



2021
2022
Waksman Institute

ANNUAL
REPORT



ABOUT US

The Waksman Institute of Microbiology is an interdisciplinary research institute of Rutgers, The State University of New Jersey, devoted to excellence in foundational life sciences research. We host investigators addressing a broad range of fundamental questions in biology, as well as the development of novel biotechnologies, to push the frontiers of scientific discovery.

Faculty research areas encompass biochemistry, cell biology, developmental biology, neuroscience, structural biology, genetics, and genomics. A distinctive feature of Waksman Institute research is our commitment to foundational studies (basic research) that generate the novel discoveries that lead to advances in scientific understanding. Consequently, we have a particular strength in core model systems that have been responsible for many fundamental advances in biology (eg *E. coli*, *Drosophila*, *C. elegans*, *Arabidopsis*, maize). At the same time, we support translation of discoveries into practical advances, as evidenced by the success of our faculty in disclosing, patenting, and licensing new technologies.

We are also actively engaged in research education through the training of undergraduate, graduate, and post-doctoral students and fellows in research, and through classroom teaching that our faculty engage in through their joint appointments with academic departments at Rutgers. We also run high school science outreach programs that introduce students in New Jersey and across the country to life sciences research.

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 or visit <http://give.rutgers.edu/WaksmanInstitute>.



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Rutgers Research and Educational Foundation

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

RREF Committee Members 2021-2022

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REPORT OF THE INTERIM DIRECTOR

Kenneth Irvine



Report of the Interim Director

Over the past academic year, we transitioned from an environment constrained by the Covid-19 pandemic to the resumption of full time, in person research. Laboratory space that we had provided to RUCDR/iBX for Covid-19 testing is now devoted to expanding new frontiers of fundamental research. I am especially pleased that we have been able to welcome back undergraduate students into our laboratories, where they can once again participate fully in our research programs. During Summer 2022, we have also been able to welcome back high school students participating in our renowned outreach programs, The Waksman Student Scholars Program and the Waksman Institute Summer Experience. Highlights over the past year include the recruitment of Nick Stavropoulos as a new, Associate Research Professor. Dr. Stavropoulos conducts NIH-funded research into mechanisms that control sleep. I am also happy to note that Dr. Juan Dong received a well-deserved promotion from Associate to full Professor. Our imaging facilities are in the process of being updated and expanded thanks to \$2 million in state funding received for the Waksman Imaging center.

The new instruments obtained for this center will provide researchers at Rutgers with powerful new capabilities for analyzing tissues, cells, and molecules. During this coming year, we look forward to hosting a celebration of our late Director, Dr. Joachim Messing, with a symposium and endowed lectureship to be held in his honor in Spring 2023.

Throughout the fiscal and educational challenges of the past year, our faculty has continued to advance the frontiers of knowledge across the breadth of the life sciences, while training the next generation of scholars and researchers. Their accomplishments, as described in this report, are impressive and inspirational. They have been ably assisted in accomplishing our mission by our outstanding administrative and facilities staff, working under the direction of Executive Director of Finance and Administration Bob Rossi.

Waksman Mission

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

Overview of the Waksman Institute

The Institute was established in part through royalties that Rutgers received from patents on antibiotics discovered in the laboratory of its first director, Selman Waksman. The most significant of these was streptomycin, the first antibiotic effective against tuberculosis, which was discovered by Waksman's student Albert Shatz. While the initial focus of the Institute was microbiology and antibiotic discovery, the Institute's research mission has evolved and broadened with advances in scientific methods and understanding over 7 decades, while remaining true to Dr. Waksman's exhortation that the Institute should be "dedicated to the free pursuit of scientific knowledge for the benefit of all mankind", devoted to the study of the simplest forms of life, and with the goal of serving as "a center where scientists may gather to work, to learn, and to teach." The Waksman Institute currently supports investigations into a broad range of fundamental questions in biology, as well as the development of novel biotechnologies, to push the frontiers of scientific discovery. Using microbial, plant, and animal models, Waksman scientists conduct research on diverse topics including morphogenesis, gene regulation, signal transduction, cancer, fertility, metabolism, sustainable energy, congenital and neurologic disorders, together with antibiotic discovery. A key aspect of research in the Institute is its

interdisciplinary nature, including faculty from multiple departments and schools, and incorporating approaches from physical and computational sciences into life sciences research. The Institute has a long history of excellence, including Rutgers's only Nobel prize winner (Selman Waksman), only Lasker prize winners (Selman Waksman and Evelyn Witkin), and recipients of a number of other major awards and prizes, including Wolf Prize winners, Howard Hughes Medical Institute Investigators, and inductees into the National Academy of Sciences.

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

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

Faculty

In the academic year 2021-2022, the Institute had sixteen resident tenured or tenure-track Lab Directors, one non-tenure track Lab Director, and seven emeriti faculty. The Institute also currently has five assistant research professors, eight visiting student/scholar researchers, ten research associates, twelve postdoctoral researchers, twenty-five technical assistants, and graduate students. The Waksman Institute's total resident population is currently 144, including thirty-five undergraduate students who did independent research during the last year in Waksman Institute labs.

Among the tenured or tenure-track Waksman faculty, five are in the Department of Molecular Biology and Biochemistry, five are in the Department of Genetics, four are in the Department of Plant Biology, 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 seventeen current Lab Directors, three are Assistant Professors, two are Associate Professors, eight are Professors, and four are Distinguished Professors, one of whom is also a Board of Governors Professor. Notable faculty awards this past year include the election of Andy Singson as a Fellow of the American Association for Advancement of Science, the Provost-Chancellor Award (2021) for Pioneering Research to Chuck Dismukes, and the selection of Annika Barber as a Scialog: Microbiome, Neurobiology and Disease Fellow.

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 over \$6 million dollars in external grant funding, and I am especially pleased to note that our newest faculty are already having success in securing external funding. We also received \$2 million in state funding for our imaging center.

ADMINISTRATION REPORT

Robert Rossi

Administration

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 two 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, shared imaging facility 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
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INFORMATION AND TECHNOLOGY REPORT

Randall Newman

The Waksman Institute employs two full time staff to maintain the computing resources and provide software and hardware support to all of our faculty, staff, and students. The 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. 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 1.2 PB 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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Daja O'Bryant, Unit Computing Specialist

BARBER LAB

Circadian Behavioral Genetics in Diverse Environmental Contexts



Dr. Annika Barber
Molecular Biology & Biochemistry

Summary

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

But many aspects of life are unpredictable, so circadian signals must be integrated with sensory cues about the flies' internal and external environment to inform behavioral choices. The Barber lab uses the fruit fly, *Drosophila melanogaster*, as a model system to investigate how neuronal networks regulating conserved behaviors integrate sensory and circadian cues to inform behavioral choices. We use the circadian clock output circuit to understand how co-transmitter signaling by small molecule neurotransmitters and neuromodulatory

peptides affects neurophysiology and circadian behaviors such as sleep, locomotor rhythms and feeding. We also seek to understand how environmental context affects circadian signaling at the molecular and circuit levels to alter physiology, behavior and ultimately other aspects of health such as longevity, and survival after traumatic injury. While our work focuses on circadian physiology, our findings elucidate fundamental aspects of how environmental contexts shape neuronal circuits to alter behavior.

Characterization of a signal integration "hub"

The *Drosophila pars intercerebralis* (PI) is an analog of the mammalian hypothalamus and regulates numerous processes including sleep, arousal, locomotor rhythms, feeding, and gene transcription in peripheral tissues. As in the hypothalamus, multiple internal and external sensory pathways converge in the PI, which then releases an array of neuropeptides that influence fly behavior. This project examines how time-of day signals are communicated to the PI by the clock neuron circuit by both fast neurotransmitters and neuropeptide signals, and investigates the role of intra-PI PI signaling in coordinating locomotor and feeding behavior. Using the novel *Drosophila* retrograde circuit tracing tool BAcTrace, we have identified intra-PI connectivity and novel inputs to the PI directly from the ventrolateral circadian pacemaker neurons (Fig. 1).

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

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

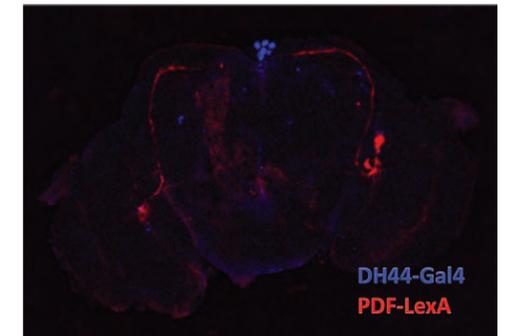


Fig. 1: Clock-to-PI retrograde tracing. Expression of the BAcTrace system in DH44+ neurons (blue) labels PDF+ clock neurons in the fly brain, indicating synaptic connectivity.

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

Development of a novel inducible gene expression system in *Drosophila*

In collaboration with the Southall Lab at Imperial College London, we have developed the Auxin-inducible Gene Expression System (AGES) an auxin-inducible degron system that is non-toxic and effective for manipulation of gene expression in larval and adult flies. AGES relies on the auxin-dependent degradation of a ubiquitously expressed GAL80, and is thus compatible with existing GAL4-driver lines. Water-soluble auxin is added to fly food at a low concentration, which induces expression comparable to uninhibited GAL4 expression. The system is suitable for induction of fluorescent protein expression in larval and adult brains, and auxin feeding has no effect on development or feeding behavior of flies. We recapitulated a classic experiment demonstrating that AGES-induced silencing of clock neurons results in rapid loss of circadian locomotor rhythms in adult flies. We are continuing to develop this system in combination with CRISPR and RNAi approaches, as well as engineering components of the degron system to allow the use of synthetic auxin analogs at even lower doses. This work was published in eLife.

Age x Diet effects on circadian function

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

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

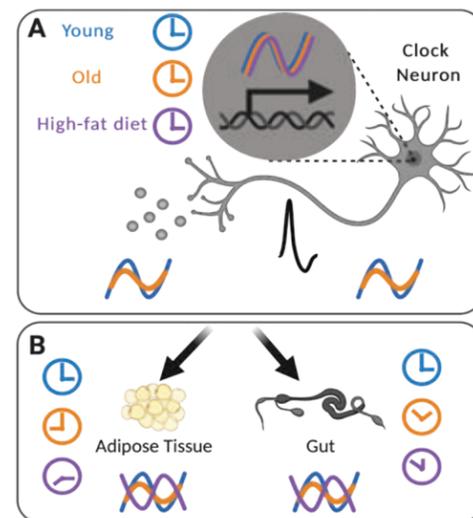


Fig. 2 Degradation of circadian signals by age and dietary challenge. (A) Aging dampens rhythms of neurotransmitter release (left) and physiological properties (right) of brain clock neurons are dampened with age, despite intact transcriptional rhythms. (B). High-fat diet induced loss of circadian phase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

Dr. Annika Barber, Assistant Professor

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Chenyue Zhao, Undergraduate Assistant

Maryem Abdelgelil, Undergraduate Assistant

Akanksha Mathivanan, Undergraduate Assistant

James Yazzo, Laboratory Technician



DISMUKES LAB

Biological and Chemical Approaches to Renewable Energy Research



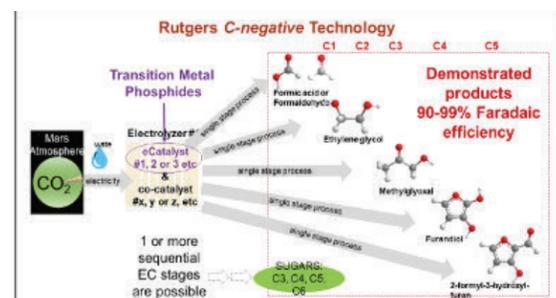
Dr. G. Charles Dismukes
Chemistry & Chemical Biology
Microbiology & Biochemistry

Summary

The Dismukes research group conducts fundamental and applied research in the areas of catalysis, renewable energy, and sustainable production of chemicals via biological and chemical approaches. The biological approach focuses on investigations of the light reactions and central carbon metabolism in photosynthetic microorganisms. The chemical approach involves synthesis of bioinspired electrocatalysts and their integration with electrolyzers and artificial photosynthetic systems. The disciplinary approaches used are genetic engineering, materials synthesis by design, electrochemistry and catalysis. The goal is to produce sustainable processes for renewable fuels and biomass production. Our laboratories are in the Waksman Institute of Microbiology and the Chemistry and Chemical Biology Laboratory at Rutgers University. Additionally, Dr Dismukes is a member of the Rutgers graduate training faculties in Chemical and Biological Engineering, Biochemistry and Microbiology, and Earth and Planetary Sciences. In the 2021-July 2022 period the group was comprised of 27 researchers and interns.

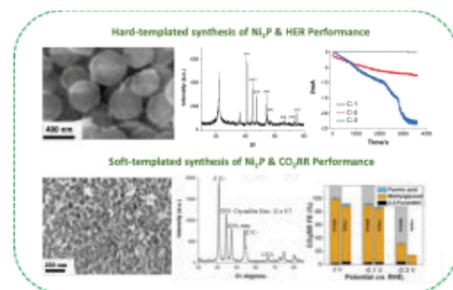
Bioinspired Electrocatalysts for Water Splitting and CO₂ Reduction. CCB.

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 CO₂ to make chemicals and food. In this project we apply the principles learned from enzymes to synthesize better heterogeneous catalysts for the generation of H₂ and O₂ from water (like Photosystem II) and CO₂ 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 and in the best cases can exceed the activity of commercial catalysts used today. Using this strategy, we have produced noble-metal-free catalysts for water oxidation (LiCo₂O₄), water reduction to hydrogen (Ni₃P₄), and selective CO₂ reduction to C₁ to C₅ products (Nickel Phosphides). The image depicts our electrolytic process for converting water and CO₂ into C₁ to C₅ products. Supported by RenewCO₂; DOE-SBIR, NA-SA-CO₂ Challenge, Rutgers Goldman Prize; Collaborations: UPenn, CU Boulder and RenewCO₂.



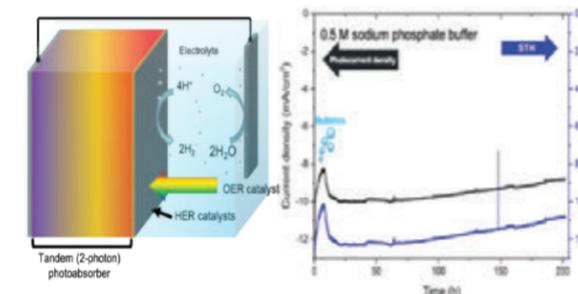
Synthesis of Transition Metal Phosphides with Defined Structural Phase, Facet and Morphology for Electrocatalysis. CCB.

In this project, we use a templating approach to synthesize nickel phosphide catalysts for hydrogen evolution and 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 frameworks to synthesize binary transition metal phosphide compounds to test as catalysts. XRD and SEM characterizations have revealed phase purity and unique morphology of the synthesized nanomaterials. These catalysts are currently being evaluated for HER and CO₂RR. HER results show high current densities can be obtained (~25mA/cm² and ~400mA/cm² for hard and soft template Ni₂P respectively). CO₂RR results show soft-templated Ni₂P catalyst can convert CO₂ to C₁, C₃ and C₄ products with high Faradaic efficiencies. Collaborator: UPenn. Support: DOE NREL-LDRP; RenewCO₂



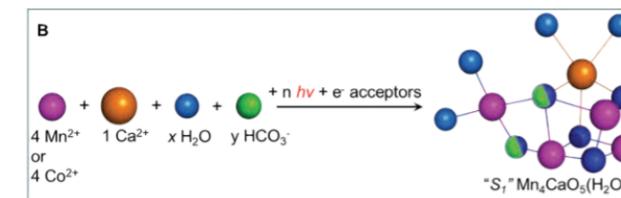
Best-in-class Platinum Group Metal-free Catalyst Integrated Tandem Junction PEC Water Splitting Devices. CCB.

Solar energy conversion to fuels requires initially the splitting of water into its elements, 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.



Why Did Nature Choose Manganese Exclusively to Make Oxygen on Earth? Waksman.

All contemporary oxygenic phototrophs split water using a single invariant cluster comprised of Mn₄CaO₅ (the WOC) as the catalyst within Photosystem II of natural photosynthesis. This cluster is unstable outside of PSII and can be reconstituted, both *in vivo* and *in vitro*, using the elemental aqueous ions and light, a process called photoassembly. Here we demonstrate the first functional substitution of manganese in any oxygenic reaction center by *in vitro* photoassembly with cobalt. Following complete removal of the inorganic cofactors from cyanobacterial PSII microcrystals (PSIIX), photoassembly with free cobalt (Co²⁺), calcium (Ca²⁺) and water (OH⁻) reassembles >90% of PSII centers. This process occurs 3-fold faster using Co²⁺ vs. Mn²⁺, due to a higher quantum yield for PSIIX-mediated photochemistry (*): Co²⁺ → * → Co³⁺Q_A⁻. The resulting reconstituted Co-PSIIX oxidizes water by the standard four-flash photocycle, though produces 4-fold less O₂ per PSII, attributed to faster charge recombination (Co³⁺Q_A⁻ ← Co⁴⁺Q_A⁻) in the catalytic cycle. Thus, the kinetic preference for Co²⁺ over native Mn²⁺ during photoassembly is offset by significantly poorer catalytic activity. Another disadvantage arises from faster photodamage of the reconstituted Co-PSIIX (3-fold faster than with the reconstituted Mn-PSIIX). This is attributed to photooxidative damage to PSII during the catalytic cycle. Manganese offers two advantages for oxygenic phototrophs that explains its exclusive retention throughout Darwinian evolution: significantly slower charge recombination (Mn³⁺Q_A⁻ ← Mn⁴⁺Q_A⁻) permits more water oxidation at low and fluctuating solar irradiation (> net energy conversion), and much greater tolerance to photooxidative damage at high light intensities (Mn⁴⁺ less damaging than Co⁴⁺). The physico-chemical basis for these differences leads us to propose changes to the coordination environment of the WOC that could create stable “inorganic mutants” with novel properties. Supported by DOE-BES. Collaboration: Arizona State U.



Bridging the gap between Markov-type and kinetic models of photosynthetic electron transport. Waksman.

Historically, two modeling approaches have been developed independently to describe photosynthetic electron/proton transport (PET) from water to plastoquinone within Photosystem II (PSII): Markov models account for losses from finite redox transition probabilities but predict no reaction kinetics, and ordinary differential equation (ODE) models account for kinetics but not for redox inefficiencies. We have developed an ODE mathematical framework to calculate Markov inefficiencies of transition probabilities as defined in Joliot-Kok-type catalytic cycles. We adapted a previously published ODE model for PET within PSII that accounts for 238 individual steps to enable calculation of the four photochemical inefficiency parameters (miss, double hit, inactivation, backward transition) and the four redox accumulation states (S-states) that are predicted by the most advanced of the Joliot-Kok-type models (VZAD). Using *only* reaction kinetic parameters without other assumptions, the RODE-calculated time-averaged (eg., equilibrium) inefficiency parameters and equilibrium S-state populations agree with those calculated by time-independent Joliot-Kok models. RODE also predicts their time-dependent values during transient photochemical steps for all 96 microstates involving PSII redox cofactors. We illustrate applications to two cyanobacteria, *Arthrospira maxima* and *Synechococcus sp.* 7002, where experimental data exists for the inefficiency parameters and the S-state populations, and historical

data for plant chloroplasts as benchmarks. *Significant findings:* RODE predicts the microstates responsible for period-4 and period-2 oscillations of O_2 and fluorescence yields and the four inefficiency parameters; the latter parameters are not constant for each S state nor in time, in contrast to predictions from Joliot-Kok models; some of the recombination pathways that contribute to the backward transition parameter are identified and found to contribute when their rates exceed the oxidation rate of the terminal acceptor pool (PQH_2); prior reports based on the assumptions of Joliot-Kok parameters may require reinterpretation. Supported by DOE-BES-PS.

Regulation of linear electron flow (LEF) in the Z-scheme from water oxidation to CO_2 reduction. Waksman.

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

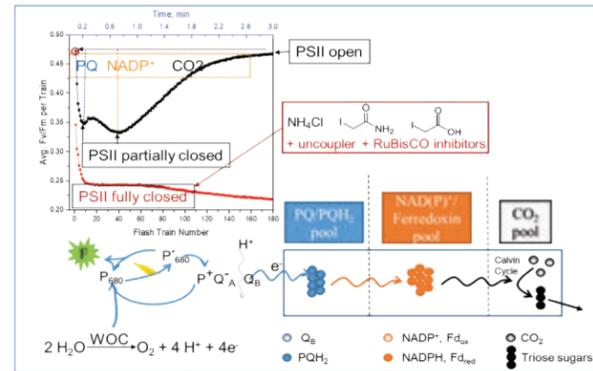
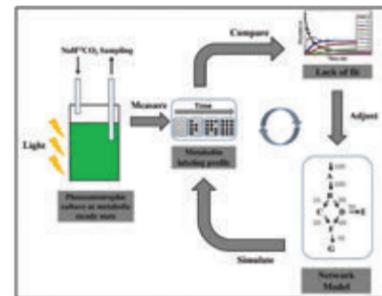


Figure 12. Changes in Chl fluorescence F_v/F_m under illumination measured by FRRF.

Photoautotrophic Carbon Fluxomics. Waksman.

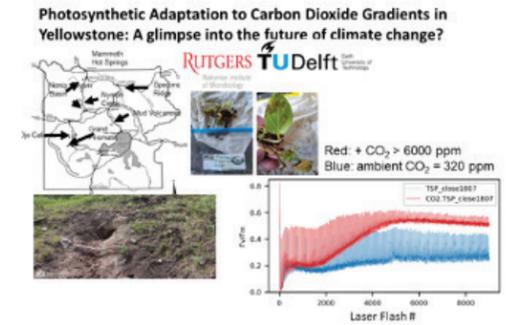
Metabolic pathways for model organisms can be found in textbooks. However, these are widely modified across the ToL and novel pathways for making carbon products abound in nature that remain to be discovered. Improving the abysmal solar to biomass efficiency of plants and algae through metabolic engineering 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 non-stationary 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 China.



Forecasting the impact of CO_2 -driven climate change on photosynthetic metabolism from the long-term adaptation of plants to naturally high CO_2 sources in the environment: Yellowstone NP

Accurate prediction of the response of primary photosynthetic productivity to anthropogenic climate change is a vital need for forecasting its global consequences on the health of the environment and on agricultural productivity. Rising carbon dioxide emissions are the main driver of climate change which has multiple influences on primary productivity (PP). These arise from complex interactions between temperature, water distribution, solar insolation, CO_2 concentration, and nutrient availability. Each of these factors influences primary photosynthetic productivity in non-linear ways such that even local ecosystem models of simple monocultures have failed to capture the dependence on these variables. To improve the predictions, we have developed a fluorescence method to measure the rate of CO_2 carboxylation in the field that bypasses the diffusion barrier to CO_2 uptake. The method monitors chlorophyll variable fluorescence yield (F_v/F_m) in response to a train of light pulses (Figure). Preliminary data were obtained for a C_3 plant (prunella

v.) in Yellowstone NP that grew above > 6000 ppm CO_2 and measured at both ambient (~320 ppm) and high (>6,000 ppm) CO_2 . Prunella plants adapt to high CO_2 by adjusting their yield of carboxylation and its rate, while also decreasing biomass production. This method offers a quantitative approach to separate the effects of changing CO_2 levels from other environmental consequences of carbon dioxide emissions. Busch-Waksman Seed. Collaboration: TU Delft.



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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 many cellular events during asymmetric cell division (Figure 1).

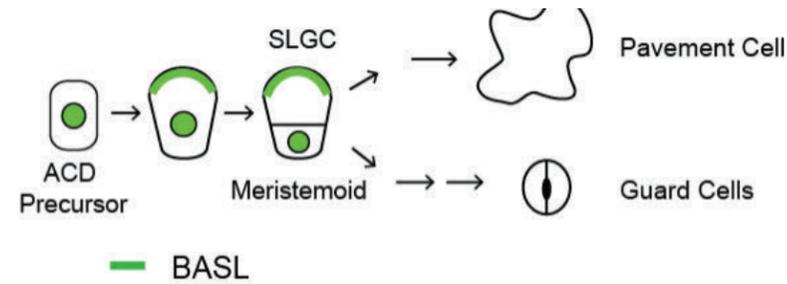


Figure 1. BASL localization and stomatal asymmetric cell fate

By using BASL as an anchor for screens to isolate genetic and physical interactors and by using the features of the protein itself as a probe for cell's ability to correctly establish polar cortical localization, our lab is in the process of building a model for plant cell polarization and its regulation in ACD. We are also interested in understanding the molecular mechanisms underlying how cell polarity orients cell division 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. During 2021-2022, we made major contributions below a few important areas in the cell polarity and asymmetric cell division field.

Membrane trafficking for polarity initiation and establishment in plant cells

The molecular mechanisms for protein polarization in plants have been extensively studied in two systems: 1) vesicular trafficking-based polarization of PIN auxin efflux carriers (membrane embedded proteins) and 2) cytoskeleton-dependent and -independent positive feedback loop-based ROP polarization (small Rho-like GTPases from plants). Polar trafficking of BASL, a non-membrane novel protein, has not been successfully connected to both pathways and might represent an unknown mechanism. Fluorescence Recovery After Photobleaching (FRAP) was performed on GFP-BASL and the recovery curves suggested that BASL dynamics is more comparable to the membrane-embedded PIN proteins, hinting the possible regulation of membrane trafficking in BASL polarization. Indeed, through the identification of physical interactors of BASL, we recently established that four members of the PRAF protein family interact with BASL and are required for the establishment of the polarity site (Wang et al., 2022 Nat. Commun) (Figure 2). The PRAF proteins are plant specific but contain phospholipid-binding domains and co-localize with small GTPases, both of which are conserved across the kingdoms in regulating membrane trafficking and delivery.

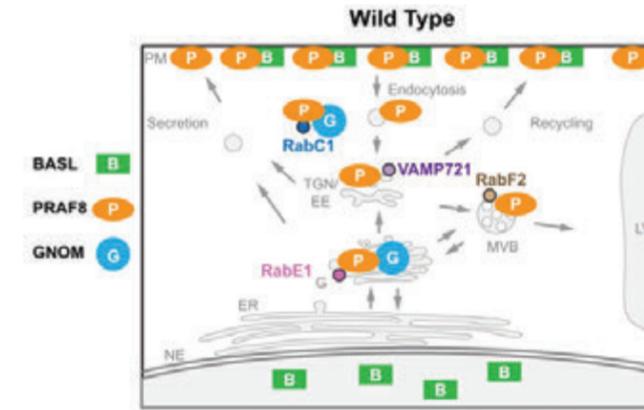


Figure 2. A working model for PRAF-mediated trafficking in establishing BASL polarity.

In wild-type plants, the polarization of BASL protein (green) in the stomatal lineage cell requires the physical partner, four PRAF proteins (orange), as well as the Golgi-localized Arf GEF GNOM (blue). The PRAF8 proteins are predominantly localized to the plasma membrane, where they may polarize together with BASL. The PRAF8 proteins may also partially associate with the Golgi, TGN/EE and a subset of endosomes/vesicles decorated by RabC1 and RabF2b. Furthermore, the PRAF proteins physically interact with GNOM, possibly leading to the association of GNOM to the RabC1- and RabE1-decorated membrane structures.

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

Our previous work showed that BASL is phosphorylated and activated by MAPK 3 and 6 (MPK3/6) and becomes 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 from other labs 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, so that stomatal ACD is promoted. Therefore, the stomatal polarity complex by scaffolding different signaling molecules could promote the division potential before an ACD and suppress the division potential after an ACD. However, how the transition of these two seemingly opposing procedures can be achieved by the same polarity complex remained a major challenge towards understanding stomatal ACD. Here, by using immunoprecipitation combined with mass spectrometry (IP-MS), we identify a family of protein Ser/Thr BSL phosphatases, as BASL partners (Guo, 2021 Nat Plants). Genetic analysis places BSL upstream of the YDA MAP kinase cascade and downstream of the plasma membrane receptors. In addition, the founding member BSL1 colocalizes with BASL in a polarized manner at the cell periphery. Interestingly, the recruitment of the BSL phosphatases in the polarity module confers a negative role to BIN2 complex but a positive role to the YDA MAPK module (Figure 3). Thus, our study reveals a crucial function of the BSL phosphatases in bridging the two opposing protein functional modules to control the balance of cell-division potential and cell-fate determination in plant ACDs. This work also represents the initiation of collaboration with the Nickels lab at the Waksman Institute.

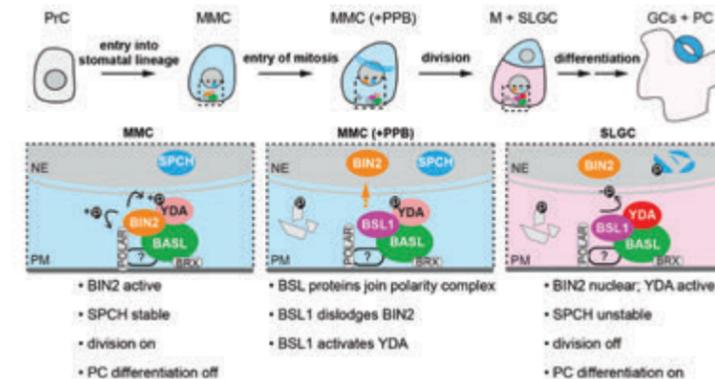


Figure 3. A working model for BSL phosphatases to function in stomatal ACD.

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

Signaling dichotomy of the BSL phosphatases in controlling stomatal fate determination

MAPK signaling modules play crucial roles in regulating numerous biological processes in all eukaryotic cells. How MAPK signaling specificity and strength are tightly controlled remains a major challenging question. In Arabidopsis stomatal development, the MAPKK Kinase YODA (YDA) functions at the cell periphery to inhibit stomatal production by activating MAPK 3 and 6 (MPK3/6) that directly phosphorylate stomatal fate-determining transcription factors for degradation in the nucleus. Recently, we demonstrated that BSL1, one of the four BSL protein phosphatases, localizes to the cell cortex to activate YDA, elevating MPK3/6 activity to suppress stomatal formation. In this study, we showed that at the plasma membrane, all four members of BSL proteins contribute to the YDA activation. However, in the nucleus, specific BSL members (BSL2, BSL3, and BSU1) directly deactivate MPK6 to counteract the linear MAPK pathway, thereby promoting stomatal formation (Figure 4). Thus, the pivotal MAPK signaling in stomatal fate determination is spatially modulated by a signaling dichotomy of the BSL protein phosphatases in Arabidopsis, providing a prominent example of how MAPK activities are integrated and specified by signaling compartmentalization at the subcellular level (Guo et al., 2022, Nat Commun).

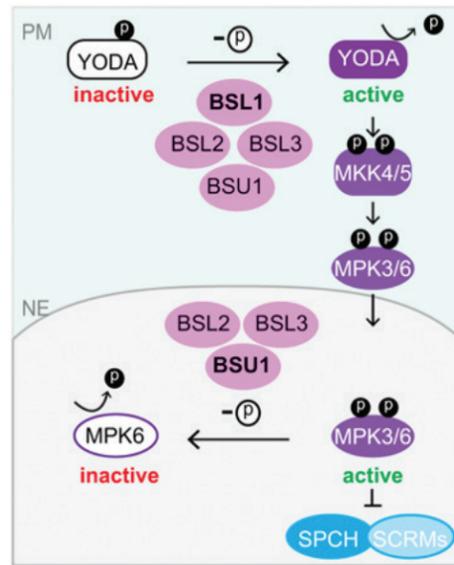


Figure 4. A working model: BSL phosphatases-based signaling dichotomy, through spatial compartmentalization of the regulation on distinct components of the linear YODA MAPK pathway, controls stomatal development in Arabidopsis. At the cell cortex close to the PM, BSL1 is a predominant regulator, together with the other three BSL phosphatases, activating the MAPKKK YODA to promote MAPK signaling. Activated MPK3/6 molecules phosphorylate the key stomatal fate transcription factors, SPCH and ICE1/SCRM, for degradation, thereby suppressing stomatal production. In the nucleus, BSU1 plays a primary role, together with BSL2 and BSL3, deactivating MPK3/6, resulting in stabilized SPCH and ICE1/SCRM, thereby promoting stomatal production.

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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 *lac* promoter by catabolite activator protein (CAP) and (2) activation of the *gal* promoter by CAP. These model systems each involve only a single activator molecule and a single activator DNA site and, as such, are more tractable than typical examples of activation in bacteria and substantially more tractable than typical examples of activation in eukaryotes (which can involve tens of activator molecules and activator DNA sites).

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

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

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

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

The transcription antitermination factor Q, which is produced by lambdoid bacteriophage during lytic infection, is one

of two classic textbook examples of regulators of gene expression that function at the level of transcription pausing and transcription termination (e.g., *Molecular Biology of the Gene*). (The other classic textbook example is the structurally and mechanistically unrelated regulator N, which is produced by bacteriophage lambda and functions in an earlier phase of lambdoid bacteriophage infection.)

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

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

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

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

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

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

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

Regulation of Transcription: Transcription-Translation Coupling.

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

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

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

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

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

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

Inhibitors of Transcription; Antibacterial Drug Discovery

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

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

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

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

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

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

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GALLAVOTTI LAB

Molecular 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). These CRMs frequently overlap with regions of accessible chromatin and can be located both proximally and distally at regions far away from genes. Such proximal and distal CRMs were for example observed in several plant architecture-related maize genes.

The overall 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, 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 developmental responses is still a major unanswered question. Recent work has highlighted how the combinatorial activity of different components of the auxin signaling pathway may be responsible for the specificity of auxin response. Auxin is perceived by the nuclear auxin receptor TIR1/AFB, part of an E3 ligase that rapidly degrades Aux/IAA co-receptor proteins and disrupts their recruitment of TOPLESS (TPL) corepressor proteins that silence transcription. The auxin-dependent degradation of Aux/IAs frees interacting activating ARF transcription factors from TPL repression, allowing them to activate downstream genes. Aux/IAs 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 Pro-

gram (PGRP) 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. Using CRISPR-Cas9 editing, we are creating mutation in specific ARF binding sites of several auxin-induced genes to functionally validate auxin transcriptional regulation in development. Furthermore, we are using a transgenic approach to modify inflorescence architecture by modulating auxin-response in tassels and ears, and we are testing whether tinkering with auxin signaling can increase grain yield.

Transcriptional repression in maize shoot development

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

The maize co-repressor REL2, a functional homolog of the TPL protein mentioned above, was originally isolated in a forward genetic screen for inflorescence defects. Mutations in the *REL2* gene give rise to pleiotropic defects throughout development, thus providing an excellent tool to study how plants use transcriptional repression mechanisms in numerous developmental processes. Notably, *rel2* mutants increase the size of meristems. Among the TFs interacting with REL2 are ZmWUS1 and ZmWUS2, two key regulators of meristem size that determine the number of rows of seeds in maize ears. We are currently investigating REL2 function in regulating the size of inflorescence meristems to determine whether this occurs via ZmWUS1/2 interaction or independently of it, using genetic and genomic approaches. This research is sponsored by a collaborative grant from the Division of Integrative Organismal Systems of the National Science Foundation.

Developing a fast and efficient maize transformation system

Key advancements in precise genome editing and novel methods for rapid generation of bioengineered crops promise to revolutionize breeding programs and increase our ability to feed and sustain human population growth. Although targeted and specific modifications of DNA sequences are now possible, several existing barriers prevent widespread adoption of editing technologies in crop species. Inefficient methods for transformation and regeneration of recalcitrant species remain major hurdles. These limitations are frequent in monocotyledonous crops, which alone provide most of the calories consumed by human populations. We have developed a new system that takes advantage of different morphogenic regulators, genes that are involved in meristem regulation or embryogenesis, and significantly accelerates the speed and efficiency of maize embryo transformation (Figure 1), enabling the generation of many CRISPR-Cas9 maize transgenic lines.

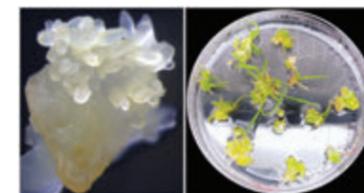


Figure 1 Legend

(Left) Cluster of somatic embryos appearing after 7 days from Agrobacterium-mediated infection of immature maize embryos. (Right) Plantlets are regenerated after ~45-50 days.

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Dr. Kenneth Irvine
Molecular Biology & Biochemistry

Summary

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. 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 organ growth is controlled have come from the identification and characterization in model systems of intercellular signaling pathways that are required for growth control. These pathways are highly conserved

among different phyla. Much of our research takes advantage of the powerful genetic, molecular, and cellular techniques available in *Drosophila melanogaster*, which facilitate both gene discovery and the analysis of gene function. We also use cultured mammalian cell models.

One major area of research involves investigations of the Hippo signaling network, which has emerged over the past 15 years as one of the most important growth regulatory pathways in animals. We study its regulation, molecular mechanisms of signal transduction, and its roles in different developmental and physiological contexts. Our investigations of how mechanical stress can influence Hippo signaling led to identification of a biomechanical pathway that links cytoskeletal tension to Hippo signaling. This mechanism contributes to feedback inhibition of growth in compressed cells and contributes to density-dependent regulation of cell proliferation. The role of density-dependent mechanical stress in modulating Hippo signaling provides a mechanism through which this pathway contributes to the regulation of organ size.

We found that biomechanical Hippo signaling can be mediated through tension-dependent recruitment of an Ajuba family protein to adherens junctions. In *Drosophila*, the Ajuba LIM protein (Jub) is recruited to adherens junctions under tension through a tension-induced conformational change in α -catenin that enables Jub binding. Once bound to adherens junctions, Jub then recruits and inhibits Warts, which is the central kinase of the Hippo signaling pathway. Our recent studies are shedding light on biochemical mechanisms that mediate the inhibition of Warts by Jub.

Additional studies have identified novel roles for Jub in modulating tension and cellular organization, which are shared with the cytohesin Steppke and the cytohesin adapter Stepping Stone, and we established that Jub and Stepping Stone together recruit Steppke 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. To investigate how Jub interacts with and regulates its distinct partners, we investigated the ability of Jub proteins missing different combinations of its three LIM domains to rescue jub phenotypes and to interact with α -catenin, Warts and Steppke. Multiple regions of Jub contribute to its ability to bind α -catenin and to localize to adherens junctions in *Drosophila* wing imaginal discs. Co-immunoprecipitation experiments in cultured cells identified a specific requirement for LIM2 for binding to Warts. However, in vivo, both LIM1 and LIM2, but not LIM3, were required for regulation of wing growth, Yorkie activity, and Warts localization. Conversely, LIM2 and LIM3, but not LIM1, were required for regulation of cell shape and Steppke localization in vivo, and for maximal Steppke binding in co-immunoprecipitation experiments. These results identified distinct functions for the different LIM domains of Jub.

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

We also investigate how cell shapes and cell behaviors are polarized by the Dachous-Fat pathway, and how this polarization influences organ shape. As one simple model, we have combined genetic analysis, live imaging, and computation image analysis to investigate cellular and molecular mechanism that govern wing shape in *Drosophila*. One unexpected outcome of these studies was the discovery that orientation of cell divisions are not required for normal wing shape. Current studies are investigating how Dachous-Fat signaling influences the shape of other *Drosophila* organs, including the leg, and defining molecular mechanisms that mediate signaling downstream of Dachous.

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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. Current efforts focus on developing a reproducible protocol for plastid transformation in the model plant *Arabidopsis thaliana*.

Agrobacterium-Mediated Transformation of the Plastid Genome

Efficient plastid transformation in *Arabidopsis thaliana* has been achieved using a recipient plant lacking a duplicated ACCase enzyme. However, obtaining fertile

transplastomic plants from the cell line is still a problem due to somaclonal variation in tissue culture cells. For *Arabidopsis* nuclear gene transformation, tissue culture limitations were overcome by direct transformation of the female gametes using the floral dip protocol and identification of transgenic events in the seed progeny. To enable floral dip transformation for plastid engineering, we decided to re-engineer *Agrobacterium* for T-DNA delivery to chloroplasts to directly transform plastids in the female gametocyte in *Arabidopsis* flowers.

During *Agrobacterium* infection, VirD2 nicks the T-DNA border sequences and covalently links to the 5' end of the T-strand via its Tyr29. The T-strand is then guided through the Type IV secretion system (T4SS) to the plant cell, where it integrates in the plant nucleus. We re-engineered VirD2 by removing its nuclear localization signals (NLSs) and fusing its N-terminus with a chloroplast-targeting transit peptide (TP). The re-engineered plastid-VirD2 (Pt-VirD2) was shown to localize to chloroplasts in a split GFP assay, in which VirD2 fused with 16 amino-acids of GFP (GFP₁₁) complemented a large GFP (GFP₁₋₁₀) already in chloroplasts (Figure 1). First, we generated multiple, independently transformed tobacco plants which accumulate GFP₁₋₁₀ at different levels dependent of the site of insertion in the nuclear genome. We then tested the GFP₁₋₁₀ expressing lines for background fluorescence under the confocal microscope. We chose the line with the lowest GFP₁₋₁₀ level (pAM407-10), in which the mean fluorescence intensity was not significantly different from the background fluorescence in wild-type chloroplasts. To test complementation in plants, the selected GFP₁₋₁₀ line (pAM407-10 line; kanamycin resistant) was transformed with the *Pt-virD2-gfp₁₁* genes (pJF7- pJF12 binary vectors; gentamycin resistant). The *Pt-virD2-gfp₁₁* genes are expressed in a Ubiquitin 1 gene promoter/terminator (*PUBQ1/TUBQ1*) cassette where they are transcribed, then translated in the cytoplasm and imported into chloroplasts (Figure 1a). Figure 1b shows red fluorescence of chlorophyll, green fluorescence when Pt-VirD2-GFP₁₁ is expressed at a high level to give a strong signal after assembly with GFP₁₋₁₀, and colocalization of the two in stoma guard cells in an image taken under bright field microscopy (Figure 1b).

Agrobacterium-mediated chloroplast transformation will be obtained by constructing vectors which encode both T-DNA and Pt-VirD2 function. Pt-VirD2 will direct T-DNA containing an antibiotic resistance marker to the plastid genome. To develop the method, transplastomic events will be identified by cocultivation of *Agrobacterium* with tobacco leaves and selecting for antibiotic resistance encoded in the vector. *Arabidopsis* flowers will then be dipped in an *Agrobacterium* culture carrying the plastid transformation vector and transplastomic *Arabidopsis* plants will be identified by germinating seed on a selective medium.

Engaging undergraduate students in research

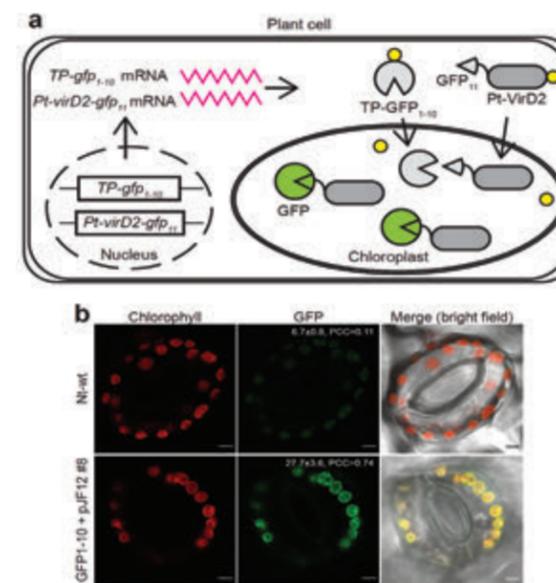


Figure 1. Split GFP assay to confirm chloroplast targeting of VirD2. (a) Schematic of split GFP assay. (b) Confocal images confirm complementation when GFP₁₋₁₀ and Pt-VirD2-GFP₁₁ (pJF12#8) are co-localized in chloroplasts.

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. Participating students during 2021-2022 from Rutgers were Julia Ferranti, Alyssa Leung, Andres Torres, and Sharanya Datta. We also collaborate with students from Farmingdale State College, a Primarily Undergraduate Institution. The collaboration is facilitated by Professor Kerry A. Lutz, who is Co-PI on the NSF Grants.

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Dr. Kim McKim
Genetics

Summary

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

Meiosis begins with pairs of chromosomes undergoing recombination events. These events not only exchange genetic information and generate diversity in the population, but they also 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-orient 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 (Figure 1). The focus of our studies is on two key steps in this process. First, the chromosomes organize the microtubules. Second, a special structure of the chromosomes, the kinetochore, regulates the attachments to the microtubules.

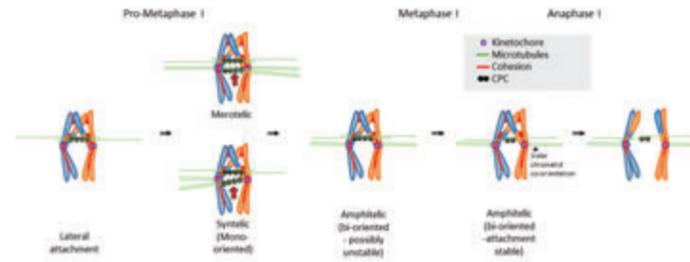


Figure 1: Bi-orientation during meiosis.

Early in pro-metaphase, lateral kinetochore – microtubule attachments involving the central spindle predominate and may facilitate separation of homologous centromeres towards opposite poles and acquiring end-on attachments. End-on attachments leading to chromosome segregation errors include merotelic or syntelic attachments. In both of these cases, the improperly attached microtubules cross through the central spindle region (indicated by a red arrow). At least in *Drosophila* oocytes, this region contains a high concentration of CPC (Figure 2). Therefore, the only end-on attachments that are destabilized by Aurora B activity are kinetochores attached to microtubules that pass through the central spindle. Thus, merotelic or syntelic attachments are oriented to conduct the CPC towards kinetochores and facilitate error correction.

The kinetochore hub: Spc105 regulates microtubule interaction and kinetochore assembly in *Drosophila* oocytes

The kinetochore is the site on the chromosomes that interacts with the microtubules. Prior works have implicated the kinetochore protein SPC105/KNL1 in promoting accurate chromosome segregation. Furthermore, attachments between the microtubules and the chromosomes is a twostep process (Figure 1). Lateral attachments enable the initial attachments while more stable end on attachments stabilize proper interactions. We have conducted a structure-function analysis of SPC105 to elucidate its functional domains and meiotic responsibilities. We found that SPC105's C-Terminal domain is necessary and sufficient for NDC80 localization and for building the rest of the kinetochore that makes end on attachments to microtubules. The rest of SPC105 regulates microtubule attachments by promoting lateral attachments to the microtubules and recruiting proteins that either prevent precocious end-on attachments or destabilize incorrect end-on attachments. Our results have provided mechanistic detail for understanding SPC105's multifaceted role in ensuring proper meiotic progression.

SPC105 recruits several proteins that regulate the attachment of microtubules to the kinetochore and cell cycle pro-

gression. One poorly understood protein in meiosis, ROD, is a component of the ROD-ZW10-Zwilch (RZZ) complex and localizes to the kinetochores. The RZZ complex is involved in attachment error correction and the spindle assembly checkpoint (SAC) during mitosis. To analyze the function of ROD in female meiosis I, we observed oocytes lacking ROD or proteins hypothesized to interact with RZZ. Female *Drosophila* depleted of ROD in the oocytes are sterile and have chromosome orientation defects, suggesting that ROD is required for correct KT-MT attachments. We found that ROD recruitment to the kinetochores is mediated by a specific region (amino acids 154-840) of kinetochore protein SPC105. Furthermore, while ROD is not required for creation of the initial KT-MT attachments, it may have a role in preventing or correcting erroneous attachments.

The microtubule organizing hub: Chromosome-directed oocyte spindle assembly depends on the Chromosomal Passenger Complex

The mechanisms ensuring accurate chromosome segregation in female meiosis requires high participation of chromosomal passenger complex (CPC). The CPC includes a catalytic subunit, Aurora B kinase, and a localization module which is composed of the inner centromere protein (INCENP), Survivin and Borealin. This complex is crucial to the assembly of the acentrosomal meiotic spindles and the regulation of kinetochore-microtubule attachments. The CPC interacts with both the chromosomes and the microtubules (Figure 2). We are investigating how the CPC is recruited to the chromosomes and how multiple microtubule binding domains within the CPC facilitate movement from the chromosomes to the microtubules.

We have proposed that Borealin recruits the CPC to the chromosomes by interacting with Heterochromatin protein-1 (HP1) (Figure 2). HP1 binds to di- and tri-methylated lysine 9 of histone H3 (H3K9me2/3). However, it remains unclear how HP1 interacts with the CPC to localize to chromosomes during meiosis. It is also unclear how HP1 pro-

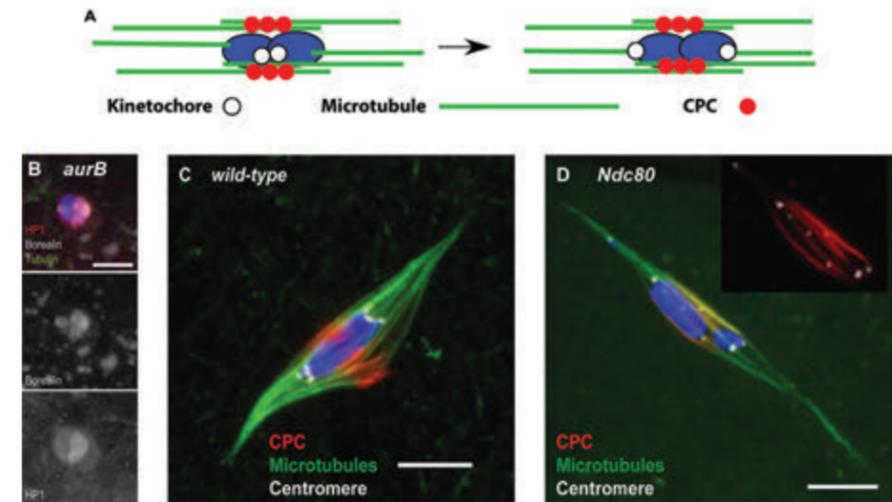


Figure 2: The kinetochores and the CPC interact with two populations of microtubules

A) In meiosis, homologous, rather than sister, centromeres are pulled in opposite directions. During prometaphase, these centromeres may be located in the central spindle region, coinciding with a high density of CPC. The antiparallel microtubules could form a bridge between homologous centromeres. B) In the absence of Aurora B kinase activity, the CPC (red) localizes to the chromosomes (blue). C) Once Aurora B is active, microtubules (green) assemble around the chromosomes and homologous centromeres (white) separate towards opposite poles, with a high concentration of CPC (red) between them. C) In the absence of end-on attachments (an NDC80-depleted oocyte), the kinetochores are only making lateral attachments, and many are still in a region of high CPC. Thus, end-on attachments may be required to move away from the region of high CPC concentration.

motes CPC activation during spindle assembly and transfer to microtubules. We are testing the hypothesis that Borealin mediates these events and promotes movement of the CPC from the chromosomes to the spindle microtubules. It is also unknown how the CPC interacts with spindle microtubules. The CPC contains three microtubule binding domains: the “spindle transfer domain” (STD) and the single α -helix (SAH) domain in INCENP and an N-terminal microtubule binding domain in Borealin. We are testing the role of each domain and HP1 in organizing a bipolar spindle and promoting accurate meiotic chromosome segregation.

Future directions: Identifying new genes required for fertility and using *Drosophila* as a platform to investigate genes implicated in human infertility.

We are performing experiment to identify new fly genes that are required for meiosis. Based on the hypothesis that meiotic genes should demonstrate elevated expression levels in meiotic tissues such as the ovary, we are using three publicly available databases containing tissue-specific expression data (RNA-seq) (FlyAtlas, FlyAtlas 2 and Modencode). Based on the analysis of these data sets, approximately 1100 genes are upregulated only in oocytes and we have prioritized 542 uncharacterized or novel genes to study. To test genes for meiotic functions, the expression of each gene is being reduced using tissue-specific RNAi. The *Drosophila* TRiP project (Harvard) has generated reagents for tissue specific RNAi of every gene. Using these publicly available shRNA lines, an RNAi screen is being performed to identify genes required in meiosis. To date, we have screened approximately 150 genes from our expression analyses by germline-specific RNAi and found 25 that cause reduced fertility, sterility, or increased nondisjunction.

Based on this system to test genes for meiotic functions, we are beginning a project to identify and characterize genes known to affect fertility in humans. Part of the rationale for this project is that meiosis in *Drosophila* oocytes is similar to mammals. In addition, we know of several genes, such as Subito (KIF20A in human) and Klp67A (Kif18A in human) that are required for meiosis or fertility in *Drosophila* and Humans. Our collaborators are identifying candidate genes from women who produce a high frequency of aneuploid embryos and have defects in fertility. Many of these identified genes are evolutionarily conserved in *Drosophila*. We will use our *Drosophila* tissue specific RNAi system to rapidly validate and assess candidate human genes. Based on previous results, we expect at least about 20% of the tested genes to have a germline phenotype. Therefore, if we test 150 candidates, we expect 20%, or about 30, to have a defect in chromosome segregation or fertility.

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NICKELS LAB

Regulation of Gene Expression in Bacteria



Dr. Bryce Nickels
Genetics

Summary

My lab seeks to address major gaps in our understanding of the mechanism of transcription and transcriptional regulation. Proper control of gene expression is critical for organismal development, cellular response to environmental signals, and the prevention of disease states. The first step in gene expression, transcription, is carried out by multi-subunit RNA polymerases (RNAPs) that are conserved in sequence, structure and function from bacteria to humans. My lab carries out the majority of our studies of transcription using bacterial RNAP as a model for understanding the mechanism of transcription and its regulation in all organisms.

We seek a quantitative understanding of each step of transcription: initiation, elongation, and termination. In addition, we seek to understand the diversity of regulatory mechanisms that link changes in cellular state to changes in the activity of RNAP. To achieve these goals, we use a comprehensive approach combining genetics, biochemistry, structural biology, genomic, and high-throughput-sequenc-

ing-based methods. In addition, we continue to develop new methods and approaches to understand the interface between DNA sequence and RNAP function, and to reexamine long-held notions of RNAP function for unexpected paradigms or unknown biology.

Promoter sequence determinants and structural basis of primer-dependent transcription initiation in *Escherichia coli* (collaboration with Richard Ebricht and Justin Kinney)

We used massively systematic transcript end readout (“MASTER”) to detect and quantify RNA 5’ ends generated by primerdependent initiation for $\sim 4^{10}$ ($\sim 1,000,000$) promoter sequences in *E. coli*. The results show primerdependent initiation in *E. coli* involves any of the 16 possible dinucleotide primers, depends on promoter sequences in, upstream, and downstream of the primer binding site, and define a consensus sequence for primerdependent initiation, $Y_{TSS2}N_{TSS1}N_{TSS}W_{TSS+1}$, where TSS is the transcription start site, $N_{TSS1}N_{TSS}$ is the primer binding site, Y is pyrimidine, and W is A or T. Biochemical and structural work show the base pair (nontemplatestrand base:templatestrand base) immediately upstream of the primer binding site ($Y:R_{TSS2}$, where R is purine) exerts its effect through the base on the DNA template strand (R_{TSS2}) through interchain base stacking with the RNA primer. Analysis of natural, chromosomally encoded *E. coli* promoters support the conclusions from MASTER. Taken together, the findings provide a mechanistic and structural description of primerdependent transcription initiation.

Structural and mechanistic basis of reiterative transcription initiation (collaboration with Richard Ebricht)

In standard transcription initiation, RNA polymerase (RNAP) synthesizes an initial RNA product complementary to the DNA template. In an alternative pathway of transcription initiation, termed “reiterative transcription initiation,” observed at promoters containing homopolymeric sequences at the transcription start site, RNAP synthesizes an initial RNA product having a 5’ sequence that contains a variable number, up to tens to hundreds, of nucleotides non-complementary to the DNA template. Using crystallography, cryo-EM, photocrosslinking, and singlemolecule DNA nanomanipulation, we show that RNA extension in reiterative transcription initiation involves slipping of RNA relative to DNA within a short RNADNA hybrid, and generates RNA that exits RNAP through a previously unobserved RNA path (“alternative RNA path”) and a previously unobserved RNA exit (“alternative RNA exit”).

Structural and mechanistic basis of σ dependent transcriptional pausing (collaboration with Richard Ebricht and Justin Kinney)

In σ dependent transcriptional pausing, the transcription initiation factor σ , translocating with RNA polymerase (RNAP), makes sequencespecific proteinDNA interactions with a promoterlike sequence element in the transcribed

region, inducing pausing. It has been proposed that σ dependent pausing involves offpathway “backtracked” states that are substrates for the transcriptcleavage factors of the Gre family, and onpathway “scrunched” states that mediate pause escape. To address this proposal, we used sitespecific proteinDNA photocrosslinking to define positions of the RNAP trailing and leading edges and of σ relative to DNA at the IPR’ promoter. The results show directly that σ dependent pausing in the absence of GreB *in vitro* predominantly involves a state backtracked by 24 bp, and that σ dependent pausing in the presence of GreB *in vitro* and *in vivo* predominantly involves a state scrunched by 23 bp. Analogous experiments with a library of 4⁷ (~16,000) transcribedregion sequences show that the state scrunched by 23 bpand only that stateis associated with the consensus sequence, T₃N₂Y₁G₊₁, (where 1 corresponds to the position of the RNA 3’ end), which is identical to the consensus for pausing in initial transcription, and related to the consensus for pausing in transcription elongation. Experiments with heteroduplex templates show that sequence information at position T₃ resides in the DNA nontemplate strand. A cryoEM structure of a complex engaged in σ dependent pausing reveals positions of DNA scrunching on the DNA nontemplate and template strands and suggests that position T₃ of the consensus sequence exerts its effects by facilitating scrunching.

Quantitative analysis of transcription start site selection in *Saccharomyces cerevisiae* determines contributions of DNA sequence and RNA Polymerase II activity (collaboration with Criag Kaplan)

DNA sequence at Transcription Start Sites (TSSs) is a key determinant of initiation by RNA Polymerase II (Pol II). To function as a TSS, an initiation compatible sequence must be specified by a promoter in an appropriate chromatin context. We report the development of a method for quantitative analysis of transcription initiation by Pol II that involves construction of DNA libraries of barcoded promoter variants, production of RNA transcripts, and analysis of transcript 5’ ends and transcript yields (Pol II MAssively Systematic Transcript End Readout, “Pol II MASTER”). Using Pol II MASTER, we measure the efficiency of transcription initiation during “promoter scanning” by *Saccharomyces cerevisiae* Pol II for ~1 million unique TSS sequences. Furthermore, we employ Pol II MASTER to determine how Pol II activity, known to widely alter TSS selection *in vivo*, alters TSS efficiencies across our promoter variants. Pol II MASTER recapitulates known critical qualities of *Saccharomyces cerevisiae* TSS -8, -1, and +1 positions while demonstrating that surrounding sequences modulate initiation efficiency over a wide range. We discover functional interactions between neighboring sequence positions, indicating that adjacent positions likely function together. We demonstrate that initiation efficiencies are altered for +1 A TSSs relative to +1 G TSSs when Pol II activity is perturbed through genetic means. Pol II MASTER provides data for predictive models of TSS initiation efficiency at genomic promoters.

Structural basis of transcription antitermination by Q λ : NusA facilitates refolding of Q λ to form a torus that extends the RNAP RNA-exit channel (collaboration with Richard Ebright)

Lambdoid bacteriophage Q proteins are transcription antipausing and antitermination factors that enable RNA polymerase (RNAP) to read through pause and termination sites. Q loads onto RNAP engaged in promoterproximal pausing at a Q binding element (QBE) and adjacent sigmdependent pause element to yield a Qloading complex, and Q translocates with RNAP as a pausingdeficient, terminationdeficient Qloaded complex. In previous work, we showed that the Q protein from bacteriophage 21 (Q21) functions by forming a “nozzle” that narrows and extends the RNAP RNAexit channel, preventing the formation of pause and termination RNA hairpins. Here, we report atomic structures of four states on the pathway of antitermination by the Q protein from bacteriophage λ (Q λ), a Q protein that shows no sequence similarity to Q21 and that, unlike Q21, requires the transcription elongation factor NusA for efficient antitermination and antipausing. We report structures of Q λ , the Q λ QBE complex, the NusAfree “preengaged” Q λ loading complex, and the NusAcontaining “engaged” Q λ loading complex. The results show that Q λ , like Q21, forms a nozzle that narrows and extends the RNAP RNAexit channel. The results further show that Q λ , unlike Q21, employs a twostage process for Q loading, involving recruitment of Q to form a preengaged loading complex, followed by NusAfacilitated reorganization of Q to form an engaged loading complex. The results establish that the threedimensional structures, the mechanisms of QBE recognition, and the mechanisms of Q loading differ for Q λ and Q21, and thus that Q λ and Q21 are not structural homologs and are solely functional analogs.

Towards a comprehensive understanding of the architecture of gene expression control in *E. coli*.

We have developed a crosslinking plus HTS method to measure RNAP occupancy termed “XACT seq” (x-link between active center and template sequencing). XACT-seq provides a direct, single nucleotide resolution readout of

the RNAP active center position relative to DNA. Use of XACT seq in combination with NET seq (native elongating transcript sequencing), which provides a single nucleotide resolution readout of RNA 3’ end positions, will enable us to define the translational register of the RNAP active center relative to the RNA 3’ end. We have optimized conditions for parallel XACT-seq/NET-seq analysis of *E. coli* cells and our in the process of analyzing data collected from wild-type cells in rich media during exponential growth.

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Summary

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

the underlying etiology of many neurological disorders and diseases, including ischemic stroke, Parkinson's Disease, and Alzheimer's Disease, are due to defects in one or more of these key neurophysiological processes. A more complete understanding of these processes will facilitate better diagnosis and treatment of multiple neurological disorders.

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

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

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

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

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

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

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

Regulators Of Mitochondrial Transport and Dynamics In Neurons.

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

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

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

Biogenic Amine Signaling Activates The UPS In Distal Epithelial Tissues.

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

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

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

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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 results were obtained during the last year.

Studies of CRISPR-Cas bacterial adaptive immunity

CRISPR-Cas (Clustered Regularly Interspersed Palindromic Repeats/CRISPR associated sequences) loci provide bacteria with adaptive immunity to phages and plasmids. We study diverse CRISPR-Cas systems from *Escherichia coli*, *Pseudomonas aeruginosa*, *Thermus thermophilus*, human pathogen *Clostridium difficile*, and thermophilic archaea both in laboratory and natural settings. In *E. coli*, we determined how plasmids targeted by CRISPR-Cas survive in the bacterial population by balancing the CRISPR interference and plasmid replication rates. As a result, subpopulations of cells bearing plasmids remain even when CRISPR defense directed against plasmids is active. In the context of natural populations such plasmid-bearing persisting cells allow clonal bacterial cultures to better cope with the unpredictable environment. These

findings have important implications for designing CRISPR-based strategies aimed at eradication of plasmids with antibiotic-resistance genes. In collaboration with Professor Westra from the University of Exeter (UK), we showed how the ability of *P. aeruginosa* CRISPR-Cas system to acquire protective spacers from infecting phages is modulated in the presence of antibiotics, again pointing at an intricate interplay of CRISPR defense and antibiotics.

Given the ongoing interest in new CRISPR-based genomic editors, we discovered the ability of Type V Cas12 editors to cleave their targets when charged with split guide RNAs. This finding considerably facilitates the use of Cas12 in diagnostic applications for determination of specific nucleic acid sequences in various samples. We also developed a new machine-learning method to identify guide RNAs with highest on-target and decreased off-target activities.

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

We bioinformatically predict and then functionally and structurally characterize bacteriophage-encoded transcription enzymes (DNA-dependent RNA polymerases) that are very distantly related to cellular enzymes. In collaboration with Professor Leiman from UTMB Galveston and the AlphaFold team we determined the structure of RNA polymerase encoded by a jumbo phage AR9 and gained insights into unique promoter recognition mechanism by this enzyme. In collaboration with Professor Lavigne group from University of Leuven in Belgium we discovered a novel phage protein that inhibits the DNA gyrase of its host *P. aeruginosa*. We also developed ONT-Seq, a new method of mapping host and viral transcripts with single-nucleotide resolution using the long-read sequencing technology from Oxford Nanopore. We plan to systematically apply this powerful method to study transcription of different viruses and their hosts throughout the infection process both in the presence and in the absence of CRISPR defense directed against different viral genes.

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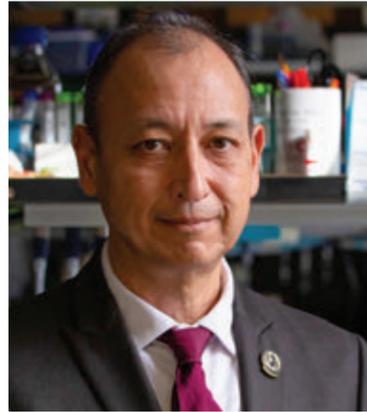
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SINGSON LAB

Reproductive Biology, Cell-Cell Interactions



Dr. Andrew Singson
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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 known as “*spe-9* class” mutants. The SPE-9 protein functions as a sperm surface ligand required for sperm to egg signaling during fertilization.

We continue to identify and characterize genes required for sperm function at fertilization taking advantage of the most up to date genetic and molecular tools. Most recently we have been using a new genome engineered balancer chromosomes in our fertility mutant screens. This allows us to screen specific segments of the genome and easily maintain mutants. This screen has turned out to be successful and we have isolated many new sterile mutant strains. With whole genome sequencing, we will know the mutated genes soon.

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). With access to new state of the art microscopes at the Waksman Institute, we have been able to observe the localization of our sperm molecules with new levels of resolution (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.

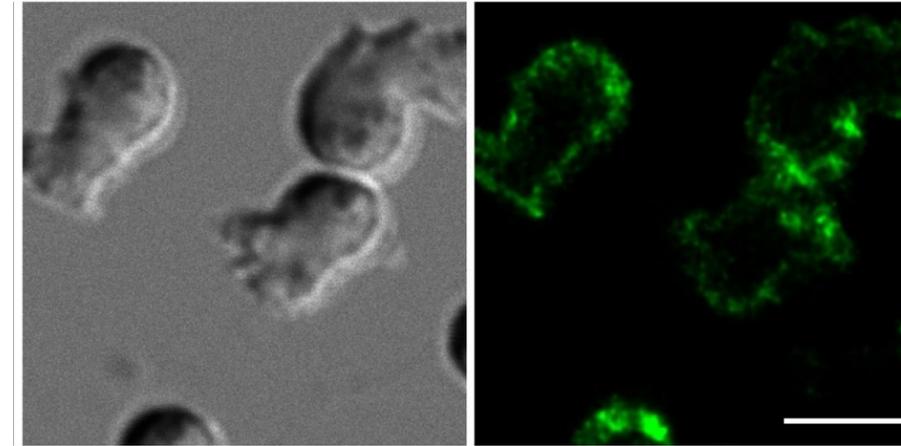


Figure 1. Although the SPE-51 protein is secreted by sperm, it remains associated with the sperm surface and is required for successful fertilization. SPE-51:NeonGreen can be detected on the surface of activated *C. elegans* sperm.

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* spermiogenesis. The *spe-24/zipt-7.1* encoded protein is a zinc transporter and demonstrates zinc as an important second messenger for sperm activation in vivo. The *spe-43* gene is a novel transmembrane protein that is alternately spliced. Further characterization of this gene will help us better understand how sperm become competent to move towards and fertilize the egg. The *spe-21* gene encodes a DHHC-CRD zinc finger membrane protein. We are characterizing the role of this protein during spermatogenesis and sperm activation. It likely regulates through lipidation the activity and localization of other sperm proteins.

Egg functions

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

bryo 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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STAVROPOULOS LAB

Sleep regulation and function



Dr. Nicholas Stavropoulos
Associate Research Professor

Summary

Sleep is a fundamental animal behavior vital for normal physiology and brain function. Although we spend one third of our lives asleep, the mechanisms that regulate sleep remain poorly understood. Elucidating these mechanisms is critical to understanding how sleep is disrupted in various neurological disorders, ranging from neurodegenerative conditions including Parkinson's and Alzheimer's to neurodevelopmental disorders such as autism. Our research is motivated by the fact that sleep is a conserved animal behavior millions of years old. Fruit flies—the evolutionary cousins of humans—sleep similarly to mammals. Sleep in flies is governed by the same neurotransmitters as in humans, including dopamine, acetylcholine, and GABA, and is modulated by drugs that alter human sleep, including caffeine and hypnotics. Critically, sleep in flies and humans alike is regulated by circadian and homeostatic mechanisms. The potential of the fly to shed light on sleep regulation is underscored by discoveries in the fly that revealed genetic and molecular underpinnings of circadian rhythms that are conserved in mammals. Among the advantages of the fly are a simpler nervous system and genome, sophisticated genetic tools, and the ability to monitor sleep

in thousands of animals in parallel. Our research leverages the fly to address unanswered questions about sleep:

- What are the genes and circuits that control sleep?
- How do alterations in genes and neurons cause sleep dysfunction?

The conserved insomniac pathway links brain development and sleep regulation

One major focus of our research is the role of “sleep genes” in brain development. An overarching theme emerging from our findings is that brain development has a critical role in shaping adult sleep patterns and pathological sleep disturbances. We found that *insomniac* (*inc*), a highly conserved gene required for normal sleep, controls the development of discrete sleep-regulatory circuits. An immediate goal is to understand the mechanisms by which *inc* shapes neuronal development.

Our studies of *inc* began with an unbiased genetic screen in which we monitored sleep-wake cycles in nearly 21,000 chemically mutagenized animals. From this screen, we identified and cloned *inc* mutations causing severely short-

ened sleep (Figure 1). We subsequently found that *inc* encodes an adaptor for the Cullin-3 (Cul3) ubiquitin ligase complex (Figure 1). Reducing *inc* or *Cul3* activity in neurons causes short sleep, as do mutations that impair the formation of Inc-Cul3 complexes. Our findings and the identification of human Cul3 mutations as high-confidence risk factors for autism, a neurodevelopmental disorder associated with perturbed sleep, establish the Cul3-Inc pathway as a model for dissecting autism-associated sleep dysfunction. Our analysis of Inc orthologs suggests that our studies will provide insights relevant to mammals. We found that the three mouse orthologs of Inc are expressed in the brain and can restore sleep to *inc* mutants, indicating that functions of Inc are conserved through ~600 million years of evolution.

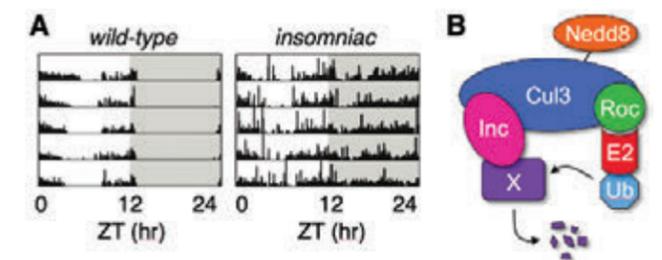


Figure 1. A) Five-day locomotor traces are shown for wild-type and insomniac mutant animals. Shading indicates night. B) Inc is an adaptor for the Cul3 ubiquitin ligase complex that ubiquitinates substrates for proteolysis (shown) or non-proteolytic regulation.

In recent studies, we found that *inc* impacts sleep through a developmental mechanism. We identified a conditional expression system suitable for manipulations of sleep and selectively restored *inc* expression to neurons in various temporal windows. We found that *inc* acts transiently during neuronal development to impact sleep in adulthood. The critical period for *inc* activity coincides with the birth and postmitotic development of many adult neurons and circuits. Using anatomical screens to map where *inc* functions, we found that *inc* impact sleep in part through the mushroom body (MB), a structure important for sleep regulation, integration of sensory stimuli, and associative learning; this result is intriguing, given that these functions are also disrupted in autism. *inc* mutants exhibit dramatic increases in MB neurogenesis and an excess of neurons that have projection defects, including axons that fail to reach their targets (Figure 2). Activation of MB neurons in adulthood strongly promotes sleep in wild-type animals but not in *inc* mutants, suggesting that developmental defects in these neurons abolish their sleep-regulatory functions. In contrast, the anatomy and function of other sleep-regulatory circuits in *inc* mutants are intact. These findings establish a model for understanding how a genetic pathway linked to autism shapes the development of specific sleep-regulatory circuits and their function in the adult brain.

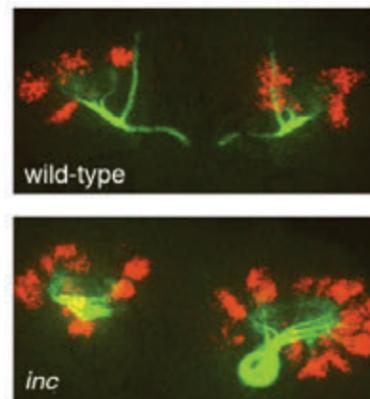


Figure 2. *inc* mutants have excess mushroom body (MB) neurons with axonal projection defects. Excess neuron clusters in *inc* mutants suggest defects in neurogenesis. Nuclei and projections are marked in red and green respectively.

Molecules and circuits that relay sleep-regulatory signals to the suppression of motor and sensory pathways

A second area of our research focuses on core attributes of sleep, locomotor inactivity and reduced responsiveness to sensory stimuli. While considerable attention has focused on sleep-regulatory circuits and molecules within the brain, how these elements ultimately impinge on motor and sensory systems remains ill-defined. In a recently completed study, we identified a neurotransmitter receptor that promotes sleep through its expression in GABAergic neurons of the ventral nerve cord (VNC), a structure that integrates motor and sensory information and which is analogous to the mammalian spinal cord (Figure 3). These findings indicate that the VNC is critical for sleep regulation and that the release of the inhibitory neurotransmitter GABA within the VNC is vital to promote and maintain the sleep state. We hypothesize that key targets of GABA release within the VNC in the context of sleep regulation include motor neurons, whose cell bodies reside in the VNC, and/or sensory pathways that transit the VNC. One future goal is to identify the motor and/or sensory targets of GABAergic VNC neurons and the circuits that lie upstream. A second goal is to understand how the activity of GABAergic VNC neurons is regulated during sleep-wake cycles. The functional parallels of the VNC and the mammalian spinal cord suggest that disruptions of conserved neurotransmitter receptors and analogous inhibitory circuits in humans may impair suppression of mo-

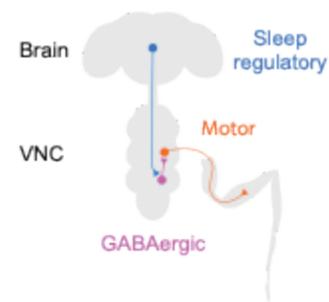


Figure 3. Model for suppression of motor neuron excitability during sleep by GABAergic VNC neurons that receive sleep-regulatory inputs from the brain.

tor and/or sensory pathways and give rise to sleep disorders. In particular, dysregulation of motor control and sensory responsiveness are associated with pathologically disrupted sleep, including in REM sleep behavior disorder, various parasomnias, neurodegenerative conditions, and neurodevelopmental disorders including autism.

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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-hydroxymethylcytosine (5hmC) in Drosophila.

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

Tet protein is detected mainly in the embryonic, larval and pupal Central Nervous system and in *Tet^{null}*, the complete loss-of-function mutation, axonal pathfinding is disrupted in the embryonic CNS and in larval and adult brains.

Previously, in collaboration with Dr. Fuks' laboratory at the Free University of Brussels, we mapped 5hmC transcriptome-wide in S2 Drosophila tissue culture cells and could show that Tet modifies ~ 1500 specific transcripts. Our working hypothesis is that Tet, mediated by its DNA-binding domain, localizes at actively transcribed target genes and controls the modification of their nascent transcripts. The 5hmC mark is then recognized by reader protein(s) that direct the association of the bound mRNA with ribosomes ultimately controlling translational levels. To test this hypothesis, we performed CHIP-Seq experiments. Bioinformatic analysis identified 2242 protein binding peaks, and 1032 of the peaks map to promoter sites. 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 guidance.

Next, we performed hmeRIP (immunoprecipitation using commercially available anti-5hmC antibody) on RNA isolated from wild type (wt) embryos as well as wt and *Tet^{null}* larval heads in order to map 5hmC transcriptome-wide. In all preparations, the distribution of modified RNAs was similar to what we had previously observed in 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 798 transcripts were at least 1.4 fold reduced in *Tet^{null}* larval heads compared to wild type. The GO term analysis of peaks identified in the hmeRIP experiments also indicated that the mRNAs of genes involved in axon guidance were most frequently identified. This observation points to an impressive correspondence between our phenotypic analysis and our genomic and transcriptomic results.

To determine if there is a link between 5hmC marks and mRNA levels, we compared the input RNA-seq from the hmeRIP experiments with the transcripts that showed the 5hmC mark. Out of 9000 total transcripts the levels of 445 were significantly increased and 115 were decreased in *Tet^{null}* brain fractions (Fig. 6A). When we compared these mRNAs with the 5hmC-modified mRNAs present in brain fractions, we found that 1716 or ~20% of the total transcripts were modified, but only 15 or 3 % of the RNAs that were upregulated in *Tet^{null}* and 13 or 11 % of the decreased mRNAs were modified (Fig. 6A, B). This result indicates that the levels of the vast majority of 5hmC modified mRNAs do not change levels in *Tet^{null}* larval heads. Thus, the 5hmC modification of the mRNAs does not appear to control the stability of transcripts. It is therefore likely that the change in levels of the mRNAs observed in *Tet^{null}* brains represent a secondary effect.

Based on the results listed above we identified Tet target genes implicated in axon guidance that showed Tet protein binding to the promoter region, and whose level of the 5hmC mark on the mRNA was reduced in *Tet^{null}* larva heads.

Two such Tet targets are Robo2 and Slit. Robo2 is the receptor of Slit and together they control axon guidance and midline repulsion. When we carefully analyzed the Tet embryonic axon phenotype, we found a midline crossing phenotype similar to that observed in slit and Robo2 embryos. Given that these two genes encode mRNAs that carry the 5hmC mark that is reduced in the *Tet^{null}* background we expect Tet to potentially control their protein levels. Indeed, both proteins were clearly reduced in brain extracts from *Tet^{null}* larval brains relative to wt. Thus we have uncovered a novel layer of regulation of the expression of the medically important SLIT/ROBO pathway.

Our results support the model we proposed above; during normal development Tet binds to target genes mediated by its DNA-binding domain and modifies nascent mRNAs thereby enhancing the translation of the modified mRNAs. This process occurs preferentially in nerve cells and can control the outgrowth of axons in the embryo and at pupal stages.

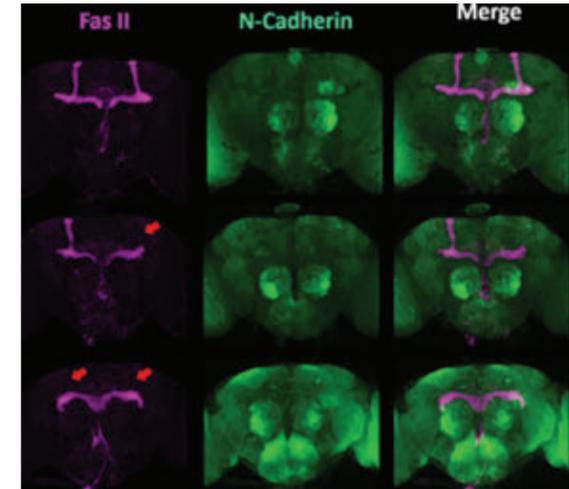


Figure legend: **Loss of mushroom body α lobe(s) in *Tet^{ΔXCC}* mutants.** Fasciclin II stains the mushroom bodies (red) N-cadherin is used as a cell marker (green). Top, wildtype brain, bottom, two different phenotypes observed in *Tet^{ΔXCC}* brains.

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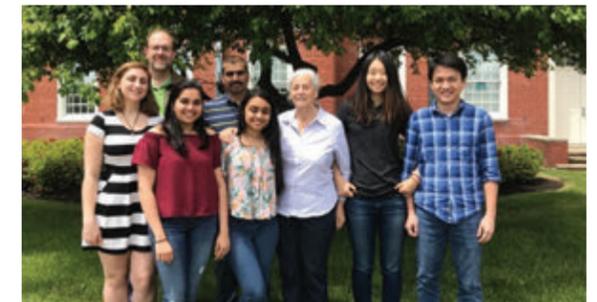
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YADAVALLI LAB

Bacterial stress response, antimicrobial resistance and biology of small proteins

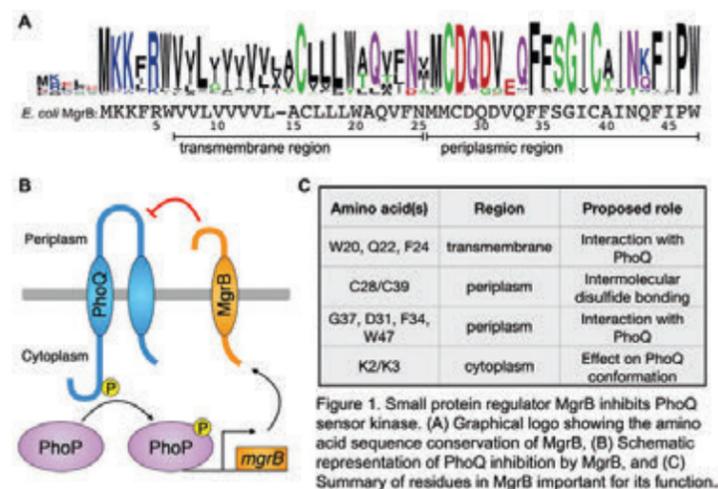


Srujana Samhita Yadavalli
Genetics

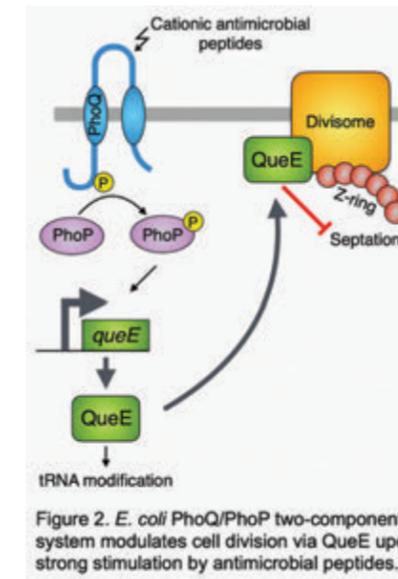
Summary

Antimicrobial resistance is an alarming problem in 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 has 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, and biochemistry to high-throughput sequencing, proteomics, and 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, and 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.



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.



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ZANDER LAB

Transcription Factor-driven Epigenome Dynamics in Plants



Dr. Mark Zander
Plant Biology

Summary

Increased crop productivity will be essential to address the imminent food demands predicted in human population models. This is increasingly difficult to accomplish because global climate change is projected to expand the geographic range of numerous pathogens, as well as shift the geographic ranges of crop suitability. To achieve this goal, we need to increase crop productivity and resilience via improved tolerance to abiotic (drought, heat) and biotic (pathogens) stresses, and to utilize marginal lands for crop production. Stress has adverse effects on plant growth and productivity (Skirycz and Inze, 2010) as a result of an extensive transcriptional reprogramming that causes rapid reallocation of metabolic resources from growth to stress tolerance response pathways. The deciphering and subsequent manipulation of molecular mechanisms underlying plant stress responses is therefore critically needed to breed more stress-tolerant crop plants ultimately ensuring our food security in the future.

The activation of stress response pathways is controlled by the interplay between master transcription factors (TFs) and the epigenome (all chemical modifications of DNA and histone proteins) that lay the transcriptional foundation for a robust stress response. A shared core component of almost all plants stress signaling pathways are master transcription factors that rapidly initiate gene regulatory networks to elicit robust stress responses. On a network level, the modus operandi of stress master has several hallmarks. First, they rapidly target other second tier TFs to amplify the transcriptional response. Second, they also target the majority of their own signaling pathway components and thirdly they initiate multiple feed-back and feed-forward loops. On a mechanistic level, master TFs are the architects of the dynamic plant epigenome. They associate with chromatin modifiers and strategically position them to shape the local epigenome environment at their target genes into either a transcriptionally permissive or restrictive state. Intriguingly, epigenome features themselves such as histone tail acetylation or DNA methylation can also instruct TF binding. This high level of regulatory plasticity within the TF-epigenome interplay allows to dynamically fine-tune transcriptional responses in a spatiotemporal manner which is of particular importance in plants since they are continuously exposed to a mélange of changing and challenging environmental conditions.

Development of a high-throughput ChIP-seq platform for plant tissues

Recent developments of next-generation sequencing-based technologies have unquestionably advanced our understanding of gene regulation in plants. Knowledge about transcription factor binding sites and occupancy of histone modifications and histone variants on a genome-wide scale is usually achieved with chromatin immunoprecipitation analysis coupled with next-generation sequencing (ChIP-seq) (Johnson et al., 2007; Ren et al., 2000). Besides significant experiment to experiment variation, another major caveat of the use of ChIP-seq in plants is the difficulty in scaling the approach to a large number of samples making it very difficult to conduct experiments with more than 20 samples. However, capturing of TF DNA binding as well as epigenome dynamics requires more complex experimental setups that can include multiple time points, TFs, epigenome features, genotypes, conditions as well as multiple cell types. To overcome these limitations, we developed a high-throughput ChIP-seq platform that works with only 100-300mg starting material allowing the processing of up to 96 samples in parallel. This low cost, high-throughput approach can be carried in a 96-well format at a cost of ~ \$30 for a single *Arabidopsis* TF or chromatin mark. This cost also applies to plants with larger genomes, as found in all crops, since plants share relatively similar numbers of genes and therefore roughly similar numbers of regulatory regions making it widely attractive for crop researchers as well.

Comprehensive analysis of the interplay between transcription factors and the epigenome

The functional relationship between transcription factors and the epigenome is highly complex depending on epig-

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

Investigating the role of the epigenome in stress responses of hemp

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

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CORE FACILITIES

Cell and Cell Products Fermentation Facility

OVERVIEW

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

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



FISCAL YEAR 2021-2022

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

For this fiscal year, the total production output maintained last year's record breaking output of around **50,000 liters/year (65 % increase from 2 years ago)** of microbe cultures of *E. coli*, *P. pastoris*, *Streptomyces* spp. and various strains of yeast and fungus. The revenue generated from all these works have continuously provided valuable support to the research goals of the Institute as a whole and will continue to do so in the years to come.

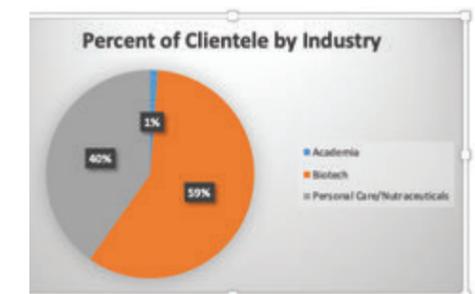
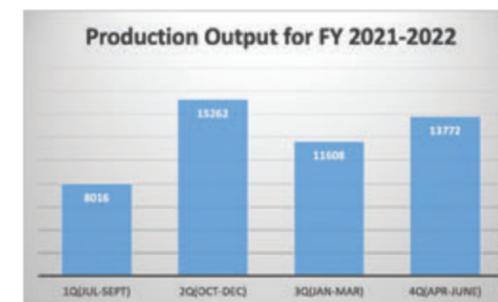
Dr. Arvin Lagda oversees and directs the overall operation of the facility, while the day-to-day projects is handled by a team of fermentation scientists led by Ms. Amanda Rodriguez (Production Manager) and supported by Dr. Sergey Druzhinin as Senior Scientist and Mr. Andrew Cloud as Laboratory Technician. The highlight for this fiscal year is the continuing implementation 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 workflow systems. This ensured product quality, safety and work efficiency.

This fiscal year, the facility obtained an almost perfect score (3.88/4) from Agilent's Supplier Evaluation for Technology, Quality, Responsiveness, Delivery and Cost (TQRDC) rating system. Moreover, a recent audit by the International Halal Foundation earned the facility a "Halal Certified facility" designation.

As part of Community outreach/education efforts, Dr. Lagda was selected by the American Society of Microbiology (ASM) as a designated mentor for the ASM Future Leaders Mentoring Fellowship program starting 2022.

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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Dr. Sergey Druzhinin, Laboratory Scientist
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Mr. Nathan Hill, Laboratory Technician



Waksman Genomics Core Facility

Waksman Genomics Core and Instrumentation Laboratory

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

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

In addition to the sequencing equipment, WGCF also offers access to Real-Time PCR on Thermo Fisher's StepOnePlus system, BioRad CFX 386 well Real Time PCR DNA shearing services using Covaris, as well as nucleic acid qualification services such as fluorometer Qubit, NanoDrop, Agilent bioanalyzer and imaging hardwares such as Typhoon Imager, GelDoc XR, LiCor Odyssey Imaging System and Tecan Plate Reader. A Beckman Scintillation Counter is also available for researchers who uses radioisotopes in their experiments.

For samples preparation, access is also available for several units of high speed refrigerated centrifuges and a couple of Ultracentrifuges. New to this year is the acquisition of a brand-new Beckman Optima XE90 Ultracentrifuge. All of which are available for the benefit of Waksman Institute faculty and researchers.

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



Bio Rad CFX



Tecan Spark



Fragment Analyzer



StepOne Plus Real Time PCR

Dr. Arvin Lagda, Core Facilities/Shared Resources

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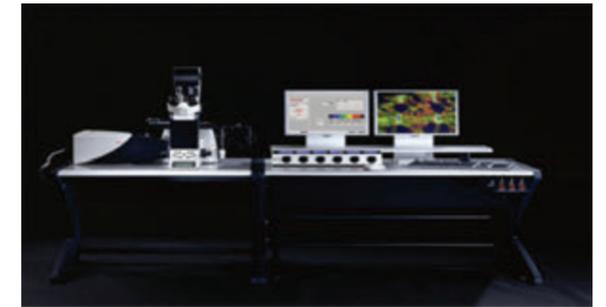
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SUPPORT SERVICES

Waksman Confocal Imaging Facility

The Waksman Institute Confocal Imaging Facility has two Leica TCS confocal microscopes, the SP5 II and the SP8, two workstations, high capacity, secure data storage for Waksman researchers, and is managed by a highly experienced and knowledgeable microscopist. Our scanning confocal microscopes are capable of high resolution imaging of labeled cell components in three-dimensional space by optical sectioning. Imaging can be done with most standard fluorophores in live or fixed samples. Both Leica TCS confocal models have inverted microscopes, spectral detection allowing for dynamic adjustment of detected emission wavelengths, sequential scanning to further reduce emission signal overlap, easy to use programs for creating Z-stacks and 3-D images, deconvolution software, multiple programs for post-imaging, and hybrid detectors for large dynamic range, increased signal sensitivity and decreased background noise, as well as standard PMT detectors, Z-stack compensation, and photon counting. Powerful and easy to use, Lightning Deconvolution on the SP8 significantly improves image resolution during acquisition by utilizing adaptive technology.



This past year, the Waksman Confocal Imaging Facility received two grants. A generous grant from the State of New Jersey enabled us to purchase three new microscopes, which will be installed over the summer and fall of 2022. These new microscopes, Leica Stellaris8 Confocal, Andor Dragonfly Spinning Disk, and Zeiss Elyra7, will add several advanced capabilities to our Imaging Facility, such as Fluorescence Lifetime Imaging Microscopy (FLIM), Super Resolution Radial Fluctuations (SRRF-Stream), optogenetics, photoablation, Lattice Structured Illumination Microscopy (SIM2), and Total Internal Reflection (TIRF). In addition to the State of New Jersey Grant, a grant from Rutgers Research Core Services allowed us to upgrade our Leica SP8 confocal microscope with a computerized scanning stage, along with Navigator, Tile Scanning, and Mark and Find software, as well as an additional hybrid detector (HyD). These upgrades allow users to quickly switch from searching image by image to seeing a full overview of the sample, increasing the viewing area up to 10,000x and imaging large tile-scans as well as multi-position experiments for single plane or high-resolution z-stacks. The additional HyD detector permits acquisition of multiple colors more rapidly without incurring the time-penalty needed to mechanically move the detector to a new spectral position.

The Waksman Institute Confocal Imaging Facility has approximately 83 trained users, primarily Waksman researchers, from 12 laboratories and is used an average of 60 hours per week (a slight reduction from previous years due to the pandemic). We are open to researchers from Rutgers University as well as other institutions and companies in the area. Our services include consultation for instrument selection, technical support and training with our microscopy expert, and individual use by trained and approved researchers. The future aim of the facility is to continue to provide exceptional imaging capabilities to Waksman researchers.

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Waksman Greenhouse

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

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



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CHARLES AND JOHANNA BUSCH FELLOWS

Predocctoral 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 hydroxymethylation of ribocytosine (5hm^rC) in mRNA and that 5hm^rC is enriched in *Drosophila* brain mRNAs. Loss of Tet at an early stage resulted in a severe defect in the *Drosophila* brain demonstrating that Tet is required for the 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 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. In addition, mutations in the human TET3 gene have been found in individuals and families, which are affected by intellectual disability, suggesting that Tet is crucial for proper human brain development. Recent results showed that Tet is required for axon projection to the right compartment of the brain and this requirement is in the early stage of brain development. RNA-seq in the Tet mutant brain revealed 1547 genes are up- and 32 genes are down-regulated. I am confirming target genes that are responsible for controlling axon projection in the early brain. To further study Tet functions in the brain, I am addressing the question of whether the Tet mutant phenotype can be rescued by altering Tet target gene levels in the brain?



Hoa Vu
Nickels Lab

Research Summary

Development of multiplexed protein-DNA photo-crosslinking as a method for analysis of transcription initiation and transcription 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 struc-

tural snapshots identified in these studies provide mechanistic insight into transcription from all sequences or whether these studies provide mechanistic insight into transcription that occurs in living cells, from sequences located on the chromosome.

My research focuses on the development of a multiplexed protein-DNA photo-crosslinking method (Bpa scanning) 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.

Bpa scanning entails formation of transcription complexes *in vivo* using a collection of 87 RNAP derivatives on three subunits (β , β' and σ) that containing a photo-crosslinker p-benzoyl-L-phenylalanine (Bpa) at specific positions. This year, I used Bpa scanning to define, in parallel, interactions of 87 Bpa-containing RNAP variants with a collection of six σ^{70} -dependent promoters that have historically been selected as model promoters for studies of RNAP-promoter structure and transcription regulation both *in vitro* and *in vivo*. We analyzed protein-DNA crosslinking with 88 Bpa-containing RNAP derivatives both on wild-type versions of each promoter, and on a library of promoter derivatives containing single base pair substitutions at each promoter position from -70 to +25 (1716 promoter variants in total). Each promoter variant was barcoded by two sequences of 8 randomized nucleotides upstream and downstream of the promoter sequence and will be introduced into plasmids coding for a Bpa containing RNAP. These Bpa-containing RNAP derivatives formed complexes with the promoter’s collection in transcription initiation and early elongation stages *in vivo*. The interactions of these derivatives and the promoters were investigated by high throughput sequencing of the crosslinking materials. The results showed that the Bpa scanning method was able to define the interactions of RNAP and promoter DNA at nucleotide and amino acid resolution during transcription initiation and early elongation in *E. coli*. I also measured RNA yield for each promoter. RNA produced by each promoter was isolated and sequenced. The downstream barcode was used to identify and map the RNA yield from each promoter. This allows us to access how promoter substitutions affect RNAP-promoter interactions and RNA yield.



Elmira Kirichenko
Irvine Lab

Research Summary

Investigating Jub dependent post-translational modification of Wts

Growth regulation is one of the fundamental questions in developmental biology. One of the most evolutionarily conserved growth regulating pathways that was first discovered in *Drosophila melanogaster* is the Hippo Signaling pathway. The central events of this signaling pathway converge to regulate the activity of the transcription co-activator Yki with the help of tumor suppressor kinases including Hippo (Hpo) and Warts (Wts) (Snigdha et al., 2019). Members of the Hippo kinase cascade undergo post-translational modifications that are critical in the regulation of their activity and function. Studies in *Drosophila* wing discs report, that depending on the cytoskeletal tension, Wts is capable of associating either with its inhibitor Jub at adherens junctions or with its activators Expanded (Ex) and Hippo (Hpo) at more apical junctions.

Activation of Wts occurs through several steps of phosphorylation, including phosphorylation of Wts by Hpo and Mats-induced Wts autophosphorylation (Avruch et al., 2012). This activation of Wts in turn negatively regulates Yki

activity (Meng et al., 2016). Although Wts-Jub interaction at AJs has been studied in the context of Yki regulation (Rauskolb et al., 2014), exactly how Jub regulates Wts activity is not well understood.

The overall goal of my project is to understand the mechanism of Wts regulation by Ajuba LIM protein Jub and the significance of Wts post-translational modification in the regulation of Hippo signaling. In my preliminary studies, I have observed that Wts forms not only a physical interaction with Jub, but also results in the appearance of an additional, slower mobility Wts band, suggesting that Wts undergoes a post-translational modification in the presence of Jub. Lambda Phosphatase treatment decreased the mobility shift caused by Jub, suggesting that the post-translational modification is phosphorylation. Mapping the modification region, I was able to observe the mobility shift using N-terminus Wts constructs in the presence of Jub, suggesting that the modification region of Wts is in the N-terminus. Since the N-terminus constructs lacked the C-terminus kinase domain, the mobility shift of the N-terminus indicated that the post-translational modification is not an autophosphorylation. Additionally, I have also observed that Wts protein expression levels become significantly reduced in the presence of Jub, suggesting that Wts degradation is promoted, possibly by the Jub-induced modification. Our results indicate that this Jub-mediated modification of Wts contributes to inhibition of Wts by Jub. Determining the functional significance of Wts post-translational modification will expand the understanding of the role of Wts in the Hippo Signaling pathway.



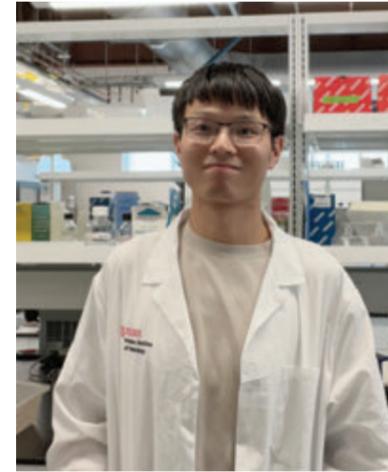
Saai Tiruchendurai Suryanarayanan
Singson Lab

Research Summary

The role of SPE-21, a palmitoyltransferase in sperm activation.

Irrespective of the species, spermatids undergo post-meiotic differentiation or spermiogenesis to gain polarity, motility and become fertilization competent. In *C. elegans*, this process, also known as sperm activation, transforms round spermatids to motile, amoeboidal spermatozoa. Upon activation, these spermatozoa produce pseudopods that help them crawl to the oocytes. Using forward genetics in *C. elegans*, we have identified a temperature sensitive allele of *spe-21* also designated as *dhhc-5*, *spe-21(as41ts)*. *spe-21/dhhc-5* encodes a palmitoyltransferase with four predicted transmembrane domains and has a DHHC-CRD motif. *spe-21(as41ts)* is a missense mutation that results in C(87)Y of the DHHC-CRD motif. Mutations in this region are predicted to affect the palmitoylation function of the protein. We also generated a genetic null allele with CRISPR, *dhhc-5(syb4299)*, which introduces a stop codon and a frameshift shortly after the transcription start site. We observed the following phenotypes in the null worms: Both *spe-21* mutant hermaphrodites and males are sterile. Their sperm fail to undergo *in-vitro* activation and

do not produce pseudopods. *In-vivo*, in comparison to the control worms, sperm are quickly lost from day 3 adult hermaphrodite spermathecae due to their inability to crawl back from the uterus, further suggesting defects in activation. It is also interesting to note that the mutant sperm appear to have off-centered nuclei and mis aligned membranous organelles (MOs). Furthermore, *spe-21(as41ts)* worms have the same phenotypes. The phenotypes in both alleles suggest possible role of palmitoylation in sperm activation. Our preliminary data using mNeon-Green tagged *spe-21* transgene reveal that SPE-21 localization is similar to MO localization in spermatids. We believe that SPE-21 interacts with other proteins and play a major role in sperm activation and fertilization by palmitoylating their MO target proteins and aiding their sperm membrane trafficking and tethering. Our discovery will be pivotal in understanding the spatiotemporal regulatory role of these enzymes in making fertilization competent sperm.



Sanqiang Zhang
Dong Lab

Research Summary

In Arabidopsis, three bHLHs -SPEECHLESS (SPCH), MUTE, and FAMA- are key transcription factors that sequentially control the specification and differentiation of guard cells on the leaf surface, making delicate pores for water vapor, carbon dioxide, and oxygen exchange between plant and the ambient environment. The mechanisms that spatial temporal switching of their expression patterns and distinctive driving forces on cell divisions are still obscure. It is also intriguing to study how hormone and environmental cues integrate these three TFs for malleable stomatal cell fate decisions and confer plant developmental plasticity facing changing climates. Dong lab and collaborators previously conducted ATAC-seq and RNA-seq through three key stages of stomatal development, I aim to explore and characterize other cell-type or cell-stage specific regulators interplaying with SPCH, MUTE, and FAMA during stomata formation.

Early studies showed that MYB3R3/4 interact with RBR1-E2F DREAM complex to regulate cell cycle progression in plants. DREAM components SOL1 and SOL2 mediate cell fate transition and cell divisions downstream of SPCH in early and late stomatal lineage. Our data indicate that meristemoid-enriched MYB3Rs are redundantly necessary to regulate cytokinesis during stomatal development. Although targeted by SPCH, some MYB3Rs could bind to SPCH

regulatory regions, which might frame a feedback regulation of the DREAM complex on SPCH function.

YODA-MKK-MAPK-WRKY33 signaling is engaged in plant response to pathogen attacks. I found a bunch of WRKYs enriched in stomatal lineage. Further study will illustrate how plants coordinate development and immunity using a shared signaling pathway.

I will continue to combine genetics, molecular, and biochemical methods to dissect the detailed roles of these novel factors in guard cell formation.



Naureen Hameed
Barber Lab

Research Summary

Neuromodulatory Signal Integration In A *Drosophila* Clock Output Region

The pars intercerebralis (PI) is a proto-hypothalamic brain region in *Drosophila* that is made up of a heterogeneous population of neurosecretory cells with diverse inputs that provide information about internal and environmental states. The diverse neuron populations in the PI regulate circadian feeding, metabolic gene transcription, circadian locomotor activity, and sleep. We identified novel clock neuron inputs to the PI, as well as intra-PI signaling that may serve to coordinate both inputs and outputs from the PI. Previously, DN1 and LNd clock neurons were shown to modulate PI neuron activity in a time-of-day dependent manner. I hypothesized additional clock-to-PI connectivity from LNV neurons. Hence, I used a genetically encoded retrograde neuronal circuit tracing tool (BACTrace) to label PDF+ LNV clock neurons upstream of PI insulin producing cells and diuretic hormone 44+ PI neurons, which indicated a physical connection. We also sought to determine whether there is physical and functional connectivity between distinct PI neuron populations. BACTrace indicated that the PI neuron populations were physically connected to each other. We further validated the

functionality of connections using a GCaMP-based stimulus-response assay, which showed that all pairs of identified PI neuron populations can modulate one another's activity. These results indicate that PI neurons integrate small molecule neurotransmitter and neuropeptide signals from upstream clock neurons via intra-PI communication to coordinate physiological and behavioral outputs. Rhythmic feeding experiments using the Fly Liquid Food Interaction Counter (FLIC) are underway to determine the effects disrupted clock and intra-PI neuropeptide signaling to other PI neuron populations have on rhythmic feeding behaviors. We endeavor to identify the specific neurotransmitters and neuropeptides involved in clock-to-PI and intra-PI signaling and to investigate how these signals regulate rhythmic behaviors.

Postdoctoral Research



Malihe Mirzaee
Maliga Lab

Research Summary

Expression of toxic proteins in tobacco chloroplasts

There is an interest to orally administer therapeutic proteins as an alternative to injection. Because of the attainable high protein levels and bioencapsulation of proteins, chloroplasts are an attractive system for the expression of therapeutic proteins for oral delivery. The problem is that some of these proteins are toxic to plant cells. We are working on a regulated system where silent transgenes can be inserted in the chloroplast genome and subsequently turned on when the expression is desired. This system utilizes transgenes expressed from a T7 RNA polymerase (T7RNAP) promoter, which is not recognized by chloroplast RNA polymerases. The transgenes are then turned on by expressing a chloroplast-targeted T7-RNA polymerase. We test the system in tobacco, where a GFP transgene has been incorporated in the chloroplast genome. We shall then turn on reporter gene expression by providing a chloroplast-targeted T7-RNAP. We are currently testing the system in tobacco, where chloroplast transformation is efficient. When operational, we shall implement the system in lettuce, where it will be used for the expression of myostatin which is normally toxic to plant cells.

Seed-specific expression of recombinant proteins

High-level protein expression in leaves impairs plant growth. To selectively express recombinant proteins in non-green plastids without impact on plant development, we took advantage of an engineered PPR10GG RNA binding protein that selectively boosts protein expression only from an engineered PPR10 binding site (BSGG). The goal is to test the potential of recombinant protein expression in seed plastids. For this, we introduced reporter genes in the plastid genome with an engineered BSGG site and transformed the nucleus with a PPR10GG transgene in a seed-specific cassette. We have found that GFP accumulates in the seed at ~1.5 % of the total soluble cellular protein, without significant impact on plant development. Our strategy exploits the seed plastids as a novel bioreactor to produce foreign proteins using a programmable PPR10GG as a tissue-specific activator of mRNA translation. This result indicates that the inducible expression system is a desirable alternative to the constitutive expression of chloroplast transgenes.

BENEDICT MICHAEL FELLOW

Predoctoral Research



Samuel Adeniyi Adeleye
Yadavalli Lab

Research Summary

I study an enzyme, QueE, that plays a role in the Queuosine(Q) biosynthesis and as a cell division inhibitor. Queuosine (Q), a hyper-modified guanosine, is a universally conserved tRNA modification in specific tRNAs' anticodon loop (G34UN). Prokaryotes synthesize Q *de novo* from guanosine triphosphate (GTP), while eukaryotes can only acquire Q from microbial or dietary sources through the salvage pathway. The role of QueE in Q-synthesis is well characterized. However, it was only in the last decade that the new function as an inhibitor of cell division was reported. When *E. coli* cells are exposed to sub-inhibitory levels of cationic antimicrobial peptides, this signal is sensed by a two-component signaling system, broadly conserved among gammaproteobacteria, including *E. coli*, Salmonella, and related species. Upon strong activation of the PhoQ/PhoP system by the antimicrobial peptide, cell division is inhibited via upregulation of QueE, which binds to the division complex leading to filamentation. However, the mechanistic details of how QueE inhibits cell division and whether this secondary function is independent of QueE's role in the tRNA modification pathway are unclear. I proposed that QueE is a moonlighting protein whose roles in cell division and Q-biosynthesis are functionally discrete. Using alanine scanning and APB-gel tRNA northern blot analyses, I have identified specific residues that affect either one, both, or

neither of the functions, supporting my hypothesis that QueE's roles in tRNA modification and antimicrobial activity peptide stress response are independent of each other. Data from site-specific *in vivo* crosslinking technique coupled with mass spectrometry identified proteins in the divisome machinery that interact with QueE. Finally, results from bacterial two-hybrid assays suggest that QueE inhibits septation by interacting with proteins involved in peptidoglycan(PG) synthesis. Additionally, filamentous cells survive fosfomycin treatment better than non-filamentous cells indicating that QueE sequesters MurA, the target of fosfomycin. We will carry out colocalization experiment and PG synthesis assays to further probe the mechanism QueE's interaction with the divisome proteins. Deciphering the mechanism of QueE's dual activities in the cell will provide insights into how RNA metabolism is interconnected to critical cellular processes such as cell division and stress responses.

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 2021-2022

Biophysical Chemistry
Chemical Bonding
Chemistry
Core Seminars in Plant Biology
Developmental Genetics
Ethical Scientific Conduct
Ethical Scientific Conduct Refresher
Genetic Analysis I
Genetic Analysis II
Inorganic Chemistry Molecular Biosciences
Microbial Biochemistry
Molecular Biology and Biochemistry
Molecular Biosciences Graduate minicourse
Plant Biology II
Plant Molecular Biology
Thesis Writing and Communication in Genetics

WAKSMAN STUDENT SCHOLARS PROGRAM

High School Outreach



Summary

With the emergence of the cyberinfrastructure 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 29 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 2021-2022 WSSP consisted of two interrelated parts: a Summer Institute (SI) and an Academic Year Program (AYP). Due to the pandemic most of these activities were conducted using an on-line, virtual, format. In July 2021, 49 students and 3 teachers from 27 different schools participated in a 9-day virtual SI. The SI program used a “flipped classroom” structure. Initially, students independently viewed videos or read notes that provided background information on molecular biology and bioinformatics and steps of the WSSP research project, then took quizzes to help them gauge their understanding of the material. Students then participated in two daily discussion seminars that went more in depth into the research project and addressed any questions they had. Students were provided with data generated by the WSSP staff and used Internet resources to process and analyze their data.

After the SI, teachers and students returned to their schools and recruited additional students who contributed to the research project during the academic year. Some teachers incorporated the project into existing research courses or advanced placement biology courses at their schools. Others conducted the project as after school clubs. These courses and clubs provided additional students beyond those who attended the SI with opportunities to conduct and contribute to the research project.

During the 2021-2022 academic year, teachers from 40 of the WSSP schools conducted the laboratory and bioinformatics sections of project in person. The WSSP provided the reagents and supplies for these schools to conduct the experiments. Four of the WSSP schools conducted the project virtually using online resources provided by project faculty. Schools that did not conduct in person laboratory activities were provided with novel DNA sequence data that was generated by the WSSP staff for each participating student to analyze on-line.

In addition to the activities based at the Waksman Institute, the WSSP also supported the program at sites beyond the central New Jersey region. Dr. Forrest Spencer at John Hopkins University, in Baltimore MD helped run the AYP for two high schools in MD. Working with the Lawrence Livermore National Laboratory, Livermore, CA, students from three schools in CA and two schools in HI participated in the program. A total of 973 high school students participated in the WSSP program during the 2012-2022 academic year

The Research Question

The 2021-2022 research project focused on identifying the genes and proteins of the duckweed, *Landoltia punctata*. Duckweeds are fresh-water aquatic plants that is used in bioremediation and can be potentially used for biofuel. Several research laboratories at Rutgers are currently investigating these plants.

To conduct the project, a plasmid cDNA library from *Landoltia punctata* was prepared by the WSSP staff. If the schools conducted the in-person laboratory activities, students grew bacterial cultures of individual clones from the cDNA library, performed minipreps of the plasmid DNA, cut the DNA with restriction endonucleases, performed polymerase chain reactions, analyzed DNA electrophoretically, had inserts from their clones sequenced, and analyzed these sequences with the aid of a computer program called the DNA Sequence Analysis Program (DSAP) that was developed by the project director and faculty. Students who did not conduct the laboratory experiments were each provided with two sequences that were generated by the WSSP staff. A total of 923 DNA sequences were generated by students conducting the laboratory portion of the WSSP at their schools. 985 different DNA sequences analyzed by the students and 651 of these sequences have been or will be submitted for publication on the National Center for Biotechnology Information (NCBI) DNA sequence database citing the students’ names as contributing authors.

WISE

The WSSP project involves both students and teachers conducting research at their high schools during the academic year. Since involvement in the yearlong program requires the participation from a teacher who has attended a WSSP SI and the support of the schools, many students from schools that are not involved in the program cannot conduct the research project. To accommodate these students, we offered summer programs called **Waksman Institute Summer Experience (WISE)** in which students perform the same research project as conducted in the WSSP. Due to the pandemic, in 2021 and 2022 these programs were all held on-line, in the same manner as the WSSP SI. One advantage to conducting a virtual WISE program (vWISE) is that we were not limited to the number of students who could attend the Program by the number of students who could work safely in the laboratory. As a result, we were able to increase the number of participants from 36 during previous in-person WISE programs to 84 in June 2021, 86 in August 2021, and 28 in Fall 2021. Students from states across the US along with several other nations were able to participate in these programs. The summer vWISE programs were conducted over a two-week period with two discussion sessions each day. The Fall 2021 vWISE program was conducted in two nightly sessions each week over a nine-week period. Students were each provided two DNA sequences and the vWISE students completed the analysis of 374 of these sequences and 312 were published on the NCBI database.

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Dr. Janet Mead, Laboratory Director

John Brick, Laboratory Assistant



PRESENTATIONS & MEETING ABSTRACTS

Waksman Institute Hosted Seminars -

Dong Juan Dong, Title Patterned Diversity: Cell Polarity and Asymmetrical Division in Plant Development ZOOM 9/28/21

McKim Anthony Choo, Leica Stellaris TauSTED super-resolution microscopy, ZOOM 10/12/21
Barber Shirley Zhang, Emory University, Title: Contextualizing the blood-brain Interface in biological time, Zoom 10/26/21

Barber Jaeda Coutinho-Budd, University of Vermont, Title: Neuron-glia and glial-glia interactions in health and disease ZOOM 12/7/21

Doong Xingyun Qi, Rutgers University, Camden, Title: Peptide ligand-regulated dynamics of the receptor kinase specifying binary cell fate on the plant epidermis ZOOM 1/21/22

Dong Michelle Facette, University of Massachusetts, Title: Processes and proteins important for asymmetrical cell division, Cook Douglas Lecture Hall 2/18/22

Barber Ian Krout, University of Rochester, School of Medicine, Title: Elucidating the role of methylmercury (MeHg) demethylation in modulating toxicity and kinetics using *Drosophila melanogaster* 3/18/22

Zander Sheng Yang He, Duke University, Title: Climate impact on the plant-microbe interactions ZOOM 3/25/22

Juan Dong, Rutgers University, Title: Creating Diversity signaling and polarity in stomatal development, ICPH Auditorium, Rutgers Newark & Zoom 4/19/22

Singson, Eric Lai, Sloan-Kettering Institute, NYC, Title: Red queen and the lost boys: Roles for RNAi to silence sex chromosome conflict during speciation, Life Science Auditorium, Busch Campus & Zoom 5/9/22

Waksman Student Scholars Programs

Virtual Waksman Student Scholars Program Summer Institute (vWSSP-SI), July 6-July 16, 2021, 49 students, 3 teachers.

Virtual Waksman Institute Summer Experience (vWISE June-20), June 21-July 2, 2020, 84 students.

Virtual Waksman Institute Summer Experience (vWISE

August-20), July 26-August 31, 2021, 86 students.

Virtual Waksman Institute Summer Experience (vWISE Fall-21), October 5-November 17, 2021, 28 students.

Barber:

McClure CD, Hassan A, Aughey GN, Butt K, Estacio-Gómez, A, Duggal A, Sia CY, Barber AF, Southall TD. (2022). An auxin-inducible, GAL4-compatible, gene expression system for *Drosophila*. Poster presented at Society for Biological Rhythms meeting, Amelia Island, Florida.

Dong:

Seminar (Virtual) at the College of Life Science, Peking University, Beijing, China 2022
Title: Polarity factors and regulators in plant asymmetric cell division.

The 2022 Joint MAS-ASPB and UMD Plant Symposium, University of Maryland, MD 2022

Title: Switching on or off: signaling control in stomatal development.

Seminar at the Department of Microbiology, Biochemistry & Molecular Genetics/Public Health Research Institute, Rutgers University at Newark, NJ 2022
Title: Creating diversity: signaling and polarity in stomatal development.

Seminar (Virtual) at the Waksman Institute, Rutgers University, NJ, 2022
Title: Patterned diversity: signaling and polarity in stomatal development.

Seminar (Virtual) at the College of Life Science, Peking University, Beijing, China 2022
Title: Polarity factors and regulators in plant asymmetric cell division.
Seminar (Virtual) for the Northwestern Agricultural and Forestry University, Yangling, China. 2022
Title: Polarity, asymmetric cell division and fate differentiation in plants.

Seminar (Virtual) for the China Agricultural University, Beijing, China. 2022
Title: Polarity factors and regulators in plant asymmetric cell division.

Dismukes:

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

Hengfei Gu, Synthesis and Interfacial Integration of Transition Metal Phosphide Catalysts on High-Performance Solar Cells for Direct Solar-Driven Water Splitting, September 2022.

Ebright:

"Structural basis of transcription and transcription-translation coupling." US-UK Online Microbial Transcription Seminar Series, 2021 (remote)

"Structural basis of transcription-translation coupling." PDB-50, Institute for Quantitative Biomedicine, Rutgers University, Piscataway, New Jersey, 2021 (remote).

"RNA polymerase: the molecular machine of transcription." Korean Society for Applied Biological Chemistry Annual Meeting, Jeju, South Korea, 2022 (plenary address; remote).

Lan, T., Ganapathy, U., Ahn, Y.-M., Sharma, S., Zimmerman, M., Molodtsov, V., Ebright, R.H., Dartois, V., Freundlich, J., Dick, T., and Aldrich, C. (2022) Rationally designed rifabutin analogs to overcome intrinsic resistance of *Mycobacterium abscessus*. American Chemical Society Spring Meeting, San Diego CA, March 20 - 24, 2022 (virtual).

Lan, T., Ganapathy, U., Ahn, Y.-M., Sharma, S., Zimmerman, M., Molodtsov, V., Ebright, R.H., Dartois, V., Freundlich, J., Dick, T., and Aldrich, C. (2022) Novel rifabutin analogs blocking *Mycobacterium abscessus* activity. Colorado Mycobacteria Conference 2022, Ft. Collins CO, May 31-June 3, 2022.

Fermentation:

Lagda, AC. "New Jersey Research Cores Partnering Conference", Rutgers University, New Brunswick, New Jersey. June 29, 2022

Lagda, AC. "The Future of Microbial Biotechnology: From Research to Regulation" USDA/IGI, Feb 02-03, 2022.

Rodriguez, A., Druzhinin, S., Cloud, A. and Lagda, AC. "Introduction to ISO22716:2007 GMP for Cosmetics Training" conducted by SGS Academy, June 13, 2022.

Gallavotti:

Gallavotti, A. "Mechanisms and manipulation of maize inflorescence architecture. Waksman Directors' Meeting. Waksman Institute, Rutgers, October 13, 2021.

Gallavotti, A., Gregory, J., Chen, Z., Feng, F., Huang, C.S., Li, M., Marand, A., Ethridge, C., Earp, T., Schmitz, R.J., Galli, M. Mapping and functional characterization of cis-regulatory variation in maize. Maize Genetics Meeting Abstract 64:P199. St. Louis, MO, March 31-April 3, 2022.

Chaudhry, A., Chen, Z., Gallavotti, A. Characterization and positional cloning of the prolific mutant *early-517**. Maize Genetics Meeting Abstract 64:P151. St. Louis, MO, March 31-April 3, 2022.

Gregory, J., Gallardo, C., Liu, X., Gallavotti, A. Boom or bust: regulation of meristem size by the REL2 corepressor family. Maize Genetics Meeting Abstract 64:P149. St. Louis, MO, March 31-April 3, 2022.

Dai, D., Mudunkothge, J.S., Galli, M., Char, S.N., Davenport, R., Zhou, X., Gustin, J.L., Spielbauer, G., Zhang, J., Barbazuk, W.B., Yang, B., Gallavotti, A., Settles, M. Paternal imprinting of dosage-effect defective1 contributes to seed weight xenia in maize. Maize Genetics Meeting Abstract 64:P118. St. Louis, MO, March 31-April 3, 2022.

Chiles, A., Taylor-Teeple, M., Nemhauser, J., Gallavotti, A., Moss, B. Altering the binding site of a plant hormone in corn proteins affect how fast they break down. Maize Genetics Meeting Abstract 64:P91. St. Louis, MO, March 31-April 3, 2022.

Irvine:

"Biomechanical regulation of organ growth", at Princeton University, Princeton NJ Dec 10, 2021

McKim:

Gordon Conference on meiosis
"Interactions between the central spindle microtubules and the kinetochores promotes homologous chromosome bi-orientation." June 2022

Maliga:

Pal Maliga, Progress in Reengineering *Agrobacterium* for T-DNA delivery to Chloroplasts, Biological Research Center, Szeged, Hungary, 12/6/2021

Pal Maliga and Aki Matsuoka, Progress in Reengineering *Agrobacterium* for T-DNA Delivery to Chloroplasts. Plant and Animal Genome Conference XXIX virtual, January 8-12, 2022

Pal Maliga, Kerry A. Lutz, Aki Matsuoka, Ana Candia, Alyssa Leung and Malihe Mirzaee, PPR10 RNA Binding

Protein for Regulated Gene Expression in Chloroplasts, Plant and Animal Genome Conference XXIX virtual, January 8-12, 2022
Pal Maliga, “Agrobacterium-mediated transformation of the plastid genome”. 2022 In Vitro Biology Meeting, June 4-7, 2022, San Diego, CA.

Aki Matsuoka, Reengineering VirD2 for T-DNA delivery to chloroplasts. Mitochondria and Chloroplasts Gordon Research Conference, Mount Snow, VT, July 17-22, 2022.

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

Ana Candia, Kerry Lutz, Aki Matsuoka and Pal Maliga (2021) Epitope Tagging of the PPR10 protein to Correlate PPR10 Protein Levels with Plastid transgene expression. 85th Annual Meeting of the Northeast section of ASPB, Oct. 30, 2021.

Aki Matsuoka, Julia Ferranti and Pal Maliga. “Reengineering VirD2 for T-DNA delivery to chloroplasts”. Mitochondria and Chloroplasts Gordon Research Conference, Mount Snow, VT, July 17-22, 2022.

Malihe Mirzaee, Alyssa Leung, Alifiya Quresh, Ana Candia, Aki Matsuoka, Kerry Lutz, and Pal Maliga. “The PPR10 RNA binding protein for seed-specific expression of recombinant proteins in tobacco plastids.” Mitochondria and Chloroplasts Gordon Research Conference, Mount Snow, VT, July 17-22, 2022.

Singson:
Frontiers in Reproduction Course Lecture, the Marine Biological Laboratories, Woods Hole MA, May 17, 2022

Stavropoulos:
Gordon Research Conference on Sleep Regulation and Function, March 2022. Invited Speaker.

Yadavalli:
Small Proteins and Epitranscriptomic Factors: Emerging mechanisms in bacterial gene regulation. Institute for Infectious and Inflammatory Diseases (i3D) 2022 Faculty Retreat, Rutgers University, Piscataway NJ (Virtual), March 2022.

Development of an in vivo photo-cross-linking method to identify small protein targets. Small Small Protein Meeting at the National Institutes of Health (NIH), Bethesda

MD (Virtual), January 2022.

Small but mighty regulators of bacterial gene expression and stress response. Biology Seminar series at the University of Delaware, Newark DE (Virtual), October 2021.

Small Proteins and Epitranscriptomic Factors: Emerging mechanisms in bacterial gene regulation. Lambda Lunch Seminar series at the National Institutes of Health (NIH), Bethesda MD (Virtual), September 2021.

Zander:
Zander M. (Invited Speaker) Transcription factor-mediated epigenome reprogramming in plant stress responses, 2022 Mid-Atlantic Society for Developmental Biology Meeting, Lehigh University, Bethlehem, PA, USA

Zander M. Transcription factor-mediated epigenome reprogramming in plant stress responses, 2022, University of Pennsylvania Plant Seminar Series, virtual

Zander M. Master Transcription Factors at Work. New Insights into MYCs and PIFs, 2021 CSHL Plant Photobiology Symposium

PATENTS & PUBLICATIONS

Patents

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

Ebright:
Ebright, R. and Ebright, Y. Antibacterial agents: dual-targeted RNA polymerase inhibitors: conjugates of benzoxazino- and spiro-rifamycins with N α -aroyl-N-aryl-phenylalaninamides. US63235616 (filed 08/20/21).

Maliga:
US11, 208665 (2021) Compositions and methods for improving plastid transformation efficiency in higher plants. Inventor: Pal Maliga

Publications:

Barber:
McClure CD, Hassan A, Aughey GN, Butt K, Estacio-Gómez, A, Duggal A, Sia CY, Barber AF, Southall TD. (2022). An auxin-inducible, GAL4-compatible, gene expression system for Drosophila. *eLife* 11: e67598. DOI: 10.7554/eLife.67598

Crespo-Flores SL and Barber AF. (2022). The Drosophila circadian clock circuit is a non-hierarchical network of peptidergic oscillators. *Curr Opin Insect Sci*. DOI: 10.1016/j.cois.2022.100944.

Dismukes:
Creating Functional Oxynitride–Silicon Interfaces and SrNbO₂N Thin Films for Photoelectrochemical Applications. Hwang, S., S.H. Porter, M. Li, R. Thorpe, A.B. Laursen, H. Gu, A. Safari, M. Greenblatt, E. Garfunkel and G.C. Dismukes. *The Journal of Physical Chemistry C*, 2022. 126(13): p. 5970-5979.

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