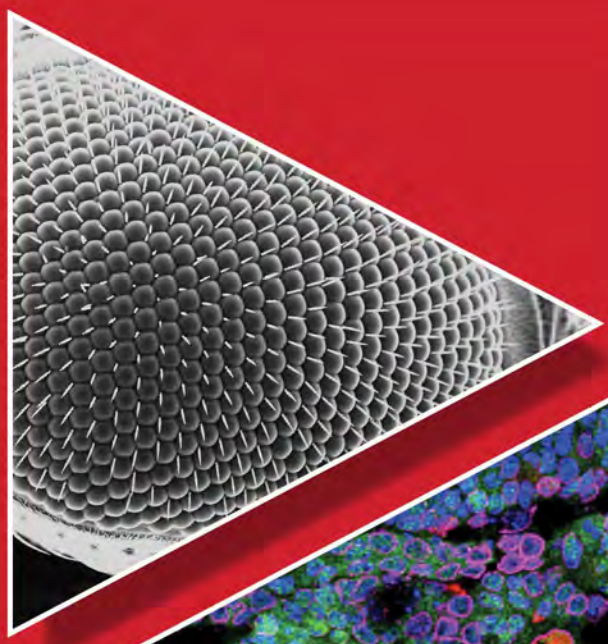

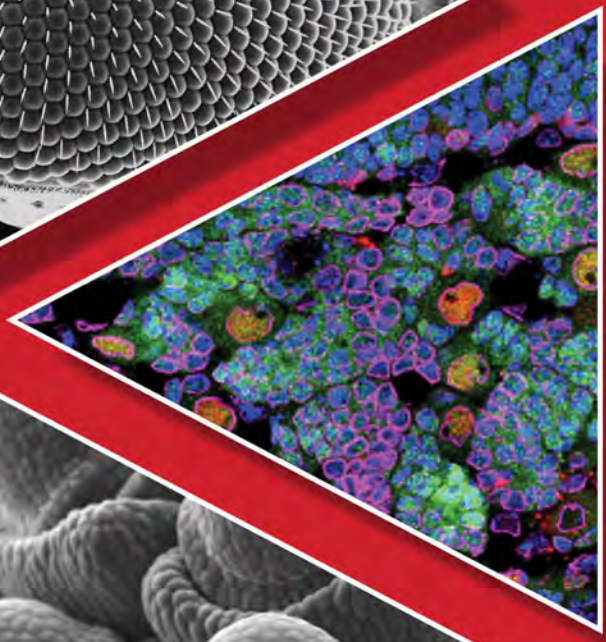

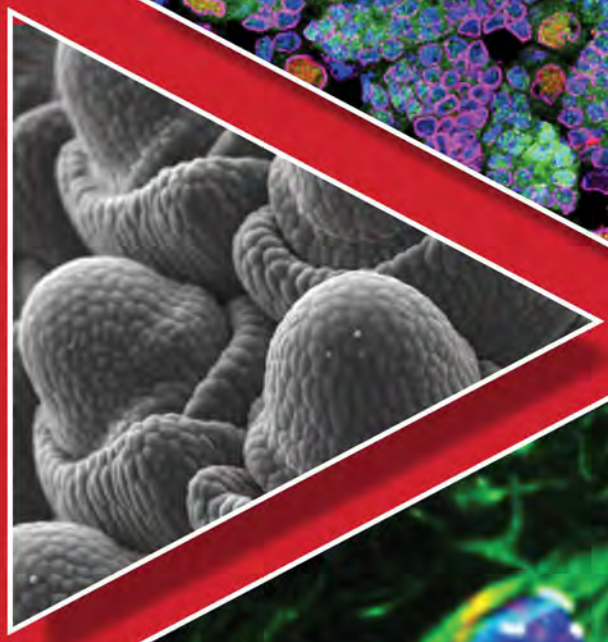

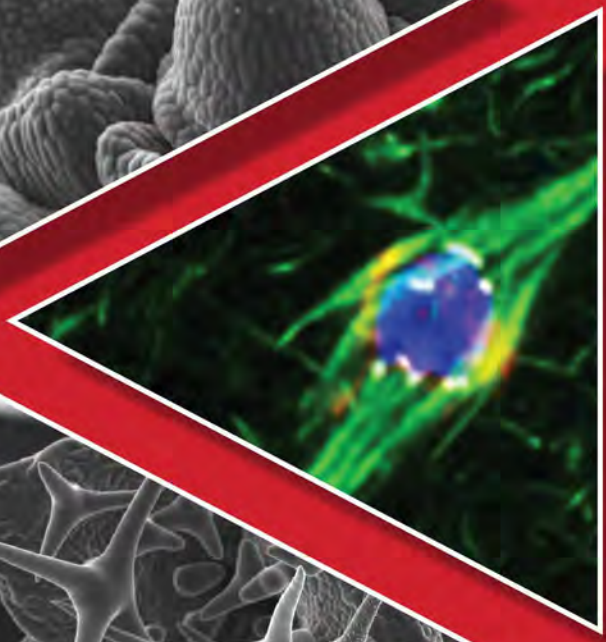

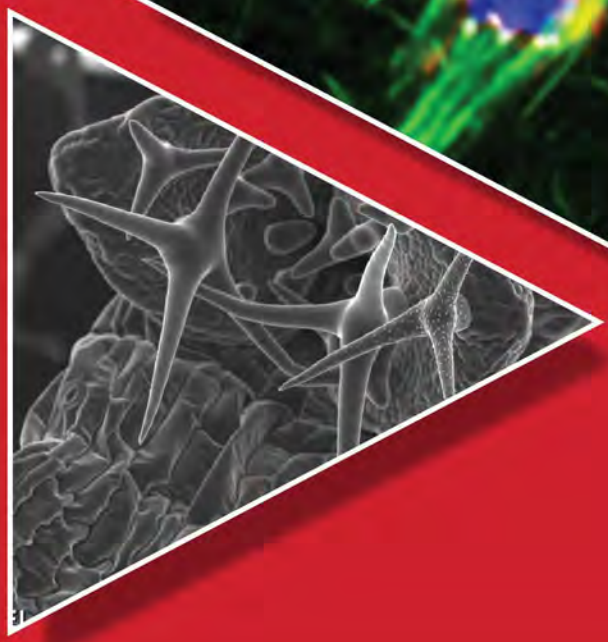

# 2019
# 2020

## WAKSMAN INSTITUTE OF MICROBIOLOGY

RUTGERS

# 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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Juan Dong
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Andrea Gallavotti
Kenneth Irvine
Pal Maliga
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Bryce Nickels
Christopher Rongo
Konstantin Severinov
Andrew Singson
Ruth Steward
Andrew K. Vershon
Srujana Samhita Yadavalli

## Non-Resident Professors

Robert Goodman

## Professors Emeriti

Richard W. Padgett
Ekkehard Bautz
Hugo Dooner
David Pramer
Robert W. Simpson
William Sofer
Evelyn Witkin

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**Robert Rossi**

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Michelle Phillips, Senior Administrative Assistant
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Erin Sorge, Grant Proposal Assistant
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Randy Newman, Core Facilities, Support Services and Information Technology Director
Daja O'Bryant, Unit Computing Specialist
Hazel Schubert, System Administrator

## Core Facilities

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Arvin Lagda, Fermentation Facility
Nanci Kane, Confocal Imaging Core Facility
Themios Chionis, Greenhouse Supervisor
Marina Druzhinina, Lab Technician
Nathan Hill, Lab Technician

## Support Services

Rosita Law, Principal Lab Technician
Shammara Scott, Principal Lab Technician
Ted Przybylski, Instrument Maker/Repairer



## 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.

## RREF Committee Members 2019-2020

Mary DiMartino
Teresa Dolan
M. Wilma Harris
Roberta Kanarick
Carole Sampson-Landers
Jhaveri Nimesh
Edgar Torres
Ronald Wilson

## Executive Officers:

Dr. Christopher Molloy Interim Chancellor- New Brunswick for Dr. Robert Barchi President & Chair
Ernie DiSandro, Interim Controller for J. Michael Gower, Executive Vice President of Finance and Administration and Treasurer of the University
Dr. Prabhas Moghe, Provost
Kenneth Irvine, Interim Director
Elizabeth Minott, Esq., Legal Counsel & Secretary
Robert Rossi, Executive Director

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Cross Section Through Drosophila Brain, Hiep Tran; SEM Spikelet Meristems in Maize Ears, Andrea Gallavotti;
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# REPORT OF THE INTERIM DIRECTOR

*Kenneth Irvine*

This has been a challenging year for the Waksman Institute. In Sept 2019 our long-time Director Jo Messing passed away unexpectedly. His sudden passing left a void in the Institute, and he is greatly missed. Over more than three decades as Institute Director, Jo influenced all aspects of the Institute, and hired all of the current faculty and staff. A tribute to Jo and his contributions to science and to Rutgers was included in last year's Annual Report. Expansion of the Institute's research space was a long-time goal for Jo, and in November 2019 an extension to the New Wing of the Institute was completed. This extension includes three large labs on three floors, plus additional space for environmental rooms, equipment, and meetings. It's unfortunate that he wasn't able to see it completed, but this new space is certainly a part of his legacy.

One highlight of the past year was the successful recruitment of a new faculty colleague. We were able to continue our search with the Department of Plant Biology and Pathology for a new hire to replace the joint faculty position held by the recently retired Hugo Dooner. Our search committee was co-chaired by Juan Dong, and Andrea Gallavotti. Through their efforts, we were successful in recruiting Mark Zander as a new tenure-track Assistant Professor. Dr. Zander comes to us from the Salk Institute in San Diego, and will be joining the Institute on January 1st, 2021. He investigates stress response in plants, including the response to altered temperatures, and how this affects immune response and growth. We also had a number of seminars, and our annual scientific retreat, hosted by the Institute, details of which are listed elsewhere in this report.

In March 2020 the Covid-19 pandemic hit New Jersey, and most of our research ground to a halt. As I write this in July 2020, research has been restarted, but in a partly empty building, as we can only operate at 50% density at this time. All of our in-person meetings, both scientific and social, have been suspended. We are especially saddened to note that much of our efforts in research education had to be suspended this summer, as no undergraduate students are allowed to conduct in-person lab research, and the Waksman Student Scholars Program could not run its usual summer lab research courses. Although Waksman does not have scientists directly engaged in Covid-19 related research, we have found ways to contribute to the global efforts to fight this disease. Our pilot plant has produced materials for Sars-CoV-2 antibody testing. We provided some of our new lab space to RUCDR to enable them to scale up their saliva test for Sars-CoV-2, to the benefit of Rutgers and the State of New Jersey. Waksman Institute members have also volunteered to help participate in conducting key steps in this testing.

A search for a new permanent director of the Waksman Institute was planned to begin this Spring, but was suspended due to the pandemic. However, an outstanding search committee has been assembled, and we anticipate that the search will begin once it is safe to travel and assemble in groups.

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.

## Overview of the Waksman Institute

**Mission**

The Waksman Institute's mission is to conduct fundamental research in the life sciences and to develop novel bio-

technologies to push the frontiers of scientific discovery. The Institute is also a catalyst for interdisciplinary university initiatives, supports life science infrastructure, and supports research education for undergraduate, graduate, and high school outreach students.

**Background**

The principal mission of the Waksman Institute is research. Although the initial emphasis of the institute at its founding was microbiology, its focus soon turned towards molecular genetics, and was later broadened to include multicellular organisms. While our founding director said at the opening of the Institute: "This Institute will devote its efforts to the study of the smallest forms of life, the microbes, wherever they are found and no matter what their activities may be," he also appreciated the dynamics of all scientific endeavors by saying: "Let this Institute serve as a center where scientists from all parts of the world may gather to work, to learn, and to teach. These Halls are dedicated to the free pursuit of scientific knowledge for the benefit of all mankind." This freedom in scientific research had enabled the members of the Institute to push the frontiers of scientific knowledge today to new levels from better nutrition to drug-resistance of infectious diseases, from cancer to birth defects. The Institute's research mission has thus evolved over almost 70 years from its initial focus on microbiology and antibiotic discovery. The Institute now supports investigations into a broad range of fundamental questions in biology. Using microbial, plant, and animal models, Waksman scientists conduct research on morphogenesis, gene regulation, signal transduction, microbial metabolism, renewable energy, cancer, fertility, and congenital and neurologic disorders, together with antibiotic discovery. A key aspect of current research in the Institute is its interdisciplinary nature, including faculty from multiple departments and schools, and incorporating approaches from physical and computational sciences together with life sciences research.

To apply advances in scientific knowledge to the benefit of mankind, the Institute continues where appropriate to seek practical and commercially viable applications of its discoveries. Historically, the institute owes its existence to the symbiotic relationship that exists between academic research institutions and the private sector. In 1939 Dr. Selman Waksman, the institute's founder and namesake, entered into an agreement with Merck & Company of Rahway, New Jersey, to study the production of antimicrobial agents by soil bacteria. Within three years, streptomycin, the first effective antibiotic against tuberculosis, was discovered by his student Albert Shatz, patented, and licensed to the pharmaceutical industry by Rutgers University. Through the patent of streptomycin, and other antibiotics discovered in Dr. Waksman's laboratories, Rutgers received approximately $16 million in royalties, which was used, in part, to build and endow the Institute.

**Organization and faculty**

The Waksman Institute is a research institute of the New Brunswick campus of Rutgers, The State University of New Jersey. The Institute reports to the Chancellor of the New Brunswick campus and receives a budget from the Chancellor's office to support the appointment of faculty, whose salary is split with the academic departments where they hold their tenure. This joint recruitment with different departments on campus facilitates faculty appointments in different disciplines and enriches the interdisciplinary research unique to the institute. The departments simultaneously receive enhanced research, instructional and service programs from their jointly-appointed Waksman-resident faculty. The faculty of the Institute also participate in multiple graduate programs, and are fully integrated into the University.

In the academic year 2019-2020, 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 111, which does not include the forty-four undergraduate students that did independent research during the last year.

Among the Waksman-resident faculty, five are in the Department of Molecular Biology and Biochemistry, five are in the Department of Genetics, three 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 fifteen current resident members, two 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 election of Pal Maliga as a full member of the Sigma Xi Scientific Research Honor Society - congratulations Pal!

To support its diverse research activities, the Waksman Institute maintains core facilities and support services, overseen by Randy Newman and Arvin Lagda. Descriptions of the facilities and services provide by each of these units are included elsewhere in this annual report.

**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 $5.6 million dollars in external grants. In addition, we received $780,000 from a patent settlement, for a total of $6.4 million in external funding. Our external funding has dropped over the past few years from an average of about $9 million, with the loss of senior faculty from Waksman including Hugo Dooner, Rick Padgett, Maureen Barr and Jo Messing. We expect this trend to reverse in the future as we continue to hire new faculty and they begin to secure external funding.

# 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.

**Robert Rossi**
**Executive Director for**
**Administration and Finance**
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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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**Information Technology Director**
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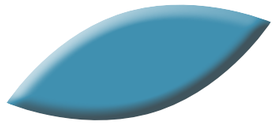
Daja O'Bryant, Unit Computing Specialist
Hazel Schubert, System Administrator

# ADVANCING OUR RESEARCH

**Animal Labs**



**Microbial Labs**

**Plant & Photosynthetic Labs**

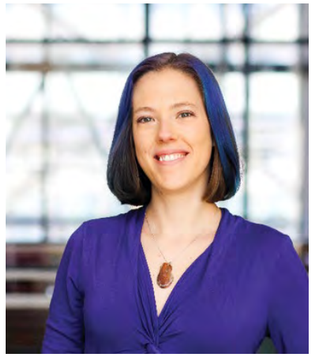
**Core Facilities**

**Support Services**



# BARBER LAB

## The molecular basis of neural signal integration in Drosophila circadian circuits



**Dr. Annika Barber**
**Molecular Biology & Biochemistry**

**Summary**
Annika Barber joined the Waksman Institute as a new Assistant Professor in January 2020. The Barber lab uses the fruit fly, *Drosophila melanogaster*, to investigate how neuronal networks integrate sensory signals using multiple molecular signals to select appropriate behavioral programs. Organisms must make behavioral decisions based on an array of both internal state cues (like hunger or time of day) and external environmental cues (like availability of food or temperature). How daily environmental cues such as time and internal drives such as hunger are coordinated to regulate behavior is poorly understood. Understanding the integration of time-of-day and nutritional state information is important to human health. For example, obesity and insulin resistance are associated with disrupted circadian activity patterns and feeding habits. Projects in the Barber lab are funded in part by NIH-NINDS R00 NS105942, Budget Period: 07/01/2020 - 06/30/2023

**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. An exciting exploratory arm of this project uses single-cell RNA sequencing of *Drosophila* neurons to identify new PI peptidergic populations regulating sleep, feeding and circadian locomotor rhythms. Using Drosophila allows detailed analysis of how diverse signals integrate within the PI at the molecular and electrophysiological level to influence behavioral choice under different environmental conditions.

**Fundamental mechanisms of neural signaling**
If we trace all the synaptic connections in the brain to generate a "map" of how neurons communicate, will we "understand the brain"? No. Brains are not computers, and organisms are not hardwired for any particular behavior. Synaptic function can be dynamically regulated to respond to changing internal and external environmental cues. Neurons accomplish this regulation through co-expression of diverse signaling molecules that can modulate synaptic function to flexibly adjust the outputs of "hardwired" circuits in response to changing internal and external environmental cues. Neurons communicating via more than one signaling molecule are common across species, and co-transmission of small molecule neurotransmitters (SMNs) together with neuromodulatory peptides offers particular opportunities for circuit flexibility.

The *Drosophila* circadian circuitry is an ideal model system to bridge this gap from genetics and physiology to behavior in rich environmental contexts. The circadian clock network in *Drosophila* is a well-studied circuit with extensive colocalization of SMNs and neuropeptides that integrates light and temperature information. The influence of specific neuronal groups and their secreted peptides in controlling aspects of circadian behavior are well-characterized. This circadian output circuit offers a robust framework to investigate conserved, neuropeptide-regulated behaviors modulated by anatomically defined neural circuits to elucidate fundamental principles of how neuromodulatory signaling alters network function and behavior. This project investigates both pre- and post-synaptic regulation of co-transmission in the Drosophila clock network and leverages this knowledge to design targeted behavioral screens to understand how co-transmission shapes circadian behavior.

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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 has involved investigations of the Hippo signaling network, which has emerged over the past decade 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. Most recently, we identified and characterized the *early girl* gene as a novel component of the Fat-Hippo signaling pathway, which acts through regulation of Dachs protein levels.

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. 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 alpha-catenin, and we recently obtained evidence that this occurs through a tension-induced conformational change in alpha-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.

We have also investigated how tissue patterning and mechanics influence morphogenesis. 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.

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Dr. Consuelo Ibar, Postdoctoral Associate
Dr. Bipin Tripathi, Postdoctoral Associate
Srividya Venkatramanan, Graduate Assistant
Deimante Mikalauskaite, Graduate Assistant
Swathi Vasudevan, Undergraduate Assistant
Tom Lehan, Undergraduate Assistant
Ahri Han, Undergraduate Assistant

# 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. The object of our research is to understand how oocytes receive the correct number of chromosomes and the mechanisms of errors that lead to aneuploidy. Using *Drosophila melanogaster* females as a model, we are studying the mechanisms that promote accurate chromosome segregation in oocytes. We are particularly interested in the protein complexes and mechanisms of meiosis and the features of the oocytes that makes them susceptible to chromosome segregation errors. Due to their unique biology, there are probably segregation mechanisms that are unique to oocytes. It is important to understand these mechanisms that may make the oocyte acentrosomal spindle susceptible to certain types of chromosome segregation errors.

In any diploid organism undergoing meiosis, the process 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. Fertilization then restores diploidy and the next generation begins. The movement of chromosomes during meiosis is driven by attachments made between the microtubules and the chromosomes.

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 critical process of bi-orientation: lateral attachments and end-on attachments (Figure 1). 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 chromosome establishes an incorrect position. End-on attachments are stronger but more permanent. In our model, end-on attachments are only established once each pair of chromosomes have bioriented and established their correct positions for segregation.

One kinetochore protein appears to be a hub for these activities, SPC105R, also known as KNL1 in other organisms. This protein recruits other kinetochore proteins while also interacting with the microtubules. Studying SPC105R and its partners will allow us to investigate how lateral attachments occur, how bi-orientation is achieved, and how the transition from lateral attachments to end-on attachments is regulated.

**Investigating how the meiotic kinetochore ensures accurate chromosome segregation**

Meiosis depends on the formation of a bipolar spindle and bi-orientation, which is the arrangement of homologous centromeres towards opposite poles. Bi-orientation is a critical part of metaphase I since it establishes how homologous chromosome pairs segregate at anaphase I. The conserved KMN network is required for kinetochore-microtubule attachments *in vivo* and is composed of three groups of proteins: SPC105/KNL1, the Mis12 complex and the Ndc80 complex. Within the KMN network, two microtubule binding activities have been identified, one with the Ndc80 complex and the other with SPC105/KNL1. We have developed a system to make germline-specific mutants of SPC105R to investigate how it integrates several different activities that promote lateral attachments while delaying end-on attachments until the chromosomes establish their correct orientation.



Our work has shown that multiple modes of kinetochore-microtubule attachment mediates bi-orientation (Figure 1). The process begins with lateral attachments that establish bi-orientation and depend on SPC105R. These are then converted to stable end-on attachments that depend on NDC80 and maintain bi-orientation. Our goal is to investigate the mechanisms of homolog bi-orientation in oocytes by studying how these components coordinate and are integrated. Specifically, the mechanisms for how lateral attachments lead to bi-orientation, and how they are converted to end-on attachments is poorly understood. We are currently investigating the effectors of lateral attachments that are downstream of SPC105R using mutational analysis and interaction studies.

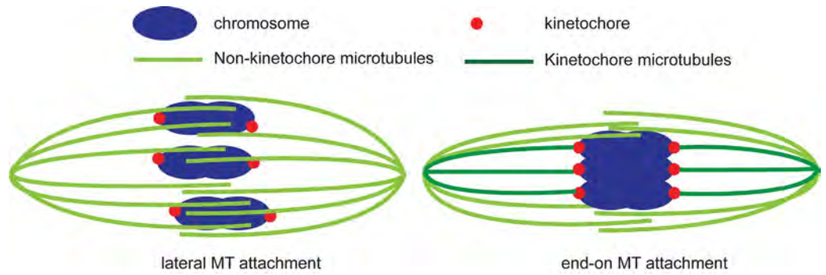


**Figure 1:** Model for how homologous chromosomes (blue) bi-orient during meiosis I. Kinetochores (red), including SPC105R, initially interact laterally with antiparallel microtubules in the central spindle (green). As the homologous kinetochores move away from each other, they form a microtubule bridge that keeps them apart. Stable end-on attachments to microtubules stabilize the bi-oriented chromosomes. The central spindle also includes several proteins (not shown) including Aurora B kinase that stabilize antiparallel microtubules and possibly interact with the chromosomes and kinetochores. Our hypothesis is that Aurora B kinase phosphorylation of SPC105R is critical for regulating bi-orientation of homologous chromosomes.

The first 40 amino acids of SPC105R includes a microtubule binding domain. It also contains two sites which can be phosphorylated by the Aurora B kinase. We hypothesize that Aurora B phosphorylation of SPC105R in this region of the protein regulates bi-orientation. We are currently testing this hypothesis by generating and analyzing a new set of mutants and tools. We propose that the lateral attachments required for bi-orientation depend on microtubule binding of the N-terminal domain of SPC105R. Furthermore, the transition from lateral to end-on attachments is associated with changes in phosphorylation of the N-terminal domain of SPC105R. Our hypothesis and associated model are outlined in Figure 2. We propose that when microtubules are interacting laterally with the kinetochore, SPC105R is phosphorylated. When end-on attachments are established, SCP105R is not dephosphorylated and a phosphatase, PP1, is recruited. This hypothesis will be tested with an SPC105R phospho-specific antibody and several new mutants of SPC105R. For example, to examine the role of SPC105R phosphorylation in bi-orientation, we will examine mutations in the Aurora B phosphorylation site. We will generate a mutant that is “phosphomimetic”, which emulates the phosphorylated state of SPC105R. If our model is correct, this mutant will have persistent lateral attachment and be unable to make end-on attachments.

**The central spindle interacts with kinetochores and promotes bi-orientation**

Our model for bi-orientation depends on lateral interactions between the kinetochores and microtubules. These microtubules are organized by several proteins that bundle them into anti-parallel arrays in the center of the spindle (Figure 2). The lateral attachments to microtubules that we propose are required for homologous chromosome bi-orientation depends on the unique structure and composition of the central spindle. 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. This may result in phosphorylation of SPC105R and lateral attachments. We propose that upon establishing correct bi-orientation, the homologous chromosomes move outwards towards the poles and leave the central spindle region. This results in loss of phosphorylated SPC105R and end-on attachments. The central spindle is emerging as a structure that can sense tension, promote error-correction and separate pairs of homologous chromosomes. These activities, mediated by Aurora B kinase, SPC105R and lateral attachments, allow the central spindle to direct reductional chromosome segregation at anaphase I.

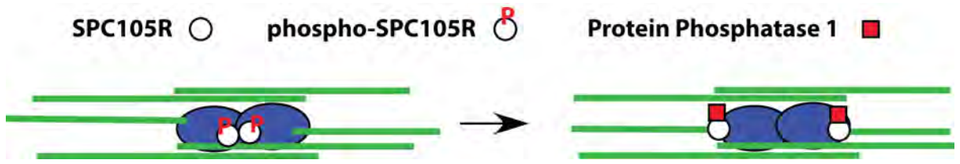


**Figure 2:** Model of bi-orientation by PP1- Spc105R interaction. Lateral attachments are associated with phosphorylated SPC105R. This may also be when the kinetochores are located in a region of high Aurora B activity, the central spindle (see Figure 1). End on attachments are associated with low phosphorylation and PP1 binding to SPC105R. This may occur when the kinetochores move towards the poles and way from high Aurora B activity.

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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 (Fig. 1). 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 (Fig. 1). 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.

**A Genetic System For Studying Neurons, Mitochondria, And Stress**

- **Trafficking of Synaptic Proteins in Neurons**
  - Synapse Formation
  - Synaptic Plasticity
- **Mitochondrial Dynamics in Neurons**
  - Motility Along Axons and Dendrites
  - Fission/Fusion
  - Mitophagy (Quality Control)
- **Neuron Stress Response & Proteostasis Mechanisms**
  - Hypoxia Response
  - Ubiquitin Proteasome System

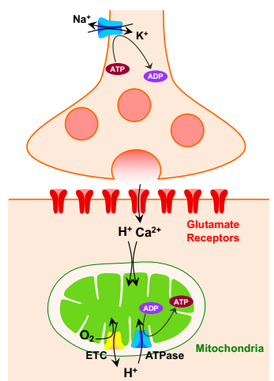


Figure 1. A Genetic System for Studying Neurons, Mitochondria, and Stress. High levels of ATP are required to maintain the membrane potential of neurons; thus, neurons rely heavily on oxidative phosphorylation and mitochondria. Hypoxic stress reduces ATP production, resulting in membrane depolarization, massive release of neurotransmitter, overactivation of neurotransmitter-gated ion channels, increased cytosolic calcium, mitochondrial dysfunction and stress, and eventually neurodegeneration.

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 (Fig. 2). 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.

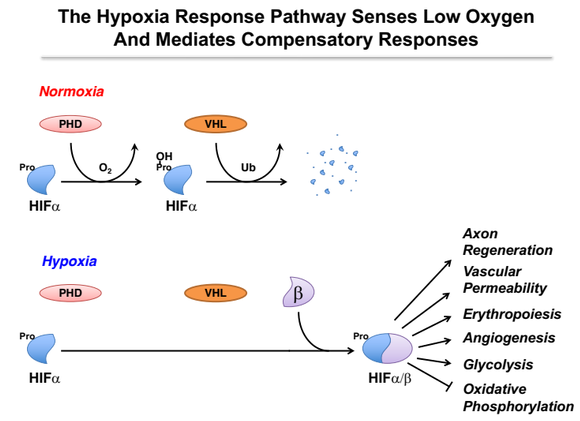


**Figure 2**. The Hypoxia Response Pathway Senses Low Oxygen and Mediates Compensatory Responses. Under normal oxygen (normoxia), a prolyl hydroxylase (PHD) enzyme uses oxygen to covalently modify specific proline side chains on the HIF alpha transcription factor. Once hydroxylated, HIF alpha becomes a substrate for the VHL ubiquitin ligase, which ubiquitinates HIF, causing its degradation by the proteasome. Under hypoxia, the PHD enzyme is inactive, preventing HIF alpha from hydroxylation and degradation. HIF alpha can then bind to HIF beta, enter the nucleus, and promote the expression of genes that offset the negative effects of hypoxic stress.

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 have broadened our studies of this pathway, and we are now conducting RNA-seq and ChIP-seq experiments to identify both HIF-1-dependent and HIF-1-independent targets of hypoxia-induced gene regulation. We have also identified over 400 unique metabolites that are regulated by this pathway and correlate with the changes in gene expression.

**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 (Fig. 3). Mitophagy can be triggered by mitochondrial stress or even by starvation (Fig. 4). Using this and other mitochondrial reporters, we are now examining how mitochondrial dysfunction contributes to a tau-based genetic model of Alzheimer's Disease.

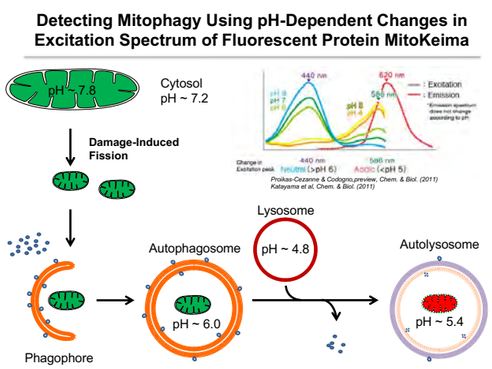


**Figure 3.** Detecting Mitophagy Using pH-Dependent Changes in Excitation Spectrum of Fluorescent Protein MitoKeima. As a quality control measure, damaged mitochondria undergo fission to generate smaller mitochondria. These mitochondria contain mitophagy receptors that recruit autophagy factors, resulting in the nucleation of a phagophore. Phagophoric membranes encapsulate the damaged mitochondria into acidic autophagosomes. These autophagosomes fuse with highly acidic lysosomes, resulting in autolysosomes, where the mitochondria are eventually digested and removed. To differentiate healthy mitochondria from mitochondria undergoing mitophagy, we employed a transgenic reporter called MitoKeima. MitoKeima emits 620 nm wavelength light. However, it is differentially excited depending on the pH. Inside healthy mitochondria, where the pH is around 7.8, MitoKeima is excited by 440 nm light. Inside mitochondria in autolysosomes, where the pH is around 5.4, MitoKeima is excited by 586 nm light. Using different filter sets, we can use this differential excitation to observe these two kinds of mitochondria separately.

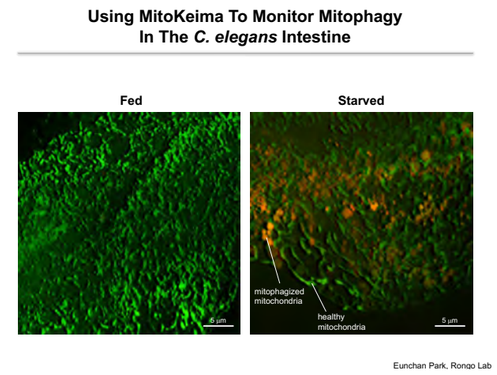


**Figure 4.** Using MitoKeima to Monitor Mitophagy in the C. elegans Intestine. Here, we have expressed MitoKeima in the C. elegans intestine. In well-fed animals, there is little mitophagy and most mitochondria are healthy, as detected by 440 nm excitation (false colored green). These mitochondria have an elongated, reticular morphology. By contrast, animals that have been starved break down many of their mitochondria through mitophagy. Mitochondria internalized in autolysosomes can be detected by 586 nm excitation (false colored red). These mitochondria have a round morphology, consistent with the autolysosomes in which they are contained.

Eunchan Park, Rongo Lab

**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 have also found that the humoral neurohormone/biogenic amine neurotransmitter dopamine promotes UPS activity in epithelia. In *C. elegans*, mechanosensory neurons release dopamine when nematodes encounter a potential bacterial food source. Dopamine in turn inhibits motoneuron activity through the dopamine receptors DOP-2 and DOP-3, resulting in a behavioral change that slows the animal down so that it can feed. We found that this released dopamine also activates the UPS in epithelial tissues, including the intestine and epidermis, through the dopamine receptors DOP-1 and DOP-4, and the cAMP-Response Element Binding Protein (CREB) transcription factor. This signaling pathway activates the expression of enzymes involved in xenobiotic detoxification (e.g., cytochrome P450 enzymes) and innate immunity, which in turn promote protein polyubiquitination. Although we do not yet understand exactly how xenobiotic detoxification activates the UPS, our results show that dopamine signaling is essential for nematodes to survive xenobiotic stress and to maintain normal proteostasis. Taken together, our results suggest that dopaminergic sensory neurons, in addition to slowing down locomotion upon sensing a potential bacterial feeding source, also signal to epithelial tissues to prepare for infection in case that potential bacterial food source turns out to be pathogenic.


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# SINGSON LAB
## Reproductive Biology, Cell-Cell Interactions


**Dr. Andrew Singson**
Genetics



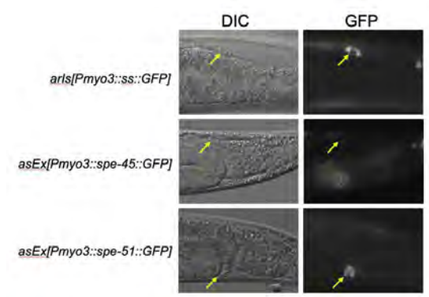
**Summary**
Reproductive success requires that two haploid cells – sperm and egg – unite to form a diploid zygote. Both sperm and egg cells must be differentiated into forms that are highly specialized for their specific roles in fertilization. After fertilization has occurred, the zygote must begin 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 molecular tools. 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.

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.

**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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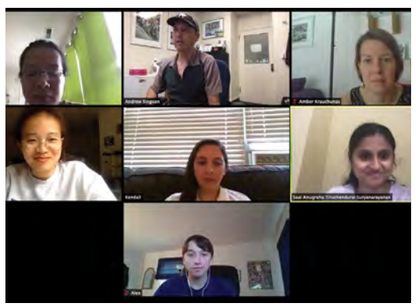



# STEWARD LAB

## 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 Drosophila by the Tet (Ten-Eleven-Translocation) protein. Tet proteins have well-documented functions in maintaining vertebrate stem cells and development and are associated with carcinogenesis and neurological disorder. 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. Fly Tet encodes two distinct proteins that are similar in organization to the vertebrate proteins**.** Both contain the enzyme’s catalytic domain, but only the larger protein contains also the conserved DNA binding domain. *Ter$^{null}$* is 100% pupal lethal.

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 nerve cells. We are studying the neuronal phenotype of Tet mutants. In Tet$^{null}$ 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, a very rare occurrence in wild type 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. In the PNS, reduction of Tet function results in defects in dendrite morphogenesis in the class IV larval sensory neurons. 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 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 771 protein binding peaks, distributed on 654 genes. Just over 50% 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 *Ter$^{null}$* 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 *Ter$^{null}$* 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. Gene ontology analyses indicates that 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. These results strongly support the idea that 5hmrC modification has a significant and positive effect on translation efficiency.

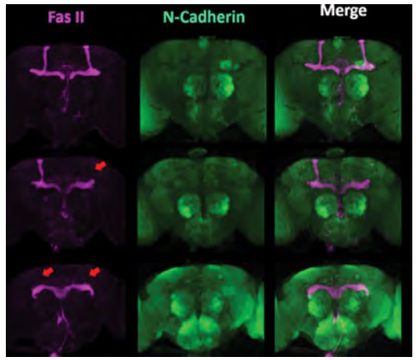


**Figure legend: Loss of mushroom body α lobe(s) in *Tet*$^{4XXC}$ mutants.** Fasciclin II stains the mushroom bodies (red) N-cadherin is used as a cell marker (green). Top, wildtype brain, bottom, two different phenotypes observed in *Tet*$^{4XXC}$ brains.


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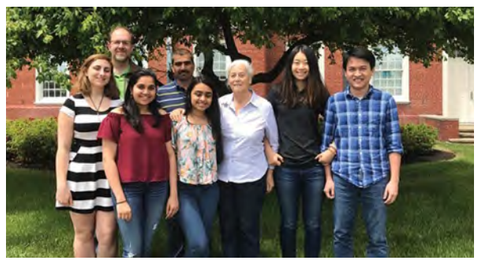



# EBRIGHT LAB

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


**Dr. Richard Ebright**
Chemistry & Chemical Biology



**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 lacpromoter 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 lavel of transcription antipausing 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 structpausing and urally 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 determinaing 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.

**Inhibitors of Transcription; Antibacterial Drug Discovery**

Bacterial RNA polymerase (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,* and human pathogen *Clostridium difficile*. 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. Together with Bryce Nickels laboratory we developed a new method to monitor short intracellular fragments in cells mounting CRISPR defence. The new method, called FragSeq, allowed us to identify and characterize "prespacers" the *in vivo* intermediates of CRISPR adaptation on their way to becoming protective spacers in CRISPR arrays.

Powerful *in vitro* fluorescent beacon methods assays are being used to determine how Cas9, a popular genomic editor, differentiates between target and non-target DNA. This research allows to better control off-target activity of genomic editors and expand their use by identifying editors with altered specificity towards PAMs(protospacer adjacent motifs). We use the diversity of CRISPR spacers to monitor adaptations of bacterial populations to viruses. In a surprising twist, we found viruses that carry miniature CRISPR arrays in their genomes and use them to compete with other viruses by charging Cas effectors of their hosts with guide RNAs that are encoded by these CRISPR arrays and recognize the competitors.

**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. Current work concentrates on three classes of RIPPs: peptide-nucleotides related to microcin C, oxazole-thiazole peptides related to microcin B, and lasso-peptides related to microcin J. Significant development of the past year was the discovery of a new class of oxazole-thiazole peptides that inhibit bacterial ribosome (a collaboration with Yury Polikanov from UIC and Jamie Cate from UC Berkeley), development of a simplified lasso-peptide biosynthetic pathway that opens way to construction of large lasso-peptide libraries for functional screenings, and uncovering a new mechanism of resistance to microcin C-like peptide-nucleotides through the function of universally conserved histidine triade proteases.

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

We identified several bacteriophage-encoded DNA-dependent RNA polymerases that are very distantly if at all related to cellular enzymes. We use biochemical and structural methods to investigate these unusual 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 UTMB). 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.


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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.

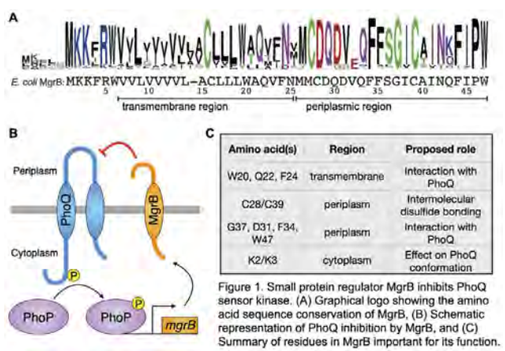


Figure 1. Small protein regulator MgrB inhibits PhoQ sensor kinase. (A) Graphical logo showing the amino acid sequence conservation of MgrB, (B) Schematic representation of PhoQ inhibition by MgrB, and (C) Summary of residues in MgrB important for its function.

**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.

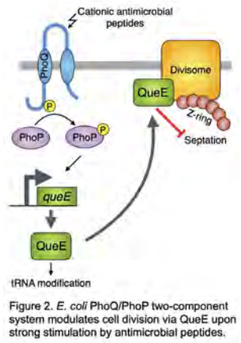


Figure 2. *E. coli* PhoQ/PhoP two-component system modulates cell division via QueE upon strong stimulation by antimicrobial peptides.


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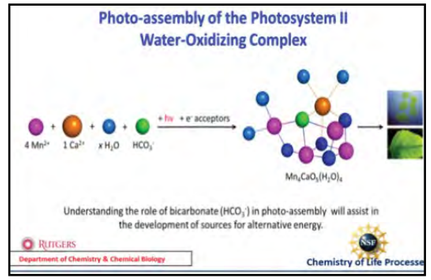
# DISMUKES LAB

## Biological and Chemical Approaches to Renewable Energy Research


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

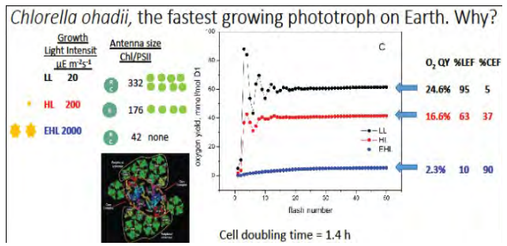


**Summary**
The Dismukes research group conducts fundamental and applied research in the areas of renewable energy production via biological and chemical approaches. Our strategy is to apply the principles of enzymatic catalysis and metabolic regulation to design bioinspired catalysts, reaction networks and microorganisms exhibiting improved performance that operate using electrical or solar energy power sources. The disciplinary approaches used are 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 Wright-Rieman Chemical Laboratory at Rutgers University. In the 2019-July 2020 period the group was comprised of 30 researchers (listed below).

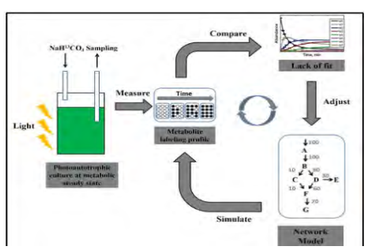




1) ***Inorganic Mutants of Photosystem II Water Oxidizing Complex and its Biogenesis.*** Waksman**.** All photosynthetic organisms on Earth use the same invariant catalyst to oxidize water. Why and can we design a better one? This project aims to understand the chemical functions of the inorganic components comprising the catalytic site (WOC). We do so by substitution of the native inorganic cofactors ($Mn^{2+}$, $Ca^{2+}$, $Cl^-$, $CO_3H^-$, $H_2O$) and examination of the consequences using novel tools designed by our lab staff. Recent advances have produced the first known substitution of native Manganese with Cobalt. Imagine what are the chemical properties? We also investigate the steps of biogenesis of the oxygenic reaction center (PSII) during the greening (etiolation) process which is sensitive to photodamage. Supported by DOE-BES. Collaborations: Arizona SU; Umea U.

2) ***Diversity of Photosynthetic Water Oxidizing Enzymes***. **Waksman.** This research investigates "photosynthetic outliers" from the field or culture collections, seeking non-classical metabolisms. We have characterized the earliest branching cyanobacterium on the tree of life[(1)], the fastest growing "Usain Bolt" of photosynthesis[(2)], metabolisms of hypercarbonate and hypersaline tolerant strains[(3)], and created transgenic strains to test numerous metabolic hypotheses[(4)]. The resulting advances in knowledge: molecular basis of light energy conversion to biomass components, non-classical metabolic pathways for biosynthesis, and novel reaction-centered based photoprotection mechanism in algae called PSII-cyclic electron flow. To achieve this, we built new instruments/methods for detection of dissolved $O_2$, $H_2$, Chl, NAD(P)H, pH, and intracellular metabolite fluxes by LC-tandem-MS[(5)]. Supported by DOE-BES. Collaborations: RU Plant Biology, TU Delft.

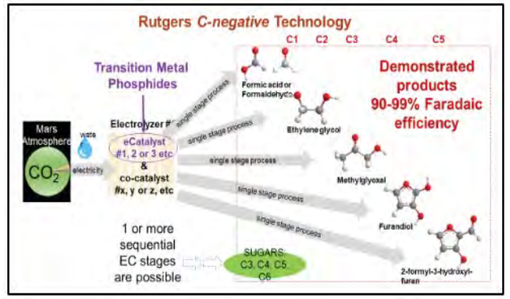



3) ***Photoautotrophic Carbon Fluxomics.*** **Waksman.** Metabolic pathways for model organisms can be found in textbooks. However, these are widely modified across the ToL and novel pathways for making carbon products abound in nature that remain to be discovered. Improving the abysmal solar to biomass 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

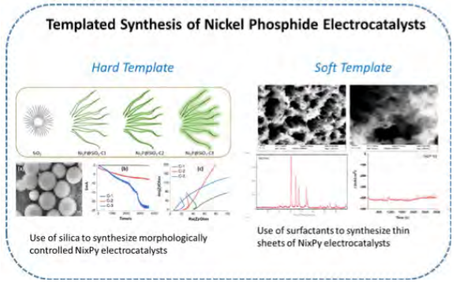


metabolic flux analysis (INST-MFA, figure) to quantitatively understand carbon flux distributions and pathway used by phototrophs during photosynthesis. It enables discovery of kinetic bottlenecks that limit efficiency, new roles for existing metabolic pathways and completely new pathways not previously known. NSF-MCB and GCEP. Collaborations: RU Plant Biology; USTC and Zhejiang U. China.

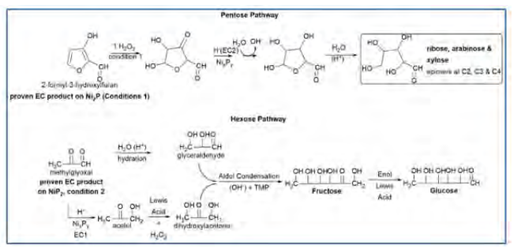
**4) *Bioinspired Electrocatalysts for Water Splitting and $CO_2$ Reduction*. CCB.** Human civilization is on a crash course to consume our natural resources and poison our environment unless we learn how to use renewable feedstocks like water and $CO_2$ to make chemicals and food. In this project we 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 CO2 reducing enzymes. The synthesized catalysts are made from earth abundant elements, exceed the activity of the best commercial catalysts used today. Using this strategy we have produced the best noble-metal-free catalysts for water oxidation ($LiCo_2O_4$), water reduction to hydrogen ($Ni_5P_4$) and $CO_2$ reduction to C1 to C5 products selectively (Nickel Phosphides). The image depicts our electrolytic process for converting water and CO2 into C1 to C5 products. Supported by the DOE-EERE-HydroGEN, NASA, NREL, Rutgers Goldman Prize and Rutgers *TechAdvance*. Collaborations: UPenn, CU Boulder, NREL.




***5) Synthesis of transition metal phosphides with defined structural phase, facet and morphology.* CCB.** In this project, we use a templating approach to synthesize nickel phosphide catalysts for hydrogen evolution and CO2 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 framework to synthesize Ni2P 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 CO2RR. Preliminary HER results show high current densities can be obtained using these catalysts (~25mA/cm$^2$ and ~400mA/cm$^2$ for hard and soft template $Ni_2P$ respectively. Support: DOE LDRP project; Collaborators: NREL

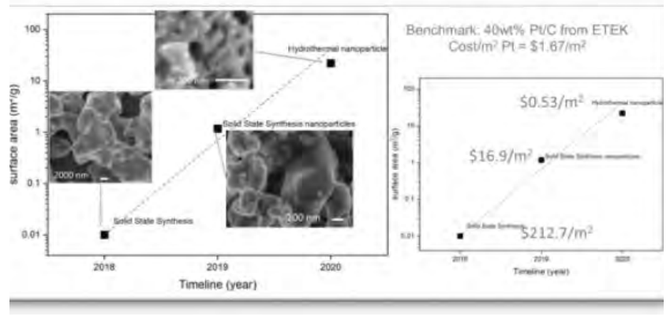



***6) Sugars from $CO_2$, Water & Electricity: NASA $CO_2$ Conversion Challenge.*** In this project, we are working on the synthesis of carbohydrates from carbon dioxide ($CO_2$) and water ($H_2O$). This work builds on our previous results wherein we have developed highly efficient electro-synthetic routes to selectively produce $C_3$ (methylglyoxal) and the $C_5$ (2-formyl-3-hydroxylfuran using nickel phosphide catalysts. Our proposed line of work to produce D-sugar involves two approaches: 1) aldol condensation of glyceraldehyde and dihyroxylacetone. 2) use of proline derived catalyst to couple methylglyoxal with aldehyde/ketone. Currently, we are working on the synthesis of proline derived chiral catalyst which is a 3 step chemical reaction involving multiple purification steps. Support: NASA.

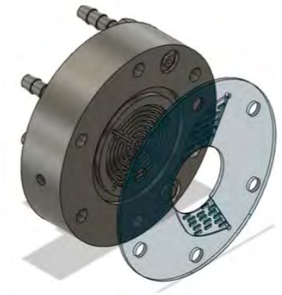




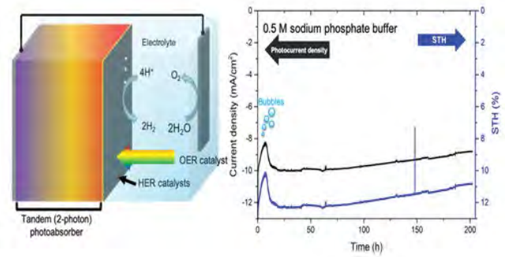

***7) Upcycling Carbon Dioxide: Ethylene Glycol from $CO_2$ & Renewable Electricity*. CCB.** In this project we apply the principles learned from enzymes to synthesize better heterogeneous catalysts for the generation of $O_2$ and protons from water (Photosystem II) and $CO_2$ conversion (acetogens and methanogens) to chemicals and fuels. Based on the previous catalyst discoveries out of the Dismukes group, this project aims to develop low cost electrolyzers and catalysts synthesis protocol. Current state of the art $CO_2$ electrocatalysts based on nickel phosphides are limited by a low current density when operated under conditions where the competing $H_2$ evolution reaction is thermodynamically controlled. This project seeks to mitigate this limitation through the synthesis of ultra-small nanoparticles using low-temperature reaction conditions. Furthermore, the project aims to investigate the feasibility of using next generation polymers as the electrolyzer material. This effort seeks to cut the projected PEM electrolyzer cost from $423/kW to less than $210 allowing the cost competitive production of carbon negative chemicals. Thus far we have developed a catalyst synthesis protocol that increases surface area by ~1100 fold and based on calculations done by RenewCO$_2$ LLC cut the catalyst cost by 400 fold over the benchmark non-PGM catalyst. Support DOE-SBIR. Subcontractor to: RenewCO$_2$ LLC.




***8) Scalable CO2 electrolyzers for the production of ethylene glycol and chlorine*. CCB.** In this project we seek to combine the production of $CO_2$ reduction products (using catalysts develop previously in the Dismukes group) with the production of chlorine and alkali using industry standard catalysts. Leveraging the conventional electrolyzer designs in the Dismukes group the project aims to develop a three-compartment electrolyzer capable of running at above ambient temperatures (<80°C) and increased pressures (<20 bar) while separating the highly corrosive $Cl_2$ evolution from the $CO_2$ reduction to carbohydrates. So far, we have developed the first protype by 3D SLA printing and have started testing the operations of the unit. Support: NSF-STTR. Subcontractor to: RenewCO$_2$ LLC.



9) ***Best-in-class Platinum Group Metal-free Catalyst Integrated Tandem Junction PEC Water Splitting Device*s**. CCB. Solar energy conversion to fuels requires initially the splitting of water into its elements, H2 and O2. 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).


By using BASL as an anchor for screens to isolate genetic and physical interactors

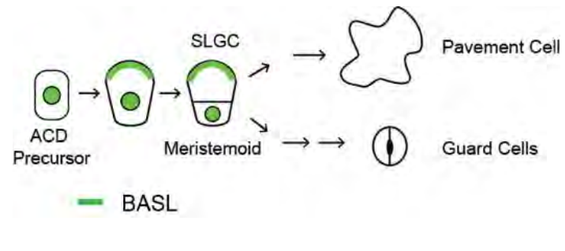


**Figure 1.** BASL localization and stomatal asymmetric cell fate

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.

**Regulatory mechanisms 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 either 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 similar to the membrane-embedded PIN proteins, hinting the possible regulation of membrane trafficking in BASL polarization. This direction is currently pursued in the lab.

**BASL scaffolds BIPP phosphatases to regulate stomatal asymmetric cell division**
Our previous work showed that BASL is phosphorylated and activated by MAPK 3 and 6 (MPK3/6) and becomes polarized 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 phosphatases, BIPPs (BASL-interacting protein phosphatases), as BASL partners. Genetic analysis places BIPPs upstream of the YDA MAP kinase cascade and downstream of the plasma membrane receptors. In addition, the founding member BIPP-1 colocalizes with BASL in a polarized manner at the cell periphery. Interestingly, the recruitment of the BIPP 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 BPP phosphatases in bridging the two opposing protein functional modules to control the balance of cell division.

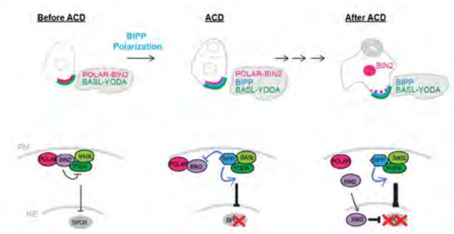


**Figure 2**. Working model for BIPP to function in stomatal ACD. BIPP proteins function as the spatiotemporal molecular switch enabling the coordination of cell division and cell-fate differentiation in stomatal ACD. BIPP proteins join the polarity complex in the ACD mother cells that are committed to cell division. Association of BIPP proteins 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.

**Proein 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. 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).

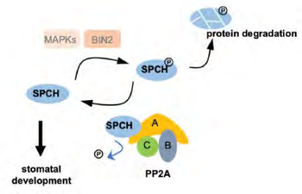


**Figure 3**. Working model for PP2A phosphatases in stomatal development.
PP2A promotes stomatal development by stabilizing the SPCH protein. PP2A might function in opposition to the identified kinases (MAPKs and BIN2) to balance the phosphorylation status of SPCH in the initiation of stomatal.


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# GALLAVOTTI LAB
## Mechanisms of Plant Development


**Dr. Andrea Gallavotti**
Plant Biology

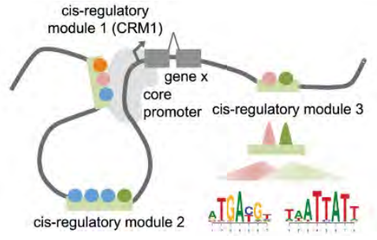


**Summary**

**Molecular Mechanisms of Plant Development**
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.


Transcriptional regulation at gene x. Colored circles represent different TFs binding to three distinct CRMs (light green bars) that can contact the core promoter via DNA looping. Motifs for two TFs are shown for CRM3.

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).

**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 devel-

opmental 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. Notable among these are two Aux/IAA proteins (BIF1 and BIF4) that work in conjunction with ARF transcription factors, and a gene that encodes a mitochondrial localized protein and affects auxin transport and homeostasis. We have also identified key regulators of meristem size that affect the number of rows of seeds in maize ears.

**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 (Figure 1), thus providing an excellent tool to study how plants use transcriptional repression mechanisms in numerous developmental processes. This research is sponsored by a grant from the Developmental Systems cluster of the National Science Foundation.

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. We are currently characterizing a series of pathways regulating meristem development and flower formation that require REL2-mediated repression by a combination of genomic, genetic and molecular approaches. One of these pathways is involved in the domestication of maize ears from its wild progenitor teosinte.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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 three areas.


**Plastid transformation in *Arabidopsis thaliana***
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. We now report *ACC2* defective lines in the RLD and Ws ecotypes, which readily regenerate plants from cultured cells. *ACC2* knockouts were obtained using CRISPR/Cas9 genome editing tools. The spectinomycin hypersensitive phenotype is characterized by the lack of shoot apex when germinated on a selective medium. This phenotype has been confirmed in both accessions, indicating that deletion of the *ACC2* gene is generally applicable to obtain spectinomycin hypersensitive plants in all species in which duplication of the ACCase pathway has been conserved. Testing plastid transformation efficiency in the *ACC2* knockout lines confirmed 100-fold elevated frequency as compared to the wild-type. Testing was accelerated by the newly developed SPEED transformation protocol (Yu et al., Plant Physiol. 181: 394-398, 2020).

**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. We are now working on re-targeting the proteins involved in T-DNA transfer. Side-stepping the tissue culture process will eliminate the need for specialized expertise to practice plastid transformation in Arabidopsis. Therefore, this research will lead to widespread applications of Arabidopsis plastid genome engineering which, combined with the available extensive genomic resources, will have a major impact on basic science and applications in biotechnology.

**Expression of recombinant proteins in chloroplasts**
The laboratory has a long tradition in the expression of recombinant proteins in chloroplasts. Most recent is development of dicistronic operons as a novel marker system for chloroplast engineering that can be used as building blocks for plant synthetic biology. The identification of transplastomic clones is based on selection for antibiotic resistance encoded in the first open reading frame (ORF) and accumulation of the reporter gene product in tobacco chloroplasts encoded in the second ORF. The antibiotic resistance gene may encode spectinomycin or kanamycin resistance based on the expression of aadA or neo genes, respectively. The reporter gene used in the study is the green fluorescent protein (GFP). The mRNA level depends on the 5' UTR of the first ORF. The protein output depends on the strengths

of the ribosome binding, and is proportional with the level of translatable mRNA. Because the dicistronic mRNA is not processed, we could show that protein output from the second ORF is independent from the first ORF (Figure 1). High-level GFP accumulation from the second ORF facilitates identification of transplastomic events under UV light. Expression of multiple proteins from an unprocessed mRNA is an experimental design that enables predictable protein output from polycistronic mRNAs, expanding the toolkit of plant synthetic biology. These data have been described in a recent publication (Yu et al., 2020 Plant Journal doi: 10.1111/tpj.14864).

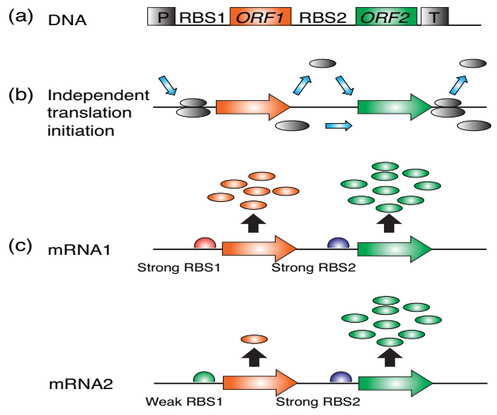


Figure 1. No translational coupling between the first and second ORF in the chloroplast dicistronic markers. Based on Figure 6 in Yu et al. Plant J. doi: 10.1111/tpj.14864
(a) Schematic map of dicistronic operons. ORF1 encodes a marker gene, the second ORF gfp. The operon has one promoter (P) and one 3'-UTR (T) for the stabilization of the mRNA.
(b) Translation initiates independently from the first and second ORFs. Shown are the small and large ribosomal subunits entering the mRNA at the ribosome entry site, and dissociating when translation of the 1st ORF is completed. Small ribosomal subunits initiate translation independently of the 2nd ORF.
(c) Protein accumulation from the first ORF has no significant impact on protein accumulation from the 2nd ORF. Protein output from the 1st ORF can be high (pMRR20) or low (pMRR21), the protein output from the 2nd ORF is always high.

**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 2019-2020 from Rutgers were Amanda Chen, Juliana DiGiacomo, Mugdha Parulekar and Alifya Quresh. From Farmingdale State College Sydney Matias and Kelly Enriquez spent the summer of 2019 at Rutgers.

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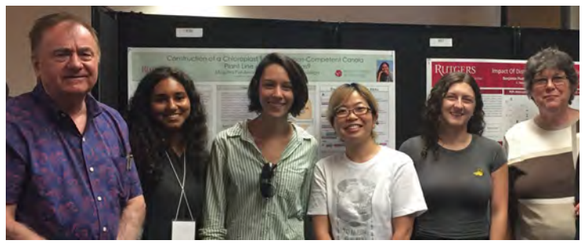
Mugdha Parulekar, Undergraduate Student
Alifya Quresh, Undergraduate Student





# 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 2019-2020**

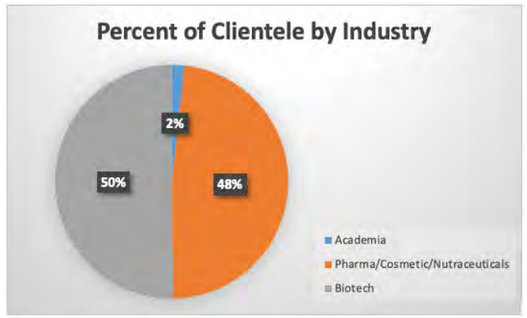
Over the past year, the scientists at the fermentation facility have completed projects for numerous biotech, pharmaceutical and cosmetic industries as well as a couple of university and university affiliates. New to this year, we have

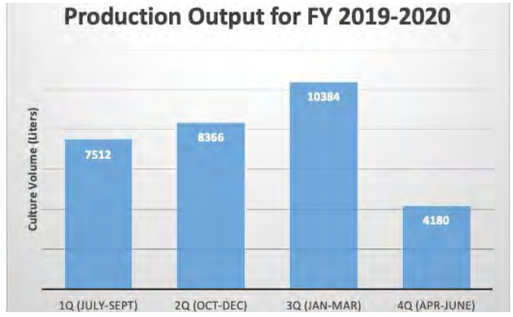
expanded our client reach to companies outside US such as Canada. For this fiscal year, the total production output exceeded last fiscal year's output and is now over **30,000 liters** of microbe cultures of *E. coli*, *P. pastoris*, *Streptomyces* spp. and various strains of yeast and fungus. The revenue generated from these work 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. Moreover, as part of the CCPFF's contribution to the Institute's scholarship mission, the facility in collaboration with Professor Henrik Pedersen (Rutgers School of Engineering) hosted a group of more than 50 high school students and conducted a tour and seminar on the basics of Fermentation and Industrial Microbiology.

Dr. Arvin Lagda oversees and directs the 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. This year, we also expanded our team by hiring Mr. Andrew Cloud as a laboratory technician. Andrew has backgrounds in basic chemistry and environmental science which supplements the current skills and expertise we have at the Pilot plant. From 2018 to late 2019 we also had the privilege to work with Mr. Joseph Troyanovich (consultant and research associate) whose decades of experience in Fermentation Science provided us with extra tools and know how to solve many of our challenges. 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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**Lab Members**

Dr. Arvin Lagda, Laboratory Director/Research Scientist
Ms. Amanda Rodriguez , Laboratory Manager/Laboratory Researcher III
Dr. Sergey Druzhinin, Laboratory Scientist/Laboratory Researcher IV
Mr. Andrew Cloud, Laboratory Technician
Mr. Joseph Troyanovich, Laboratory Researcher



# Waksman Genomics Core Facility

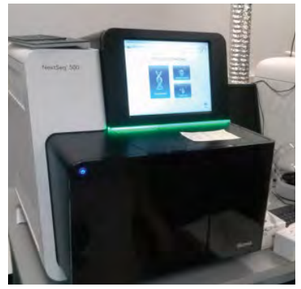
Waksman Genomics Core and Instrumentation Laboratory (WGCF) provides access to high-throughput next generation sequencing and molecular biology instruments to the Waksman Institute's researchers as well as Rutgers research community. Since 2008, the core offers single read, paired-end, and multiplex 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 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 and Agilent bioanalyzer.

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.

MiSeq



NextSeq500

## Dr. Arvin Lagda, Core Facilities/Shared Resources

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Dr. Dibyendu Kumar, Manager, Research Associate
Dr. Yaping Feng, Bioinformatics Research Scientist, Research Associate
Dr. Min Tu, Lab Operations Scientist, Postdoctoral Associate

# 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, 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. An exciting upgrade, Lightning Deconvolution, was added to the SP8 this year, significantly improving image resolution during acquisition by utilizing adaptive technology.

The Waksman Core Facility has approximately 63 trained users, primarily Waksman researchers, from fourteen laboratories and is used an average of 65 hours per week. 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. The future aim of the facility is to continue to provide exceptional imaging capabilities to Waksman researchers.

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Laboratory Researcher IV
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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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# TRAINING FUTURE LEADERS

## Predoctoral Fellowships

## Graduate & Undergraduate Courses

## High School Outreach/WSSP



# 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. To further study Tet protein, I am addressing two questions. Does Tet DNA binding domain have its own non-enzymatic function which distinct from its catalytic domain? And how Tet domains involve in modifying *Drosophila* mRNA?

I hypothesize that Tet regulates transcription by recruiting epigenetic modifiers to its target gene via the DNA binding domain. I am analyzing $Tet^{AXXC}$ and $Tet^{YRA}$ transcriptome and testing some epigenetic regulators which are potentially Tet partners. To study the mechanism of how Tet modifies RNA, I am testing 5hmrC level in mRNA of $Tet^{AXXC}$ and $Tet^{YRA}$ brains by LC-MS/MS. I am also coordinating with people in the lab to test if Tet binds to RNA and identify Tet complex with modified RNA.

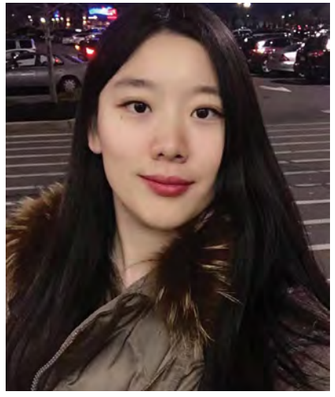
Hoa Vu
Nickels Lab

**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 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 RNAP derivatives, each having a photoactivatable crosslinking agent incorporated at a single, defined site in RNAP that, upon photoactivation forms covalent crosslinks with DNA; photoactivation to initiate covalent crosslinking of RNAP to DNA; and use of highthroughput sequencing to define the crosslink position on the DNA and the site in RNAP that crosslinked to the DNA. I am using this method to analyze the dynamics of RNAP-DNA interactions during transcription initiation, initial transcription, transcription elongation and transcription pausing in vivo, in *Escherichia 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.





Lu Wang
Dong Lab

**Research Summary**

**Connected function of BIP2 and GNOM controls stomatal cell polarity for stomatal asymmetric cell division**

Stomata are epidermal structures that modulate gas exchange between a plant and its environment (Dow and Bergmann, 2014). During stomata development, asymmetric cell divisions create two daughter cells that differ in size, fate, location, and cellular components, which underlies correct patterning of a well-organized and functional stomatal system. The protein BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE (BASL) is polarized to control stomatal asymmetric division (Dong et al., 2009). However, how BASL polarity is initiated and maintained in the cell cortex remains largely unknown.

My project has been focused on the molecular mechanism by which the peripheral membrane protein BASL is polarized in the stomatal lineage cells undergoing asymmetric cell division. We identify the BIP2 proteins as BASL physical interactors. Florescent protein tagged BIP2 proteins are localized to plasma membrane, cytoplasm, and endomembrane system. The loss-of-function *bip2* quadruple mutants lead to defects in BASL polarization and abnormal stomatal ACD. Interestingly, the developmental defects of the *bip2* quadruple mutant highly resemble the ARF GEF *gnom* mutant and similarly, *GNOM* is also required for BASL polarization. The ARF GEF GNOM was known for endocytic recycling-mediated protein polarization in plants. We further show that BIP2 interacts with GNOM and their physical association may occur at the trans-Golgi network and recycling endosomal vesicles in the plant cells. Quantitative stomatal phenotyping of *gnom;bip2-4t;5c;8c;9c* quintuple mutant is similar to *bip2-4t;5c;8c;9c* quadruple mutant or *gnom* mutant, supporting BIP2 and GNOM function in the same genetic pathway. Thus, we propose that the connected function of BIP2 and GNOM in endosomal recycling is required for the peripheral membrane protein BASL to polarize.

# BENEDICT MICHAEL FELLOW

## Predoctoral Research

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.



# 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 2019-2020

Advanced Inorganic Chemistry
Biological Clocks In Genetics, Physiogy & Behavior
Chemistry
Core Seminars in Plant Biology
Genetic Analysis II
Genetics and Cell Biology of Fertilization
Microbial Biochemistry
Molecular Biology and Biochemistry
Molecular Biology of Gene Regulation & Development
Molecular Biosciences
Plant Molecular Biology
Plant Molecular Biology II

# 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 27 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 2019-2020 WSSP consisted of two interrelated parts: a Summer Institute (SI) and an Academic Year Program (AYP). In July 2019, 30 students and 4 teachers from 30 high schools attended a 10-day SI at the Waksman Institute. The SI consisted primarily of daily seminars and laboratory activities that focused on molecular biology and bioinformatics. The laboratory sessions introduced students and teachers to the content, background information, and laboratory procedures that were needed to carry out the research project at their schools during the academic year. Students and teachers used Internet resources to process and analyze their data. Some of the seminars that were presented dealt with bioethical issues and career opportunities in the fields of science, technology, engineering, and mathematics (STEM).

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. The Biology Equipment Lending Library (BELL), which was previously established with generous support from the Toshiba America Foundation and the Johnson & Johnson Foundation, enabled the WSSP to loan expensive equipment to the schools so that many students could conduct the research project during the AYP.

Four after school workshops were conducted at the Waksman Institute during the school year. In these sessions, students presented their results from their research efforts and discussed the problems they encountered while working on the project at their schools. These sessions were also used to review the background material, to update all the participants in changes in laboratory protocols and share new findings in molecular biology. In previous years, near the end of the academic year, each school team presented their research findings at a poster session to which scientists, school administrators, and parents were invited. Because of the pandemic the poster session was cancelled in spring 2020.

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 4 high schools in MD. A two-week summer Institute for 4 teachers and 16 students was conducted at the Lawrence Livermore National Laboratory, Livermore, CA, and 4 teachers and 40 students conducted investigations during a summer session in Waipahu, HI. As a result of these extended outreach activities and the growth in the number of schools participating locally in New Jersey, a total of 1211 students from 49 different high schools in NJ, MD, PA, CA and HI participated in, and contributed to, the WSSP this past year.

**The Research Question**

The 2019-2020 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. During the summer and continuing throughout the academic year, the 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. From the 2019-2020 SI and AYP, over 1800 plasmid clones were purified and 1422 were sequenced. Due to the school closures, several schools were not able to complete the laboratory experiments on their clones. To date, 1020 DNA sequences have been analyzed by the students. 543 DNA 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 program requires the participation of the teacher and 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 two 10-day summer programs called **Waksman Institute Summer Experience (WISE)** in which students perform the same research project as conducted in the WSSP. In June and August 2019, 36 and 34 students, respectively, participated in WISE and each of the students successfully purified and analyzed a novel DNA sequence and published their findings on the NCBI database. Due to the success of WISE and the demand from students wanting to participate in the program we had planned to offer two two-week WISE summer institutes during the 2020 summer. However, due to restrictions preventing on campus sessions we plan to offer two one-week on-line bioinformatic sessions in the summer of 2020 for 60 students in each session.

**Dr. Andrew Vershon, Director WSSP, Professor**

**Susan Coletta, Educational Director**
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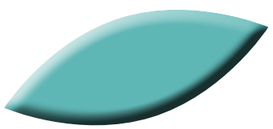
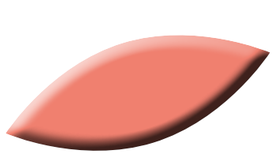
**Dr. Janet Mead, Laboratory Director**
John Brick, Laboratory Assistant

# SHARING OUR DISCOVERIES

## Waksman Annual Retreat



## Presentations & Meeting Abstracts



## Patents & Publications



# WAKSMAN ANNUAL RETREAT

**Rutgers University Inn & Conference Center**
**September 6, 2019**

## Presentations

- **Zhiyong Zhang** - Messing lab: Transcriptional regulation of storage protein and starch synthesis in maize endosperm.
- **Zongliang Chen** - Gallavotti lab: The Barren inflorescence3 mutant is caused by ectopic expression of a tandem duplicated ZmWUS1 gene.
- **Qiguo Yu** - Maliga lab: Engineered PPR10 RNA-Binding Protein for Transgene Regulation in Plastids.
- **Hiep Tran** - Steward lab: Tet dioxygenase controls neuronal development through 5-hydroxymethylation of ribocytosine in mRNA
- **Srividya Venkatramanan** - Irvine lab: Investigating the role of LIM domain proteins in regulation of Hippo signaling and the cytoskeleton
- **Jessica Fellmeth** - McKim lab: The role of Cenp-C in kinetochore building and chromosome segregation
- **Lu Wang** - Dong lab: BIP2 proteins function in membrane trafficking and the establishment of stomatal cell polarity
- **Amber Krauchunas** - Singson lab: The molecular underpinnings of the C. elegans fertilization synapse
- **Yaping Feng** - Genomics Core Facility: Omics data-mining
- **Jeremy Bird** - Ebright/Nickels labs: Functional roles and regulatory sensitivities of RNA 5' capping with NAD(H)
- **Konstantin Severinov** - Severinov lab: Off-target activity of CRISPR-Cas13 leads to cell death and abortive phage infection

## POSTER SESSION

- All authors listed on individual posters if not included below-

**Barr**

- Kade Power, R. O'Hagan; A. Gu; J. Walsh; M. Morash; S. Bellotti; W. Zhang; M. Barr: Determining the function of the kinase NEKL-4 in cilia
- Kumar Tiger; J. Wang; J. Walsh; M. Barr: Determining the function of globin gene GLB-28 in ciliated sensory neurons

**Dismukes Lab**

- Charles Dismukes: Forecasting climate adaptation strategies of photosynthetic organisms by learning from adaptive responses to CO2 concentration gradients in Yellowstone National Park
- Apostolos Zournas and Charles Dismukes: Screening to find the Usain Bolt of photosynthetic carbon fixation

**Dong Lab**

- Dongmeng Li and Juan Dong: Endosomal BIP2 proteins link PI3P to stomatal polarity
- Xiaoyu Guo and Juan Dong **:** BASL scaffolds BPP phosphatases to regulate stomatal asymmetric cell division

**Ebright Lab**

- David Degen**,** Herrman, J., Feng, Y., Müller, R., and Richard Ebright: The macrolide-glycoside antibiotics disciformycin and gulmirecin: inhibit bacterial RNA polymerase through interactions with the RNA polymerase CBR/AAP site
- Vadmin Molodtsov, Chengyuan Wang, Kaelber, J., and Richard Ebright: Structural basis of bacterial transcription-translation coupling. Gordon Conference on Mechanism and Regulation of Microbial Transcription
- Shuya Yang, Nova, I., Mazumder, A., Laszlo, A., Derrington, I., Gundlach, J., and Richard Ebright: Fractional-nucleotide translocation in sequence-dependent pausing by RNA polymerase: single-molecule picometer-resolution nanopore tweezers (SPRNT)
- Yu Liu, Winkelman, J., Yu, L., Pukhrambam, C., Zhang, Y., Nickels, B., and Richard Ebright: Structural and mechanistic basis of reiterative transcription initiation
- Chih-Tsung Lin, Ebright, Y.W., Liu, Y., Lin, W., Zhang, Y., Degen, D., Talaue, M., Wu, P., Zhang, Q. and Richard Ebright: Dual-targeted inhibitors of bacterial RNA polymerase

**Gallavotti Lab**

- Lei Sun, Zongliang Chen, Weibin Song and Andrea Gallavotti: Comprehensive reverse genetic analysis of the maize AUXIN RESPONSE FACTOR gene family
- Xue Liu, Mary Galli and Andrea Gallavotti: ADHERENT1 encodes a 3-ketoacyl CoA synthase that regulates cuticle formation in maize
- Mary Galli, Zefu Lu, Robert Schmitz, Carol S. Huang and Andrea Gallavotti: Mining transcriptional cis-regulatory modules in the maize genome
- Jason Gregory, Mary Galli and Andrea Gallavotti: Topographic analysis of RAMOSA ENHANCER LOCUS2 b-propeller loops in mediating TF partner binding

**Genomics Core Facility**

- Min Tu, Yaping Feng and Dibyendu Kumar: Opportunities and Challenges with Omics Data

**Maliga Lab**

- Lisa LaManna, Mugdha Parulekar, and Pal Maliga: A Multiplex CRISPR/Cas9 Gene Editing Platform to Obtain acc2-Knockouts in Brassica napus
- Aki Matsuoka, Juliana DiGiacomo, and Pal Maliga: Target Excision by Direct Export of a Site-Specific Recombinase from Agrobacterium to Tobacco Chloroplasts

**McKim Lab**

- Janet Jang and Kim McKim: Regulation of Meiotic Spindle Assembly and Cohesion in *Drosophila* Oocytes by PP2A
- Jay Joshi and Kim McKim: SPC105R is required for lateral microtubule attachments and maintaining sister centromere cohesion during meiosis in *Drosophila* oocytes
- Neha Changela and Kim McKim: Regulation of kinetochore-microtubule attachments by the Rod protein in *Drosophila* oocytes.

**Messing Lab**

- Paul Fourounjian and Joachim Messing: Flowering across the Lemnaceae
- Jiaqiang Dong and Joachim Messing: The recessive *dek23* mutant is uncovered by VarMap pipeline to encode an importin protein functioning during maize seed development.
- Yin Li and Joachim Messing: Characterization of phenotypic diversity of stem sugar in Sorghum
- Zhiyong Zhang and Joachim Messing: Towards celiac-safe bread

**Nickels Lab**

- Kyle Skalenko and Bryce Nickels: Mechanism of primer dependent transcription initiation



**Rongo Lab**

- Mehul Vora and Christopher Rongo: A MultiOmics approach to understand the hypoxia response pathway in *C. elegans*
- Mehul Vora and Christopher Rongo: Mimicking Human Marfan and Marfan-like Syndrome Mutations Alters Trafficking of the Type II TGFβ Receptor in *C. elegans*
- Jaegal Shim and Christopher Rongo: SURO-2/TMEM39 facilitates collagen secretion in collaboration with COPII
- Eunchan Park and Christopher Rongo: Monitoring Mitophagy in *C. elegans* using Mito-Keima

**Severinov Lab**

- Ishita Jain and Konstantin Severinov: Decoding the mechanism of CRISPR interference provided by RNA-guided RNA-targeting Cas13a effector

**Singson Lab**

- Xue Mei and Andrew Singson: A secreted immunoglobulin domain-containing protein, SPE-51, is required for sperm function at fertilization

**Yadavalli Lab**

- Sam Yadavalli: Bacterial Gene Regulatory Networks And Stress Response

# PRESENTATIONS & MEETING ABSTRACTS

**Waksman Institute Hosted Seminars**
Rutgers & Multi University/Professional, 50th Anniversary of the Discovery of Sigma Factors

Matthew Brooks, PhD, NIH Postdoctoral Fellow, Center for Genomics and Systems Biology, New York University, Network Walking: Charting nitrogen gene regulatory networks and their crosstalk with photosynthesis

Brad Nelms, PhD, Postdoctoral Fellow, Department of Biology, Stanford University, Cell differentiation at the boundary between generations

Jacob Brunkard, PhD, Plant and Microbial Biology and USDA Plant Gene Expression Center, University of California, Berkeley, TORning over a new leaf: specialized roles of TARGET OF RAPAMYCIN in coordinating plant metabolism

Fionn McLoughlin, PhD, Postdoctoral Research Associate, Department of Biology, Washington University of Saint Louis, Protein quality control during abiotic stress: interplay between sequestration, refolding and degradation

Mark Zander, PhD, Postdoctoral Researcher, The Salk Institute for Biological Studies, Plant Molecular and Cellular Biology Laboratory, Epigenomic regulation of phenotypic plasticity

Silva Ramundo, PhD, Postdoctoral Researcher, Department of Biochemistry and Biophysics, University of California, san Francisco, Dissecting the signaling network of the chloroplast unfolded protein response

**Waksman Student Scholars Programs**
Waksman Student Scholars Summer Institute, Waksman Institute, Rutgers University, July 8-July 19, 2019

Waksman Institute Summer Experience (WISE June-19),

Waksman Institute, Rutgers University, June 20-July 3, 2019.

Waksman Institute Summer Experience (WISE August-19), Waksman Institute, Rutgers University, July 29-August 9, 2019.

**Barber:**
Barber AF, Fong SY, Kolesnik A, Sehgal A. (2020). How neuropeptides and fast neurotransmitters interact to transmit time-of-day signals that regulate circadian feeding. Poster presented at Society for Biological Rhythms Meeting (virtual).

**Dismukes:**
2019 Gordon Research Conference: Photosynthesis, Grand Hotel, Newry, ME, July 23-28.

2019 DOE Hydrogen and Fuel Cell Technologies Research, Development &Demonstration; AMR.

**Dong:**
2020 Seminar at the Biology Department, University of Massachusetts, Amherst, MA
Title: "Polarity factors and regulators in stomatal asymmetric cell division."

2020 Seminar at the Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee, Knoxville
Title: "Polarity factors and regulators in stomatal asymmetric cell division."

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

2020 Symposium for Plant Molecular Biology, Peking University Title: The polarized molecular switch for plant asymmetric cell division.
2020 Seminar at the Department of Biology, McGill University, Montreal, Canada. Title: Polarity factors and regulators in plant asymmetric cell division.

2019 Shanghai Plant Reproduction Symposium, Shanghai Jiaotong University, China. Title: Cortical polarized protein phosphatases regulate plant asymmetric cell division.

2019 FASEB Mechanisms in Plant Development, Olean, NY Title: Cortical polarity segregates opposing functions of protein phosphatases in stomatal asymmetric cell division.

2019 Annual Meeting for Plant Cell Dynamics, Penn State University, PA. Title: Membrane signaling and trafficking in cell polarity and asymmetric division.



**Ebright:**
"Therapeutics for drug-resistant bacteria: arylmyxopyronins." Centers of Excellence in Translational Research Review Meeting, National Institute of Allergy and Infectious Diseases, Rockville Maryland, 2020.

"Structural basis of transcription antitermination by Q." Symposium on 50th Anniversary of Sigma Factors, Rutgers University, New Brunswick, New Jersey.

"Antibacterial drug discovery targeting bacterial RNA polymerase: myxopyronin (Myx)," Symposium on 75th Anniversary of Streptomycin, Rutgers University, New Brunswick, New Jersey.

"Antibacterial drug discovery targeting bacterial RNA polymerase: myxopyronin (Myx)." Genetics of Industrial Microorganisms Meeting, Pisa, Italy, 2019 (keynote address).

"Structural basis of transcription antitermination by Q." Gordon Research Conference on Mechanisms of Microbial Transcription, Bates College, Maine, 2019.

Herrman, J., Degen, d., Feng, Y., Müller, R., and Ebright, R.H. (2019) The macrolide-glycoside antibiotics disciformycin and gulmirecin: inhibit bacterial RNA polymerase through interactions with the RNA polymerase CBR/AAP site. Gordon Conference on Mechanism and Regulation of Microbial Transcription. Bates College, Maine, July 28-August 2, 2019.

Winkelman, J., Pukrambam, C., Zhang, Y., Taylor, D., Shah, P., Ebright, R.H., and Nickels, B. (2019) RNA polymerase profiling identifies pause sites in initial transcription. Gordon Conference on Mechanism and Regulation of Microbial Transcription. Bates College, Maine, July 28-August 2, 2019.

Bird, J., Kuster, D., Nickels, B., and Ebright, R.H. (2019) Functional roles and regulatory sensitivities of RNA 5' capping with NAD(H). Gordon Conference on Mechanism and Regulation of Microbial Transcription. Bates College, Maine, July 28-August 2, 2019.

**Gallavotti:**
Gallavotti, A. TBD. Northeast Regional Meeting of the Society for Developmental Biology, Woods Hole, MA, April 3-5, 2020 (meeting cancelled).

Gallavotti, A. Genetic and genomic dissection of maize inflorescence architecture. University of Georgia Plant Center Retreat, Unicoi State Park, October 24-25, 2019.

Gallavotti, A. A tandem duplication at the maize WUSCHEL1 locus is responsible for major architectural rearrangements in inflorescence meristems. FASEB The Mechanisms in Plant Development Conference, Olean, NY, July 28-August 2, 2019.

Chen, Z., Li, W., Gaines, C., Buck, A., Song, W., Galli, M., Gallavotti, A. A tandem duplication of the maize WUSCHEL1 gene promotes major architectural rearrangements in inflorescence meristems. Maize Genetics Conference Abstract 62:T14. Kailua-Kona, Hawai'i, HI, March 12-15, 2020 (meeting cancelled).

Dong, Z., Naing, T., Xu, Z., Galli, M., Gallavotti, A., Dooner, H., Chuck, G. Necrotic upper tips1 is a florally induced NAC transcription factor that pormotes water movement by fortifying protoxylem cell walls. Maize Genetics Conference Abstract 62:T28. Kailua-Kona, Hawai'i, HI, March 12-15, 2020 (meeting cancelled).

Craven, T., Yu, H., Ramos Baez, R., Chen, Z., Gallavotti, A., Nemhauser, J., Moss, B. Maize auxin response circuits recapitulated in yeast. Maize Genetics Conference Abstract 62:T38. Kailua-Kona, Hawai'i, HI, March 12-15, 2020 (meeting cancelled).

Armstrong, A., Taylor-Teeples, M., Galli, M., Nemhauser, J., Moss, B., Gallavotti, A. Assessing the impact of mutated AUX/IAA proteins in maize inflorescence development. Maize Genetics Conference Abstract 62:P101. Kailua-Kona, Hawai'i, HI, March 12-15, 2020 (meeting cancelled).

Griffin, B., Galli, M., Montes-Serey, C., Gallavotti, A., Walley, J.W. REL2 acetylation in plant pathogen interactions. Maize Genetics Conference Abstract 62:P131. Kailua-Kona, Hawai'i, HI, March 12-15, 2020 (meeting cancelled).

Liu, X., Strable. J., Bourgault, R., Galli, M., Chen, Z., Dong, J., Molina, I., Gallavotti, A. ADHERENT1 is a 3-KetoacylCoA synthase required for maize cuticle development and organ separation. Maize Genetics Conference Abstract 62:P149. Kailua-Kona, Hawai'i, HI, March 12-15, 2020 (meeting cancelled).

Wu, H., Galli, M., Zhan, J., Dannenhoffer, J.M., Yadegary, R., Gallavotti, A., Becraft, P.W. Investigation of NKD1, NKD2 and OPAQUE2 interaction on gene netwrok associated with maize endosperm development. Maize Genetics Conference Abstract 62:P214. Kailua-Kona, Hawai'i, HI, March 12-15, 2020 (meeting cancelled).

Gallavotti, A., Huang, C.S., Krogan, N., Li, M., Feng, F., Galli, M. Mapping and functional characterization of cis-regulatory variation in plants. Maize Genetics Conference Abstract 62:P219. Kailua-Kona, Hawai'i, HI, March 12-15, 2020 (meeting cancelled).

**Genomics Core Facility**
Lagda, AC. "New Jersey Core Facilities Showcase" Cook Student Center, Rutgers Busch Campus, New Brunswick, New Jersey. October 11, 2019

Lagda, AC. "2019 Regional Academic Drug Discovery Symposium" McDonell Hall, Princeton University, Princeton, New Jersey. November 1, 2019

Lagda, AC. "Leadership and Management in Core Facilities" Kellogg School of Management, Northwestern University, Chicago, Illinois. November 11-15, 2019

**Irvine:**
July 28 – Aug 10 2019 Invited lecture at KITP Workshop on "Morphogenesis in Animals and Plants: Search for Principles", UCSB, CA.

June 22-26, 2020 EMBO Virtual Meeting on Molecular Biology and Development of Drosophila

**Maliga:**
Pal Maliga "Engineered PPR10 RNA-binding protein for tissue-specific expression of recombinant proteins in potato tuber amyloplasts". Plant Biology 2019, The Annual Meeting of the American Society of Plant Biologists. San Jose, CA, August 2-7, 2019.

Pal Maliga: "Target Excision by Direct Export of a Site-Specific Recombinase from Agrobacterium to Tobacco Chloroplasts" 40th Annual Crown Gall Conference, October 25-27, 2019, University of Missouri, Columbia, MO

Pal Maliga: "Chloroplast Engineering: the Path to Commercial Products". AgWest Bio, Saskatoon, Canada, December 3, 2019,

Pal Maliga: Direct Export of a Site-Specific Recombinase from Agrobacterium to Tobacco Chloroplasts. International Plant & Animal Genome XXVII Conference, January 11-15, 2020, San Diego, CA.

Pal Maliga: Engineering the Chloroplast Genome of Canola Using Non-Transgenic Approaches. Cibus, San Diego, CA. January 10, 2020.

Qiguo Yu, Alice Barkan and Pal Maliga "Engineered PPR10 RNA-binding protein for tissue-specific expression of recombinant proteins in potato tuber amyloplasts". Plant Biology 2019, The Annual Meeting of the American Society of Plant Biologists. San Jose, CA, August 2-7, 2019.

Aki Matsuoka and Pal Maliga: "Target Excision by Direct Export of a Site-Specific Recombinase from Agrobacterium to Tobacco Chloroplasts" 40th Annual Crown Gall Conference, October 25-27, 2019, University of Missouri, Columbia, MO

Qiguo Yu, Tarinee Tungsuchat-Huang, Alice Barkan and Pal Maliga: "Synthetic Operons Tune Chloroplast Transgene Expression with Dynamic Range". Plant Synthetic Biology (SynBio2019) Conference, San Jose, CA, August 7-9, 2019

Lisa LaManna, Qiguo Yu, Megan E. Kelly, Kerry Ann Lutz, and Pal Maliga "New Tools for Engineering the Arabidopsis Plastid Genome". 3rd International Conference on Plant Synthetic Biology, Bioengineering, and Biotechnology, October 4-6, 2019, Cambridge, UK

**McKim:**
February 4, 2019: Regulation of Meiotic Spindle Assembly, Sister Chromatid Cohesion and Biorientation in Drosophila Oocytes New York Academy of Sciences, NY.

**Rongo:**
Rongo, C. Title: Uncovering Novel Mechanisms of Hypoxic Stress Response Using C. elegans. Presented at the Department of Biology, University of Texas at Arlington, Mar. 5, 2020.

**Singson:**
Frontiers in Reproduction Course Lecture, the Marine Biological Laboratories, Woods Hole MA (Delayed one year due to pandemic).

Invited Discussion Leader, Gordon Research Conference on Fertilization and Activation of Development.

Princeton Day School REx Program Lecture: "Research on fertility using worms."

**Yadavalli:**
Bacterial stress response to antimicrobial peptides. 2020 Annual Microbiology Symposium, Rutgers University



# PATENTS & PUBLICATIONS

## Patents

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

**Ebright:**
Ebright, R., Freundlich, J., Mittal, N., Jaskowski, M, and Shen, J. (2019) Inhibitors of bacterial RNA polymerase: arylpropanoyl, arylpropenoyl, and arylcyclopropanecarboxyl phloroglucinols. US Patent US10450292.

**Maliga:**
10,563,212 (2020) Intercellular transfer of organelles in plant species for conferring cytoplasmic male sterility. Inventors: Pal Maliga, Zora S. Maliga, Csanad Gurdon, Gregory Thyssen

## Publications:

**Barber:**
King AN, Schwarz JE, Hsu CT, Barber AF and Sehgal A. A circuit mechanism underlying suppression of circadian signals by homeostatic sleep drive. bioRxiv, p. 2020.04.29.068627. doi: 10.1101/2020.04.29.068627.

Barber AF and Sehgal A. (2020) 'Monitoring electrical activity in Drosophila circadian output neurons' in Brown, SA (ed.) Circadian Clocks: Methods and Protocols. New York: Springer Verlag

Barber AF and Sehgal A. (2018) Preview: Cold temperatures fire up circadian neurons. Cell Metabolism 27: 951-953

King AN, Barber AF, Smith AE, Dreyer AP, Sitaraman D, Nitabach MN, Cavanaugh DJ, & Sehgal A. (2017). A peptidergic circuit links the circadian clock to locomotor activity. Curr Biol 27: 1915-27

Barber AF, Erion R, Holmes TC and Sehgal A. (2016). Circadian and feeding cues integrate to drive rhythms of physiology in Drosophila insulin producing cells. Genes Dev 30: 2596-2606

Covarrubias M, Barber AF, Carnevale V, Treptow W, and Eckenhoff, RG. (2015) Mechanistic Insights into the Modulation of Voltage-Gated Ion Channels by Inhalational Anesthetics Biophys J 109: 2003-11

Barber AF, Carnevale V, Klein ML, Eckenhoff RG, and Covarrubias M. (2014) Modulation of a voltage-gated Na+ channel by sevoflurane involves multiple sites and distinct mechanisms. Proc Natl Acad Sci USA 111: 6726-31

Raju SG, Barber AF, Lebard DN, Klein ML and Carnevale V. (2013) Exploring Volatile General Anesthetic Binding to a Closed Membrane-Bound Bacterial Voltage-Gated Sodium Channel via Computation. PLOS Comput Biol 9: e1003090

Barber AF, Carnevale V, Raju SG, Amaral C, Treptow W and Klein ML (2012) Hinge-bending motions in the pore domain of a bacterial voltage-gated sodium channel. BBA Biomembranes 1818: 2120-25.

Barber AF, Liang Q, Covarrubias M. (2012) Novel activation of voltage gated K+ channels by sevoflurane. J Biol Chem 287: 40425-32

Barber AF, Liang Q, Amaral C, Treptow W and Covarrubias M. (2011) Molecular mapping of general anesthetic sites in a voltage-gated ion channel. Biophys J 101:1613-22

**Dismukes:**
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

'Birth defects' of photosystem II make it highly susceptible to photodamage during chloroplast biogenesis. Shevela, D., G. Ananyev, A.K. Vatland, J. Arnold, F. Mamedov, L. Eichacker, G.C. Dismukes and J. Messinger, Physiol. Plant 2019 May;166(1):165-180. doi: 10.1111/ppl.12932.

Crossing the Thauer limit: rewiring cyanobacterial metabolism to maximize fermentative H2 production. Kumaraswamy, G.K., A. Krishnan, G. Ananyev, D.A. Bryant and G.C. Dismukes, Energy & Environmental Science, 2019,12, 1035-1045.

The Catalytic Cycle of Water Oxidation in Crystallized Photosystem II Complexes: Performance and Requirements for Formation of Intermediates. Ananyev, G., S. Roy-Chowdhury, C. Gates, P. Fromme and G.C. Dismukes, ACS Catalysis, 2019. 9(2): p. 1396-1407.

Creating stable interfaces between reactive materials: titanium nitride protects photoabsorber–catalyst interface in water-splitting photocathodes. Hwang, S., S.H. Porter, A.B. Laursen, H. Yang, M. Li, V. Manichev, K.U.D. Calvinho, V. Amarasinghe, M. Greenblatt, E. Garfunkel and G.C. Dismukes, Journal of Materials Chemistry A, 2019. 7(5): p. 2400-2411

**Dong:**

Bian C, Guo X, Zhang Y, Wang L, Xu T, DeLong A, Dong J. Protein Phosphatase 2A promotes stomatal development by stabilizing SPEECHLESS in Arabidopsis. Proc Natl Acad Sci U S A. doi: 10.1073/pnas.1912075117.

Xue X, Bian C, Guo X, Di R and Dong J. 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. Cell polarity: Regulators and mechanisms in plants.
J Integr Plant Biol. 62(1):132-147. doi: 10.1111/jipb.12904.

Ge Z, Zhao Y, Liu MC, Zhou LZ, Wang L, Zhong S, Hou S, Jiang J, Liu T, Huang Q, Xiao J, Gu H, Wu HM, Dong J, Dresselhaus T, Cheung AY, Qu LJ. LLG2/3 Are Co-receptors in BUPS/ANX-RALF Signaling to Regulate Arabidopsis Pollen Tube Integrity. Curr Biol. 29(19):3256-3265.e5. doi: 10.1016/j.cub.2019.08.032.

Yang K, Zhu L, Wang H, Jiang M, Xiao C, Hu X, Vanneste S, Dong J, Le J. A conserved but plant-specific CDK-mediated regulation of DNA replication protein A2 in the precise control of stomatal terminal division. Proc Natl Acad Sci U S A. 116(36):18126-18131. doi: 10.1073/pnas.1819345116.

Guo X, Dong J. To Divide or Differentiate: It Is about Scaffolding. Trends Plant Sci. 24(6):481-484. doi: 10.1016/j.tplants.2019.03.007.

**Ebright:**

Panduwawala, T., Iqbal, S., Thompson, A., Christensen, K., Genov, M., Pretsch, A., Pretsch, D., Liu, S., Ebright, R.H., Howells, A., Maxwell, A., and Moloney, M. (2019) Functionalised bicyclic tetramates derived from cysteine as antibacterial agents. Org. Biomol. Chem. 17, 5615-5632.

Talbot, G., Jezek, A., Murray, B., Jones, R., Ebright, R.H., Nau, G., Rodvold, K., Newland, J., and Boucher, H. (2019) The Infectious Diseases Society of America's 10 × '20 initiative (ten new systemic antibacterial agents FDA-approved by 2020). Clin. Infect. Dis. 69, 1-11.

Yin, Z., Kaelber, J., and Ebright, R.H. (2019) Structural basis of Q-dependent antitermination. Proc. Natl. Acad. Sci. USA 116, 18384-18390.

Ebright, R.H., Werner, F., and Zhang, (2019). RNA polymerase reaches 60: transcription initiation, elongation, termination, and regulation in prokaryotes. J. Mol. Bio. 431, 3946-3946.

Li, L., Molodtsov, V., Lin, W., Ebright, R.H., and Zhang, Y. (2020) RNA extension drives a stepwise displacement of an initiation-factor structural module in initial transcription. Proc. Natl. Acad. Sci. USA 117, 5801-5809.

Winkelman, J., Pukhrambam, C., Zhang, Y., Shah, P., Taylor, D., Ebright, R.H., and Nickels, B. (2020) XACT-seq comprehensively defines the promoter-position and promoter-sequence determinants for initial transcription pausing. Mol. Cell (in press)

Winkelman, J., Nickels, B., and Ebright, R.H. (2020) The transition from transcription initiation to transcription elongation: start-site selection, initial transcription, and promoter escape. In RNA Polymerase as a Molecular Motor, Second Edition. Eds. Landick, R., Wang, J., and Strick, T. (RSC Publishing, Cambridge, UK) (in press).

**Gallavotti:**

Liu, X., Bourgault, R., Strable, J., Galli, M., Chen, Z., Dong, J., Molina, I., Gallavotti, A. The FUSED LEAVES1-ADHERENT1 regulatory module is required for maize cuticle development and organ separation. BioRxiv 2020, https://doi.org/10.1101/2020.02.11.943787.

Baez, R.R., Buckley, Y., Yu, H., Chen, Z., Gallavotti, A., Nemhauser, J., Moss, B.L. "A synthetic approach reveals a highly sensitive maize auxin response circuit". Plant Phys 2020 (182), 1713-1722.

Ricci, W., Lu, Z., Ji, L., Marand, A., Ethridge, C., Murphy, N., Noshay, J., Galli, M., Mejia-Guerra, M.K., Colome-Tatche, M., Johannes, F., Rowley, M., Corces, V., Zhai, J., Scanlon, M., Buckler, E., Gallavotti, A., Springer, N., Schmitz, R., Zhang, X. "Widespread long-range cis-regulatory elements in the maize genome". Nature Plants 2019 (5), 1237-1249.

Liu, Q., Galli, M., Liu, X., Federici, S., Buck, A., Cody, J., Labra, M., Gallavotti, A. "NEEDLE1 is a mitochondria localized ATP-dependent metalloprotease required for thermotolerant maize growth" Proc Natl Acad Sci USA 2019 (116), 19736-19742.

**Irvine:**

Crespo-Enriquez, I., Hodgson, T., Zakaria, S., Cadoni, E., Shah, M., Allen, S., Al-Krishali, A., Mao, Y., Yiu, A., Petzold, J., Villagomez-Olea, G., Pitsillides, A.A., Irvine, K.D., and Francis-West, P. 2019. Dchs1-Fat4 regulation of osteogenic differentiation in mouse. Development 146, p. dev176776.

Ibar, C. and Irvine, K.D. 2020. Integration of Hippo-YAP signaling with metabolism. Dev Cell, in press.

Li, R., Ibar, C., Zhou, Z., Irvine, K.D., Liu, L., Lin, H. 2020. E2 temperature and Gamma distribution of polygonal planar tessellations. arXiv preprint arXiv:2002.11166.

Venkatramanan, S., Ibar, C., Irvine, K.D. TRIP6 is required for tension at adherens junctions. bioRxiv. doi.org/10.1101/2020.04.19.049569.

**Maliga:**

Yu, Q., Lamanna, L.M., Kelly, M.E., Lutz, K.A. and Maliga, P. (2019) New Tools for Engineering the Arabidopsis Plastid Genome. Plant Physiol. 181: 394-398. DOI:10.1104/pp.19.00761

Yu Q, Tungsuchat-Huang T, Verma K, Radler MR, Maliga P (2020) Independent translation of ORFs in dicistronic operons, synthetic building blocks for polycistronic chloroplast gene expression. Plant J. doi: 10.1111/tpj.14864

**McKim:**

Wang, L.I., DeFosse, T., Battaglia, R.A., Wagner, V., Radford, S.J. and McKim, K.S. Acentrosomal spindle assembly and chromosome bi-orientation in Drosophila oocytes depends on interactions between HP1 and the Chromosome Passenger Complex. In preparation

Jang, J.K., Gladstein, A. Sisco, Z. and McKim, K.S. Regulation of Meiotic Spindle Assembly and Cohesion in Drosophila Oocytes by Protein Phosphatase 2A. In preparation.

Wang LI, Das A, McKim KS. Sister centromere fusion during meiosis I depends on maintaining cohesins and destabilizing microtubule attachments. PLoS Genet. 2019 15(5):e1008072

**Nickels:**

XACT-seq comprehensively defines the promoter-position and promoter-sequence determinants for initial-transcription pausing. Winkelman JT, Pukhrambam C, Zhang Y, Shah P, Taylor DM, Ebright RH, and Nickels BE. Molecular Cell (2020) in press.

Functional determinants of a small protein controlling a broadly conserved bacterial sensor kinase. Yadavalli SS, Goh T, Carey JN, Malengo G, Vellappan S, Nickels BE, Sourjik V, Goulian M, and Yuan J. Journal of Bacteriology (2020) doi: 10.1128/JB.00305-20

Universal promoter scanning during transcription initiation in Saccharomyces cerevisiae.
Qiu C, Jin H, Vvedenskaya IO, Abante-Llenas J, Zhao T, Malik I, Visbisky AM, Schwartz SL, Cui P, Čabart P, Han KH, Lai WK, Metz RP, Johnson CD, Sze SH, B. Pugh F, Nickels BE, and Kaplan CD. Genome Biology (2020) 21:132.

The transition from transcription initiation to transcription elongation: start-site selection, initial transcription, and promoter escape. Winkelman JT, Nickels BE, and Ebright, RH. in RNA Polymerase as a Molecular Motor, Second Edition. Eds. Landick, R., Wu, J., and Strick, T. (RSC Publishing, Cambridge, UK) (2020) in press.

CapZyme-Seq: a 5'-RNA-seq method for differential detection and quantitation of NAD-capped and uncapped, 5'-triphosphate RNA. Vvedenskaya IO and Nickels BE. STAR Protocols (2020) 1, 100002.

**Rongo:**

NeMoyer, R., Mondal, A., Vora, M., Langenfeld, E., Glover, D., Scott, M., Lairson, L., Rongo, C., Augeri, D.J., Peng, Y., Jabbour, S.K., and Langenfeld, J. (2019) Targeting bone morphogenetic protein receptor 2 sensi-

tizes lung cancer cells to TRAIL by increasing cytosolic Smac/DIABLO and the downregulation of X-linked inhibitor of apoptosis protein. Cell Communication and Signaling. Nov 19;17(1):150. PMID: 31744505.

Joshi, K., Matlack, T.L., Pyonteck, S., Menzel, R., and Rongo, C. (2020) Biogenic amine neurotransmitters promote eicosanoid production and protein homeostasis. bioRxiv 2020.02.25.964189.

**Severinov:**
Zyubko, T., Serebryakova, M., Piskunova, J., Metelev, M., Dubiley, S., Severinov, K. (2019) Efficient in vivo synthesis of lasso peptide pseudomycoidin proceeds in the absence of leader and leader peptidase. Chem. Sci., 14, 1619-1627

Maikova, A., Kreis, V., Boutserin, A., Severinov, K., and Soutourina, O. (2019) Using endogenous CRISPR-Cas system for genome editing in the human pathogen Clostridium difficile. Appl. Env. Microbiol., 85, e01416-19

Shmakov, S. A., Faure, G., Makarova, K. S., Wolf, K. I., Severinov, K. V., and Koonin, E.V. (2019) Systematic prediction of functionally linked genes in microbial genomes. Nat. Protocols, 14, 3013-3031

Shiriaeva, A., Savitskaya, E., Datsenko, K. A., Vvedenskaya, I. O., Fedorova, Y., Morozova, N., Metlytskaya, A., Sabantsev, A., Nickels, B. E., Severinov, K., and Semenova E. (2019) Detection of spacer precursors formed in vivo during primed CRISPR adaptation. Nat. Comm., 10, 4603

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