WAKSMAN INSTITUTE OF MICROBIOLOGY

ANNUAL REPORT

2022 2023

RUTGERS

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.

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Kenneth Irvine, Interim Director
Elizabeth Minott, Esq., Legal Counsel & Secretary
Robert Rossi, Executive Director

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REPORT OF THE INTERIM DIRECTOR

Kenneth Irvine

Report of the Interim Director

Throughout the year, our faculty continue 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.

Our research labs train students throughout the year, but summer is a particularly busy time as we host multiple in person sessions of our high school science outreach programs, the Waksman Student Scholars Program and the Waksman Institute Summer Experience. We also have many undergraduates working full time in laboratory research, including some with fellowships supported by the Waksman Institute. We are especially pleased to continue our partnership with the Rutgers in Science and Engineering (RISE) program, which is devoted to expanding opportunities for diverse students to participate in research.

Highlights of the past year include a special symposium in April in honor of our late Director, Dr. Joachim Messing. The symposium featured talks by trainees and colleagues of Dr. Messing, headlined by the inaugural Joachim Messing lecture in Molecular Genetics – an annual, endowed lecture that will bring an outstanding scientist to campus each year. The inaugural Messing lecture was delivered by Dr. Marja Timmermans, from the University of Tubingen. Also in the Spring, we hosted the inaugural Evelyn Witkin lecture, presented by Dr. Bonnie Basler from Princeton. A special treat was the in-person attendance at the lecture of Dr. Witkin, just after her 101st birthday. At the end of the semester, Waksman Institute lab Director Drew Vershon was awarded the Ernest E. McMahon-Class of 1930 Award in recognition of his leadership of our high school science outreach programs. This is a special award that recognizes an individual or a group within Rutgers University that has made a significant and creative contribution to the extension of the educational resources of the University to the people of New Jersey. This summer, Dr. Andrea Gallavotti was promoted to full Professor – congratulations Andrea! This past year we also completed updates to our imaging facilities, which now operate five state-of-the-art microscopes as Rutgers core facilities.

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. Key research areas investigated by Waksman scientists encompass Developmental and Reproductive Biology, Microbes and Drug-Resistant Infectious Disease, Neuroscience and Neurological Disorders, and Environmental Sustainability and Climate Change. A key aspect of research in the Institute is its interdisciplinary nature, including faculty from multiple departments and schools, and incorporating approaches from physical and computational sciences into life sciences research. The Institute has a long history of excellence, including Rutger's only Nobel prize winner (Selman Waksman), only Lasker prize winners (Selman Waksman and Evelyn Witkin), and recipients of a number of other major awards and prizes, including Wolf Prize winners, Howard Hughes Medical Institute Investigators, and inductees into the National Academy of Sciences.

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 provide by each of these units are included elsewhere in this annual report.

Faculty

In the academic year 2022-2023, 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 three assistant research professors, four visiting student/scholar researchers, six research associates, sixteen postdoctoral researchers, twenty-three technical assistants, and eighteen graduate students. The Waksman Institute's total resident population is currently 148, including forty-one 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, ten 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 Ernest E. McMahon-Class of 1930 Award to Dr. Drew Vershon, in recognition of his leadership of our high school science outreach programs.

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 $6.7 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.

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, microscopy, greenhouse facility services, and specialty repair of equipment. The staff for these core central services is kept to the minimum necessary for operational support, and part-time employees are utilized as appropriate.

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

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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).

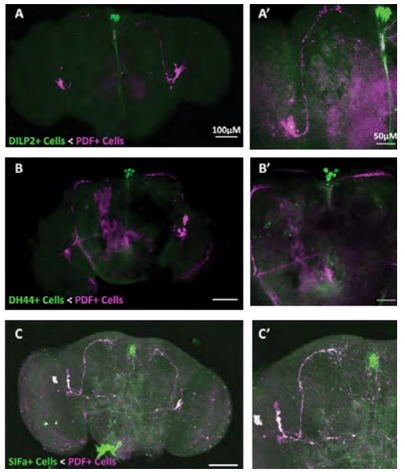

Fig. 1: Clock-to-PI retrograde tracing. Expression of the BAcTrace system in (A, A') DILP2+; (B, B') DH44+ and (C, C') SIFa+ PI neurons marks the PI neurons in green, and labels the PDF+ clock neurons as upstream synaptic partners in magenta.

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 integrate s 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, including the key circadian neuropeptide, pigment dispersing factor (PDF). Knockout of the PDF receptor in Drosophila insulin producing cells results in disruption of diurnal eating patterns, and evening-specific overfeeding,

confirming a role for PDF in clock output signaling to the PI.

Sex-specific roles for neuropeptides in clock output signaling

Across phylogeny, sex differences in circadian phenotypes and responses to circadian perturbations are evident, despite minimal sex differences in the brain molecular clock, and in timekeeping within brain clock neurons. This suggests that sex differences in circadian biology arise from clock outputs – that is signals from the brain clock to the periphery – and in tissue-specific responses to those clock output signals. Understanding sex differences in circadian output signaling is critical to developing personalized chronotherapeutic interventions to both prevent and treat disorders with sexually dimorphic risks and outcomes. A CRISPR-based knockout screen of clock circuit neuropeptides found that deletion of three key neuropeptides in the fly brain clock uniquely affects mated female flies, but not males or unmated females. Research in the lab is currently investigating the mechanisms for sex- and mating-status-based differences in circadian output signaling in the fly clock.

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

Sleep and circadian rhythm disorders are a major feature of traumatic brain injury (TBI); TBI patients not only experience acute insomnia and sleep disorders, but also show delayed rhythms of core body temperature and melatonin secretion for years after injury. Symptoms observed in TBI patients, such as mood disorders and PTSD, are associated with changes in sleep and rhythmic physiology. At present the field lacks a mechanistic understanding of how sleep and circadian rhythms changes are driven by traumatic injury, and whether these changes are protective or maladaptive. Using a head-specific fruit fly traumatic injury paradigm (Fig. 2A), we have found that flies exhibit chronic sleep changes after mild and moderate injury that persist for at least a week (Fig 2 B, C). TBI drives fragmentation of night-time sleep, although sleep deprivation assays demonstrate that flies can still sense accumulated sleep need. We are currently conducting a candidate knock-down screen of genetic targets that play a role in stress and inflammatory responses in neurons and glia to identify molecular pathways that drive sleep changes after TBI. We are also testing how pharmacological interventions that improve sleep after injury could protect against the long-term sequelae of TBI.

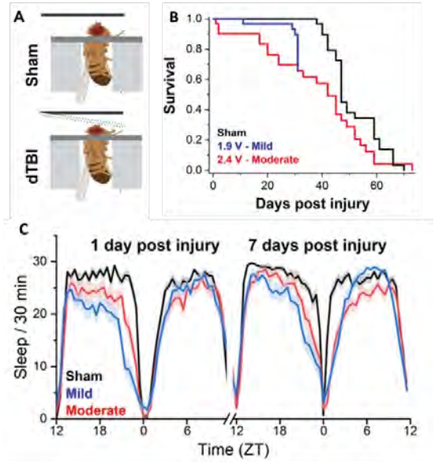

Fig. 2: Sleep disorders after TBI in Drosophila.
(A) Method of calibrated closed-head compression injury using deflection of a piezoelectric plate. (B) Mild and moderate injury reduce lifespan fly lifespan without acute mortality. (C) Sleep is reduced, particularly at night, in mild (blue) and moderately (red) injured flies compared to sham controls.

Development of a novel inducible gene expression system in Drosophila

In collaboration with the Southall Lab at Imperial College London, we 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. We validated that in neurons, this system is also compatible with reversible RNAi knockdown and irreversible CRISPR knockout. We have gone on to develop AGES2, a higher-specificity iteration of the AGES system that engineers a binding pocket in the E3 ligase of the system to bind to the auxin analog 5-phenyl-IAA with high specificity. AGES2 reduces the necessary dose of induction agent, with the goal of reduced leak of expression and fewer off-target effects of induction agent feeding. We are currently validating this tool for use in neurons and glia in behavioral experiments

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. 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 when flies age with a high-fat diet. Age related changes in circadian function and metabolism are highly conserved in *Drosophila*, allowing us to examine how clock function in peripheral tissues regulates life- and health-span in dietary challenge.

We have optimized a high-fat diet (HFD) that allows flies to survive for up to six weeks (Fig. 3A), and found that aging on a high-fat diet reduces circadian behavioral rhythmicity more than aging alone (Fig. 3B), suggesting additive effects of dietary stress and aging. At the molecular level, dampening of clock gene expression occurs with aging for genes such as *timeless* (Fig. 3C) , however a high-fat diet actually rescues the rhythm of *timeless* expression and instead dampens rhythms of another clock gene, *period.* The tight links between diet, circadian function, and life/healthspan suggest a diet x clock effect on regulating circadian phase synchrony through unknown mechanisms. Further analysis of tissue-specific changes in clock gene expression with aging and dietary stress will allow us to examine how clock function in peripheral tissues regulates life- and health-span in dietary challenge.

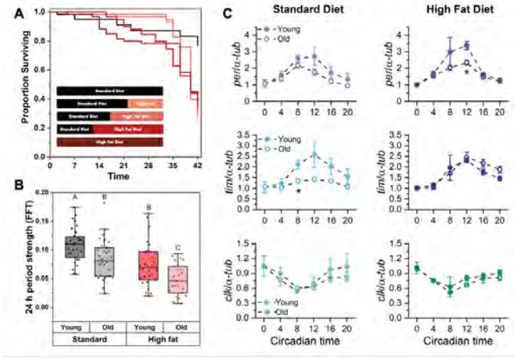

Fig. 3: Additive effects of aging and high-fat diet on circadian physiology.
(A) Feeding flies a high-fat diet consisting of 20% lard reduces lifespan compared to standard diet, even when fed for just two weeks. (B) Activity monitoring showed that while 4-week old flies have reduced rhythm strength compared to young (1-week old) flies, aged flies fed HFD have even further reduction in rhythm strength. flies. (C) On a standard diet (left) rhythms of clock gene expression in fly heads dampen with age. When flies were maintained on HFD for their full lifespan (right) timeless gene expression rhythms maintained high amplitude into old age.

Dr. Annika Barber, Assistant Professor

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Evan Cirone, Undergraduate Assistant
Vinithra Kathirvel, Undergraduate Assistant
Shambhavi Gupta, Undergraduate Assistant

DISMUKES LAB

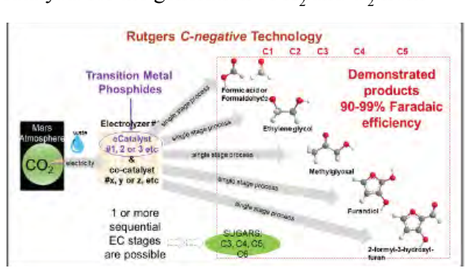
Biological and Chemical Approaches to Renewable Energy Research

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

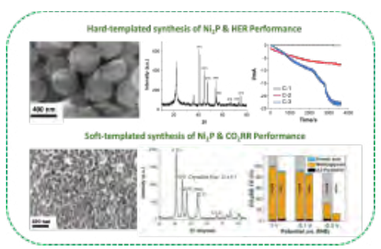
Summary

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 2022-June 2023 period the group was comprised of 29 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 CO_2 to make chemicals and food. In this project we apply the principles learned from enzymes to synthesize better heterogeneous catalysts for the generation of H_2 and O_2 from water (like Photosystem II) and CO_2 conversion (like acetogens and methanogens). Bioinformatics and data science tools are used to determine the chemically relevant attributes for catalysis by the CO_2 reducing enzymes. The synthesized catalysts are made from earth abundant elements and in the best cases can exceed the activity of commercial catalysts used today. Using this strategy, we have produced noble-metal-free catalysts for water oxidation ($LiCo_2O_4$), water reduction to hydrogen (Ni_5P_4), and selective CO_2 reduction to C_1 to C_5 products (Nickel Phosphides). The image depicts our electrolytic process for converting water and CO_2 into C_1 to C_5 products. Supported by RenewCO$_2$; DOE-SBIR, NA-SA-CO$_2$ Challenge, Rutgers Goldman Prize; Collaborations: UPenn, CU Boulder and RenewCO$_2$.

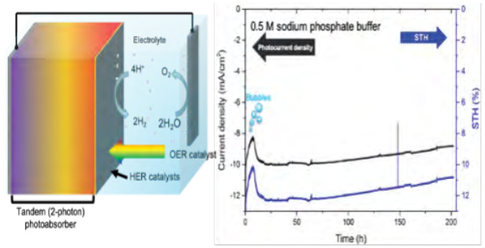


Synthesis of Transition Metal Phosphides with Defined Structural Phase, Facet and Morphology for Electrocatalysis. CCB.
In this project, we use a templating approach to synthesize nickel phosphide catalysts for hydrogen evolution and CO_2 reduction reactions. We aim to develop robust protocols to synthesize morphologically controlled and crystalline nanocatalysts that can electrochemically produce sustainable chemicals. So far, we have used silica as a hard template and cetyltrimethylammonium bromide as the soft template which act as frameworks to synthesize binary transition metal phosphide compounds to test as catalysts. XRD and SEM characterizations have revealed phase purity and unique morphology of the synthesized nanomaterials. These catalysts are currently being evaluated for HER and CO2RR. HER results show high current densities can be obtained (~25mA/cm^2 and ~400mA/cm^2 for hard and soft template Ni_2P respectively. CO_2RR results show soft-templated Ni_2P catalyst can convert CO_2 to C_1, C_3 and C_4 products with high

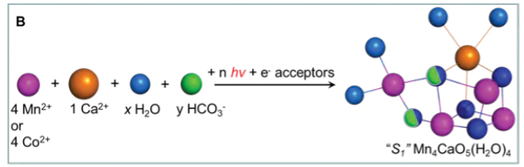


Faradaic efficiencies. Collaborator: UPenn. Support: DOE NREL-LDRP; RenewCO$_2$

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



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 (*): $Co^{2+}\rightarrow *\rightarrow Co^{3}+Q_A$-. The resulting reconstituted Co-PSIIX oxidizes water by the standard four-flash photocycle, though produces 4-fold less O_2 per PSII, attributed to faster charge recombination (Co^3+Q_A-$\leftarrow Co^4+Q_A$-) in the catalytic cycle. 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 ($Mn^3+Q_A\leftarrow Mn^{4+}Q^{A-}$) permits more water oxidation at low and fluctuating solar irradiation (> net energy conversion), and much greater tolerance to photooxidative damage at high light intensities (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 CO2 reduction. Waksman.

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

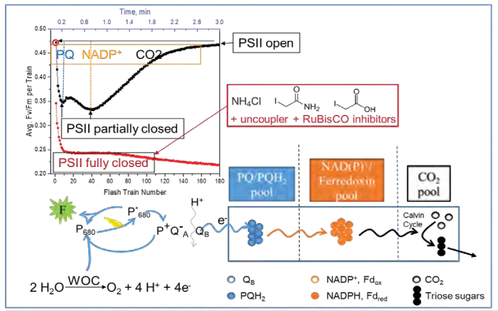
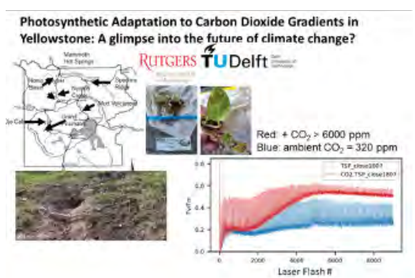


Figure 12. Changes in Chl fluorescence Fv/Fm under illumination measured by FRRF.

How photosynthesis adapts to a climate stressed environment: Long-term adaptation of a C3 plant to extreme CO_2 levels in Yellowstone NP.

those found near geothermal sources in Yellowstone, a natural laboratory of extreme climate conditions? CO_2 is a vital ingredient of photosynthesis, underlying biomass productivity across the planet. Elevated CO_2 levels can boost photosynthetic efficiency, but it is also toxic at higher levels, inhibiting biomass production. Whether photosynthesis can favorably adapt to such CO_2 extremes over prolonged periods of time is as yet unknown. Here, we investigate how photosynthetic metabolism changed in a C_3 plant adapted to growth at 12x ambient CO_2 over several decades in field conditions. We find that adaptation to such high CO2 levels lowers net carboxylation flux (Calvin cycle) and results in highly stunted growth and chlorosis.. Availability of photosynthetic light-induced energy (NADPH & ATP) for carboxylation is short-lived, possibly because it is diverted from carboxylation to homeostasis and repair. Our results demonstrate there are limits for elevated CO2 becoming more harmful than beneficial for plant growth. Busch-Waksman Seed. Collaboration: TU Delft.



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DONG LAB

Cell Polarity and Asymmetric Division in Plants

Dr. Juan Dong
Plant Biology

SUMMARY

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

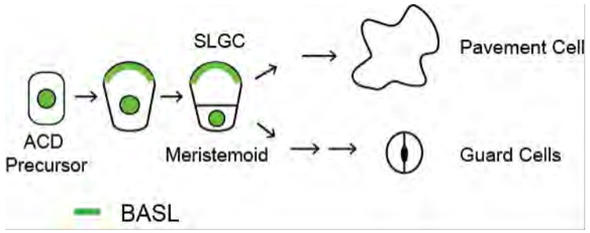

Figure 1. BASL localization and stomatal asymmetric cell fate

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 plant and how differential daughter cell fates are specified by the cortical polarity complex. Our work focuses on the identification of additional polarized proteins and of mutants that display specific subsets of polarity defects. At a larger extent, some of our work also include the characterization of novel regulators in stomatal development in Arabidopsis. Through collaborative effort, we also expand our research interest towards understanding the mechanical and physiological features of functional stomatal guard cells in plants. 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-embeded 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.

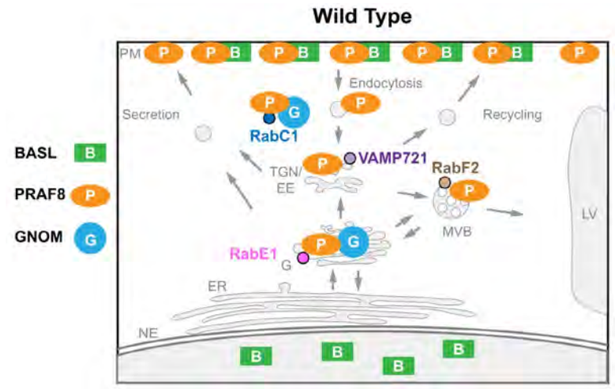

Figure 2. A working model for PRAF-mediated trafficking in establishing BASL polarity.
In wild-type plants, the polarization of BASL protein (green) in the stomatal lineage cell requires the physical partner, four PRAF proteins (orange), as well as the Golgi-localized Arf GEF GNOM (blue). The PRAF8 proteins 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 RabE1d-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.

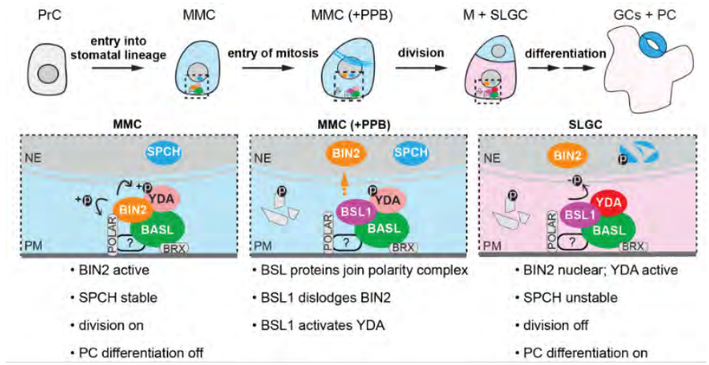

Figure 3. A working model for BSL phosphatases to function in stomatal ACD.
The BSL proteins function as the spatiotemporal molecular switch enabling the coordination of cell division and cell-fate differentiation in stomatal ACD. BSL1 join the polarity complex in the ACD mother cells that are committed 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).

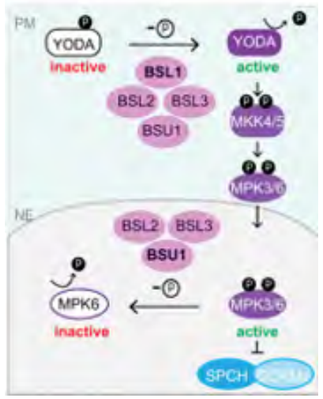

Figure 4. A working model: BSL phosphatases-based signaling dichotomy, through spatial compartmentalization of the regulation on distinct components of the linear YODA MAPK pathway, controls stomatal development in Arabidopsis.
At the cell cortex close to the PM, BSL1 is a predominant regulator, together 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/SCRMs, 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/SCRMs, 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 cryogenic electron microscopy (cryo-EM) and 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, elongation, and termination:

(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.

(5) 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: (i) translocation of the RNAP active center relative to DNA (by scrunching in initial transcription; by stepping in transcription elongation); (ii) binding of the incoming nucleotide; (iii) formation of the phosphodiester bond; and (iv) release of pyrophosphate.

(6) When RNAP reaches a termination site or receives a termination signal, RNAP stops synthesizing RNA, releases the RNA product, and dissociates from DNA.

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, transcription termination, 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 cryo-EM, x-ray crystallography, FRET, and photocrosslinking methods to define structures of trapped intermediates in transcription initiation, elongation, and termination. In addition, 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 in transcription initiation, elongation, and termination.

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

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

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

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

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

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

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

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

Results for both Qlambda 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

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. 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 overal goal is to obtain a highly integrated view of how multiple TFs contribute to the control of certain transcriptional programs. This is important because a significant percentage of trait-associated variants in crop species lie within non-coding regions and likely affect TF binding. Our goal is to explore how *cis*-regulatory variation contributes to phenotypic diversity in maize and other species, 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 regulation or meristem size in maize inflorescences

Maize inflorescences, the tassel and the ear, are formed by the activity of a main apical meristem, called the inflorescence meristem. In ears, the size of inflorescence meristems determines how many rows of seeds are formed and is therefore an important component of seed yield. We have identified two classes of genes that regulate inflorescence meristem size, a small family of transcriptional corepressors called REL2/RELKs, and two paralogous transcription factors (TFs) called ZmWUS1 and ZmWUS2.

Transcriptional corepressor proteins do not bind DNA directly, but instead interact with DNA-binding TFs that act in specific developmental and signaling pathways and suppress transcriptional output. We are currently investigating REL2 and RELKs function in regulating the size of inflorescence meristems to determine whether this occurs via ZmWUS1/2 interaction or independently of it, using genetic, genomic and transgenic approaches. By leveraging our knowledge on the molecular mechanisms regulating meristem activity, we are testing whether certain genetic combinations increase seed yield in maize F1 hybrids (Figure 1). 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, enabling the generation of many CRISPR-Cas9 maize transgenic lines. This new technology is already accelerating the pace of discovery in our lab and provides an invaluable tool for functional analysis.

Figure 1 Legend
By using specific combinations of the *rel2* mutant, we showed that it is possible to increase seed yield in maize F1 hybrids. 12 and 20 indicate the number of rows of kernels in normal (left) and mutant (right) ears.

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IRVINE LAB

Developmental Biology

Dr. Kenneth Irvine
Molecular Biology & Biochemistry

Summary

Our research investigates relationships between tissue patterning, growth and morphogenesis in developing and regenerating organs and 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 shape. 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 insights into how organ growth and shape are controlled have come from the identification and characterization in model systems of intercellular signaling pathways that are required for growth and morphogenesis. 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.

Over the past year we have investigated multiple topics related to control of growth, tissue mechanics, Hippo signaling, Ds-Fat signaling, and morphogenesis. The Hippo signaling network has emerged 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 is 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. This mechanism contributes to feedback inhibition of growth in compressed cells and to density-dependent regulation of cell proliferation, which contributes to the regulation of organ size.

In addition to mediating regulation of Hippo signaling by cytoskeletal tension through interaction with Warts (Wts), we found that Jub also participates in feedback regulation of junctional tension through regulation of the cytohesin Steppke (Step). We then examined how Jub interacts with and regulates its distinct partners by investigating the ability of Jub proteins missing different combinations of its LIM domains to rescue *jub* phenotypes and to interact with α-catenin, Wts and Step. Co-immunoprecipitation experiments identified a specific requirement for the second LIM domain (LIM2) for binding to Wts. However, in vivo, both LIM1 and LIM2, but not LIM3, were required for regulation of wing growth, Yki activity, and Wts localization, implying that LIM1 has activities distinct from Wts binding that contribute to Wts regulation. Conversely, LIM2 and LIM3, but not LIM1, were required for regulation of cell shape and Step localization in vivo, and for maximal Step binding. These results identified distinct functions for the different LIM domains of Jub.

While there is a single Ajuba family protein in *Drosophila* (Jub), there are three in mammals (AJUBA, LIMD1 and WTIP). When we characterized links between mechanical forces and Hippo signaling in mammalian cells, we discovered conservation of the Jub biomechanical pathway and a role for this pathway in cell density-dependent regulation of mammalian Hippo signaling. However, we found that only LIMD1, but not AJUBA or WTIP, is required for tension-dependent regulation of Lats in mammalian cells.

Our studies of morphogenesis have included analysis of the Ds-Fat signlaing network, and of spectrins. Spectrins are membrane cytoskeletal proteins generally thought to function as heterotetramers comprising two α-spectrins and two β-spectrins. They influence cell shape and Hippo signaling, and it had been suggested that they provide an alternate (ie Jub-independent) platform for biomechanical regulation of Hippo signaling. We investigated this in wing discs, but we found that spectrins regulate Hippo signaling through the Jub pathway due to their influence on cytoskeletal tension. Unexpectedly, our results also established that *Drosophila* β-heavy Spectrin (β_H-Spec, encoded by *karst*) localizes and functions independently of α-Spectrin, implying that it does not function within an α-β heterotetramer. Instead, we found that β_H-Spec co-localizes with F-actin and myosin, and that it reciprocally regulates and is regulated by myosin. In vivo, in vitro, and in silico experiments indicate that β_H-Spec and myosin can directly compete for binding to F-actin. This competition can explain the influence of β_H-Spec on cytoskeletal tension and myosin accumulation. It also provides new insight into how β_H-Spec participates in ratcheting mechanisms associated with cell shape change.

The Dachsous (Ds)-Fat signaling network controls organ growth and shape. For example, the adult *Drosophila* wing has an elongated shape, which becomes more round upon loss of Ds or Fat. It had been proposed that this was due to loss of oriented cell divisions, but we discovered that this could not be the explanation as randomizing cell division orientation does not alter wing shape. Instead, our recent results identify two distinct mechanisms that are involved in the influence of Ds on wing shape. First, loss of Ds or Fat alters the initial shape of the developing wing primordia in a manner consistent with eventual formation of a more rounded adult wing. Second, Ds-Fat alters patterns of tissue tension in the larval wing disc, which we hypothesize alters responses to stresses that occur during pupal development.

Ds and Fat are each transmembrane cadherin family proteins. Signaling downstream of Fat has been intensively investigated, but signaling downstream of Ds is less well understood. To gain insight into this, we initiated structure-function studies of the Ds intracellular domain (ICD). Using CRISPR and RMCE, we made a series of ICD deletions and have evaluated their impact on Ds function. This identified two regions that significantly impact Ds, one that leads to smaller wings and another that leads to larger, rounder wings. In biochemical experiments, we have identified motifs within the ICD that bind to key pathway components including Dachs, Sple, and Lft, which helps explain some of the phenotypes observed with these deletions.

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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* and developing protocols for RNA-guided engineering of plastid genome using Cas9. This year we highlight a project on the expression of recombinant proteins in seed plastids.

PPR10 RNA binding-protein for exploring protein expression in tobacco seed plastid

To test recombinant protein accumulation in dry seed plastids, we employed a two-component regulatory system (Figure 1). The system uses an engineered PPR10 RNA binding protein, PPR10^{GG}, that recognizes only the engineered BS^{GG} binding site. The introduced two component system is orthologous to the native tobacco PPR10 system because the tobacco PPR10 protein does not bind BS^{GG}, the engineered maize binding site and the engineered maize PPR10^{GG} protein does not bind BS^{Nt}, the tobacco *atpH* mRNA. The first component (PPR10^{GG}) is expressed from the napin A (PnapA) seed-specific promoter. The second component is a *gfp* reporter gene with an engineered BS^{GG} binding site upstream of the AUG translation initiation codon that would respond to activation by the engineered PPR10^{GG}. Transplastomic plants carrying the BS^{GG}:*gfp* construct were then transformed with the nuclear *PnapA:PPR10^{GG}* gene. Plants representing 17 independently transformed lines were transferred to the greenhouse and GFP accumulation was tested in dry seed. GFP could be readily detected on immunoblots in two out of the 17 lines. PPR10^{GG} was also detectable in the same two lines. Accordingly, in seed the P*napA* promoter transcribes *PnapA:PPR10^{GG}* mRNA facilitating translation of GFP in seed plastids.

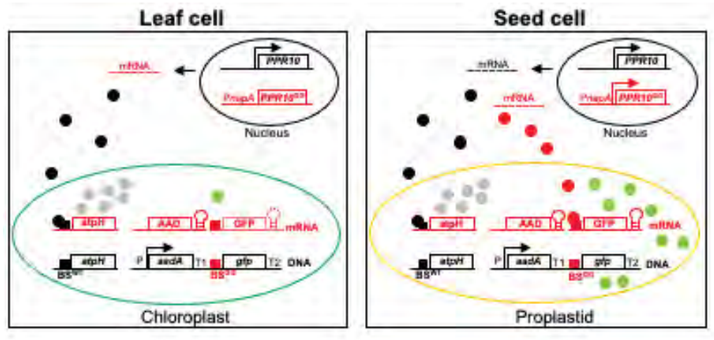

Figure 1. **Two-component systems for seed-specific expression of reporter proteins.**
In the leaf, the nuclear *PPR10^{GG}* is not expressed from the seed-specific napin A promoter (PnapA). Therefore, the reporter transgene with the BS^{GG} binding site is expressed at the baseline level. In the seed, the *PPR10^{GG}* gene is transcribed from the napin A promoter, the mRNA is translated on cytoplasmic ribosomes, and imported into the chloroplast, where it activates the translation of the reporter gene by binding to BS^{GG}.

Protein accumulating in seed protein bodies is heavily glycosylated. Protein expressed in seed plastids creates a second pool of recombinant protein which is free of glycosylation. The absence of glycosylation and the stability of protein in seed make attractive to express bacterial vaccine antigens in seed plastids.

Engaging undergraduate students in research

Engaging undergraduate students in research is part of the broader impact of research activity supported by the National Science Foundation. We integrate research and education by training undergraduates to facilitate full participation of women and underrepresented minorities in STEM fields. Participating students during 2022-2023 from Rutgers were Julia Ferranti, Alyssa Leung, Sharanya Datta and Shaunak Kinare. 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. Rahim Khan, an undergraduate student from the Lutz laboratory conducted Arabidopsis research in the Maliga laboratory supported by NSF funds in the RISE Summer Program.

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MCKIM LAB

Molecular Genetics of Meiotic Recombination and Chromosome Segregation

Dr. Kim McKim
Genetics

Summary

Meiosis is a critical part of gametogenesis. It is the process that puts the correct amount of genetic information into sperm and eggs. 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-orients on a bipolar meiotic spindle such that when the cell divides, the chromosomes move in opposite directions and the chromosome complement is reduced in half (Figure 1).

Errors in meiosis lead to aneuploidy, or an abnormal chromosome number, in the gametes. Aneuploidy is a leading cause of spontaneous abortions and infertility in women and also causes diseases such as Down, Turner or Klinefelter syndromes. 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. 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.

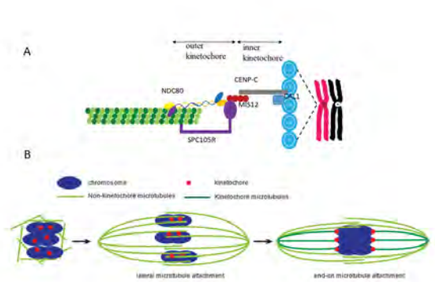

Figure 1: Structure of the kinetochore and bi-orientation in meiosis
A) The kinetochore is the most important part of the chromosomes that interacts with the spindle microtubules. The centromere is defined by the specialized histone CENP-A/ CID (blue). This is linked to the kinetochore by CENP-C (grey). Spc105R makes lateral interactions with microtubules and recruits the NDC80 complex, which makes stable end-on attachments to microtubules. B) Spindle assembly begins with assembly of the microtubules around the chromosomes. Early in pro-metaphase, lateral kinetochore – microtubule attachments involving SPC105R and the central spindle predominate. In *Drosophila oocytes*, this region contains a high concentration of CPC (Figure 2). The central spindle may facilitate separation of homologous centromeres towards opposite poles before acquiring stable end-on attachments. In this model, end-on attachments in the central spindle are destabilized by CPC activity. Improper formation of end-on attachments leads to chromosome segregation errors.

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

The kinetochore is the site on the chromosomes that interacts with the microtubules. Furthermore, attachments between the microtubules and the kinetochores is a two-step process (Figure 1). Lateral attachments enable the initial

attachments because it is more efficient for a kinetochore to find the side of a microtubule than the end of one. This is followed by more stable end on attachments stabilize proper interactions. This transition is critical because if a chromosome has attached to the wrong microtubules, it is easier to correct if the attachment is lateral rather than end-on. We have conducted a study of kinetochore protein SPC105 to elucidate its role in regulating microtubule attachments and progression through meiosis. One third of SPC105 (the 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. Another third of SPC105 regulates cell cycle progression. A relatively small part of SPC105R (about 300 out of 2000 amino acids) is required for promoting lateral attachments and is required to correct errors in the transition to end on microtubule attachments. This region also appears to recruit proteins that either prevent precocious end-on attachments or destabilize incorrect end-on attachments. Thus, our results have provided mechanistic detail for understanding SPC105's role in ensuring accurate meiotic chromosome segregation.

We have identified two proteins that are recruited by SPC105 and may be required for avoiding errors and forming the correct attachment of microtubules to the kinetochores. These proteins include the kinase MPS1 and the ROD-ZW10-Zwilch (RZZ) complex, both of which depend on SPC105R for localization to the kinetochores. The RZZ complex is involved in attachment error correction and the spindle assembly checkpoint (SAC) during mitosis. 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 the 300 amino acid region required for accurate attachment of kinetochores to microtubules and chromosome segregation. ROD, and by extension MPS1, is not required for creation of the initial KT-MT attachments, but may have a role in preventing or correcting erroneous attachments.

Organizing microtubules around the chromosomes: Chromosome-directed 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.

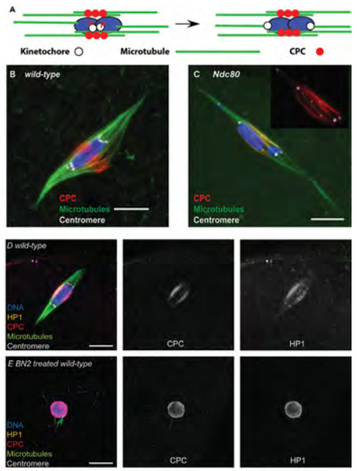

Figure 2: The kinetochores and the CPC interact with two populations of microtubules
A) In meiosis, homologous centromeres are pulled in opposite directions. During prometaphase, these centromeres may be located in the central spindle region, coinciding with a high density of CPC. Later they move towards the poles and make end-on attachments. B) A wild-type spindle with CPC (red) in the center of the spindle between homologous centromeres (white) separated towards opposite poles. C) In the absence of NDC80, centromeres fail to make end on attachments to microtubules and only make lateral attachments. D) In wild-type oocytes, the chromatin protein HP1 (yellow) moves to the microtubules with the CPC (red). E) When the kinase activity of the CPC (Aurora B) is inhibited by the drug BN2, the CPC and HP1 localizes to the chromosomes (blue).

We are investigating the hypothesis that Incenp and Borealin direct the CPC to the chromosomes by interacting with Heterochromatin protein-1 (HP1) (Figure 2). HP1 is known best as a chromatin protein that that regulates chromosome structure and gene expression. However, we have shown it is an active component of the spindle assembly process, moving from the chromosomes to the microtubules as the spindle assembles. However, it remains unclear if and how HP1 recruits the CPC to the chromosomes during meiosis, and if it has a role in spindle assembly and function. Both Incenp and Borealin have potential HP1 interaction sites. We are investigating the function of these domains in the movement of the CPC from the chromosomes to the 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 of these domains and HP1 in organizing a bipolar spindle and promoting accurate meiotic chromosome segregation.

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

We are performing an 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 publicly available databases containing tissue-specific expression data (FlyAtlas, FlyAtlas 2 and Modencode). Based on the analysis of these data sets, approximately 1100 genes are upregulated only in oocytes. 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 250 genes by germline-specific RNAi and found approximately 50 that cause reduced fertility, sterility, or increased nondisjunction.

Based on this system to test genes for meiotic functions, we have initiated 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 in *Drosophila* and have been shown to cause fertility defects in Humans. Our collaborators are using multiple data sets to identify candidate genetic variants that cause altered frequencies of aneuploidy in human embryos. Many of these identified genes are evolutionarily conserved in *Drosophila.* We are using our *Drosophila* tissue specific RNAi system to rapidly validate and assess candidate human genes. Our goal is to identify and characterize the types of genetic alterations that cause fertility defects in humans

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NICKELS LAB

Regulation of Gene Expression in Bacteria

Dr. Bryce Nickels
Genetics

Summary

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. Our research focus is the mechanism and regulation of transcription by bacterial RNA polymerase (RNAP), which serves as a model for understanding the mechanism of transcription and its regulation in all organisms.

Our work has provided fundamental insight into the mechanism of transcription initiation, transcription elongation, and transcriptional regulation, particularly in the areas of transcription start site selection, primer-dependent initiation, use of metabolites as non-canonical initiating nucleotides, and transcription pausing. To perform our studies, we use an approach that combines conventional methods (e.g. molecular biology, genetics, and biochemistry) with cutting-edge high-throughput sequencing (HTS) methods (e.g. massively-parallel transcriptomics and massively-parallel protein-DNA photo-cross-linking). Use of these HTS-based methods has enabled us to probe mechanistic aspects of transcription and transcriptional regulation, both in vitro and in vivo, that would be otherwise inaccessible.

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RONGO LAB

Stress, Mitochondrial Dynamics, and the Central Nervous System

Dr. Christopher Rongo
Genetics

Summary

Our research philosophy is based on curiosity driven science, where we use the simple genetic model organism *C. elegans* as an in vivo system, combined with the most appropriate technical approaches, to answer specific questions in biomedical science. Using *C. elegans*, which are small (1 mm), transparent nematodes (worms), we address questions regarding how neurons respond to hypoxic stress, how they regulate responses to that stress in other tissues, and how the regulation of mitochondrial dynamics and quality control plays a fundamental role in brain function, homeostasis, and aging. Our lab is currently doing research on the following four inter-related topics listed below.

Oxygen And The Hypoxia Response Pathway

Oxygen is the terminal electron acceptor in the Electron Transport Chain of mitochondria, which uses it to maximize energy and ATP production. Hypoxia is a condition of low oxygen levels that disrupts energy production and overall metabolism. Hypoxia and the body's response to hypoxia play a role in multiple human diseases, including ischemic stroke, myocardial infarction, hypertension, chronic kidney disease, COPD, and cancer. Understanding how tissues and cells respond to hypoxia is critical to the development of new therapeutic approaches for treating these diseases (Figure 1). It is also important to understand how diverse organisms evolved and adapted to hypoxic environments.

Questions About Hypoxia That We Are Addressing: How do cells sense hypoxia? What are the factors that mediate a response to hypoxia? Does hypoxia cause different effects in diverse tissue types? How does the hypoxic response operate holistically over multiple tissues? What exactly is that response? How does the response offset hypoxic damage?

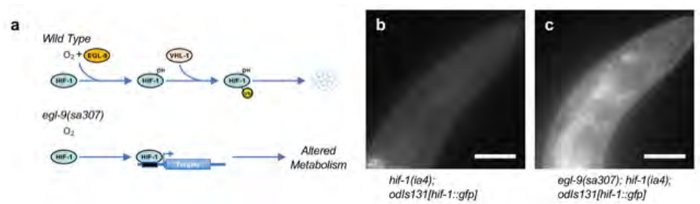

Figure 1. Identifying Genes Directly Regulated By HIF-1.
Animal cells respond to hypoxia by employing the hypoxia response pathway. (a) Diagram of the hypoxia response pathway in C. elegans. Under normal oxygen conditions, the transcription factor HIF-1 is hydroxylated by EGL-9, ubiquitinated by VHL-1, and degraded by the proteasome. In egl-9(sa307) mutants or under hypoxic conditions, HIF-1 remains stable and regulates the transcription of target genes whose expression alters metabolism. (b,c) HIF-1::GFP fluorescence in the indicated genotypes under normoxia. HIF-1::GFP is degraded in wild-type animals, but is stable and accumulates in the nuclei of egl-9 mutants. Scale bar indicates 30 microns.

Mitochondrial Dynamics

Mitochondria are the sites of oxygen respiration, oxidative phosphorylation, and energy production. More than just the "powerhouse of the cell," mitochondria also mediate lipid metabolism, cytosolic calcium buffering, cofactor synthesis, apoptosis, and necrosis. They are incredibly dynamic organelles that undergo fission, fusion, intracellular motility (transport), and multiple forms of quality control. Although mitochondria possess their own genome, most of the proteins that reside in mitochondria are encoded by the nuclear genome and must be imported across the double membrane structure of the mitochondria. Dysfunctional mitochondria play a role in multiple human diseases, including ischemic stroke, Parkinson's Disease, Alzheimer's Disease, ALS, Leigh Syndrome, optic atrophy, and cancer. Mitochondrial function and regulation are going to be key elements for addressing these diseases.

Questions About Mitochondrial Dynamics That We Are Addressing: What mediates mitochondrial motility in complex cells like neurons? How and why do mitochondria undergo fission and fusion? How are nuclear-encoded proteins imported into mitochondria? How are these mitochondrial dynamics regulated?

Mitophagy and Mitochondrial Quality Control

Mitochondria are essential for cells that need to maximize ATP production. Neurons in the brain are particularly reliant on mitochondria for their energy needs, which are tremendous due to ATP required to maintain the electrochemical membrane potential that mediates neuronal communication. Yet, mitochondria are a potential threat to cells, as they produce free radicals and reactive oxygen species as byproducts of oxidative phosphorylation. The resulting oxidative stress can damage cells and result in neurodegeneration. Unlike many cells of the body, neurons are not easily replaced by stem cells and thus must survive and endure mitochondrial oxidative stress for decades. Neurons and other cells employ quality control mechanisms to offset potential damage from rogue mitochondria. One such mechanism is mitochondria-selective autophagy (mitophagy), which recognizes rogue mitochondria, engulfs them in augophagosomal membranes, and digests them following fusion with lysosomes (Figure 2). Failure to remove offensive mitochondria is associated with aspects of aging and diseases like Parkinson's Disease.

Questions About Mitophagy That We Are Addressing: How do cells recognize a rogue mitochondria from a functional one? What are the factors that mediate mitophagy? How does mitophagy differ in different tissue types or during aging? How is mitophagy regulated? What is the link between mitophagy and neurodegeneration?

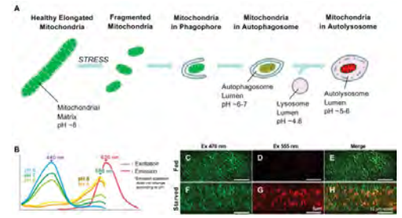

Figure 2. MitoKeima reports mitophagy in vivo.
We have generated transgenic strains of C. elegans that express the genetically encoded fluorescent protein MitoKeima, which is localized to mitochondria and can allow researchers to discern mitophagic mitochondria from healthy mitochondria. (A) Cartoon of the mitophagy process. The mitochondrial matrix pH of a healthy mitochondrion (green) is 8. Stress (e.g., hypoxia) induces mitochondria fission and mitophagy (engulfment by phagophoric membranes) to form autophagosomes, which become acidified. Autophagosomes fuse with lysosomes (pink), exposing their contents(in this case, mitochondria)to acidic pH of around 5-6(red). (B) MitoKeima can exist in two forms, each with its own excitation wave length. The basic form (blue and green lines) excites around 440 nm, whereas the acidic form (yellow and orange lines)excites around 586 nm. Both forms emit at 620 nm (red lines). Adapted from Katayama, H. et al., (2011) Chemistry & Biology 18, 1042-1052. (C-E) Under well-fed conditions, transgenic nematodes expressing MitoKeima in their intestine have localized mitochondrial fluorescence excited by (C) 470 nm but not a (D) 555 nm laser, indicating little baseline mitophagy. (F-H) Twenty fours of starvation induces mitophagy, with a mix of healthy mitochondria excited by (F) 470 nm light and mitophagic mitochondria excited by(G) 555 nm light. We are using these MitoKeima strains to identify genes that mediate or regulate mitophagy.

Transcription Factors And Their Targets

Our studies of the hypoxia response pathway have recently focused on the pathways terminal effector: the transcription factor HIF-1. An ultimate goal in studying any signaling pathway that regulates gene expression at the transcriptional level is to identify and characterize all of the transcriptional targets of the pathway. To that end, we are using

RNA-seq, ChIP-seq, and other Omics techniques to identify all the target genes regulated by HIF-1. We are employing novel computational approaches to separate true targets from background noise and the technical artifacts that often arise when one uses these technical approaches. We are beginning to collaborate with other *C. elegans* researchers to use these same approaches to identify transcriptional targets of other interesting transcription factors and signal transduction pathways in the worm.

Questions About Transcription Factors That We Are Addressing: Where do transcription factors bind in the genome? What computational tools can we use to identify their true regulatory targets? How does the transcriptional profile activated by a signal transduction pathway change over time? What is the relationship between transcription factor binding, the regulation of gene expression, and changes in the epigenetic chromatin landscape?

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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.

CRISPR-Cas (Clustered Regularly Interspersed Palindromic Repeats/CRISPR associated sequences) loci provide bacteria with adaptive immunity to foreign genetic elements such as phages and plasmids. CRISPR-Cas systems function by acquiring (adapting) fragments of foreign DNA into CRISPR arrays and then using the acquired information to destroy foreign nucleic acids once they appear inside immunized cells.

We study CRISPR-Cas systems from *Escherichia coli*, *Thermus thermophilus* and human pathogen *Clostridium difficile*.

In *E. coli*, we studied how prespacers – fragments taken from foreign DNA – become protective spacers in the chromosomal CRISPR arrays. Spacers are of defined size and so fragments of foreign DNA must be accurately processed before they can be incorporated in the arrays. Using Frag-Seq, a powerful procedure previously developed in our laboratory that allows one to monitor short DNA fragments inside the cell, we identified prespacers in cells undergoing CRISPR adaptation. Using a collection of *E. coli* mutants we identified nucleases required for generation of spacers. This work was performed with cells undergoing the highly efficient primed CRISPR adaptation, a process that happens when cells encounter a genetic element to which they have earlier acquired CRISPR immunity. We are now designing highly sensitive experimental and computational approaches to study prespacers during naïve adaptation, a much less efficient process that occurs in cells that encounter a foreign genetic element for the first time. We have also designed sophisticated biochemical and biophysical approaches that allow us to reconstruct the spacer generation process by the Type I-E CRISPR-Cas system of *E. coli in vitro*.

We used *E. coli* as a chassis strain to study rare Type VI CRISPR-Cas systems. In contrast to other types, these systems exclusively target foreign RNA. In collaboration with Jennifer Doudna lab from the University of California, Berkeley we demonstrated that upon the recognition of RNA targets, diverse Type VI CRISPR-Cas systems start to specifically cleave cellular tRNAs at their anticodon loops. This leads to the inhibition of protein synthesis and cessation of cell growth. This mechanism of action allows RNA targeting Type VI CRISPR-Cas systems to efficiently protect cells from mobile genetic elements with DNA genomes.
In collaboration with Olga Soutorina laboratory from the University of Saclay in France we discovered a large new family of anti-CRISPR proteins encoded by *C. difficile* phages. These proteins target Cas proteins of the host bacterium Type I-B CRISPR-Cas system rendering it inactive. *C. difficile* is resistant to most commonly used antibiotics and phage therapy has been considered as a promising alternative for treatment. *C. difficile* CRISPR-Cas system is highly active and protects it from phages in the highly crowded environment of the gut. Our discovery of phage-encoded inhibitors of *C. difficile* CRISPR-Cas should allow rational selection of phages for therapeutic applications that will overcome the resistance of the host.

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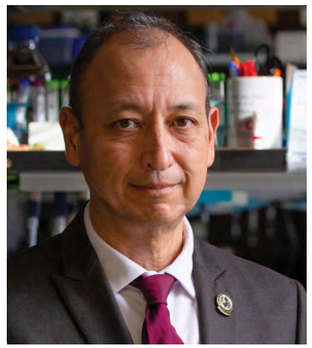
SINGSON LAB

Reproductive Biology, Cell-Cell Interactions



Dr. Andrew Singson
Genetics

Summary

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

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

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

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

Sperm function

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

We continue to identify and characterize genes required for sperm function at fertilization taking advantage of the most up to date genetic and molecular tools. Most recently we have been using a new CRISPR/Cas9 genome engineered balancer chromosome-based fertility mutant screen. 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 are uncovering the nature of the mutated genes.

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. Our work on understanding the role of these genes in normal fertilization pathways is ongoing. 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-13 is a novel small single pass transmembrane molecule of 130 amino acids. SPE-13 adds to a growing list of small transmembrane molecules that have been implicated in gamete interactions or cell fusion events.

Figure 1 shows the localization of SPE-13::GFP in a spermatid (Figure 1 A, B) and a mature spermatozoon (Figure 1 C,D). This movement of SPE-13::GFP from vesicles in spermatids to the plasma membrane in mature sperm places it in a position where it can directly interact with other sperm surface molecules as well as with egg cell surface molecules during fertilization.

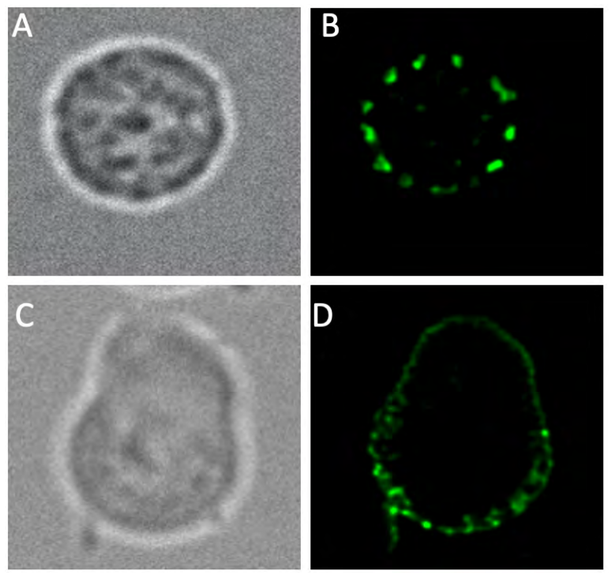


Figure 1. SPE-13::GFP localizes to Membranous Organelles (MOs) in spermatids (A, B) and can diffuse to the plasma membrane in mature and motile spermatozoa. Light micrographs (A, C) or fluorescent imaging of SPE-13::GFP. In motile spermatozoa we note a significant amount of signal is still associated with fused MOs.

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 considers 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 discovered that 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. We recently discovered the *egg-7* gene. The EGG-7 protein has homology to human PGM3 that is a phosphoacetylglucosamine mutase. Mutations in PGM3 are implicated in numerous human diseases including immunodeficiency and teratoma formation and is one of

about 130 known congenital disorders of glycosylation. This is a particularly exciting discovery because glycosylation has long been considered to be fundamental aspect of mammalian sperm-egg recognition.

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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?

Conserved genetic pathways that link brain development and sleep regulation

One focus of our research is how particular genes act during brain development to shape sleep patterns in adulthood, including in pathological sleep disturbances. We found that *insomniac* (*inc*), a highly conserved gene required for normal sleep, controls the development of discrete sleep-regulatory circuits. We continue to study the mechanisms by which *inc* and other genes link aspects of neuronal development to the function of sleep-regulatory circuits in the adult brain.

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 shortened 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

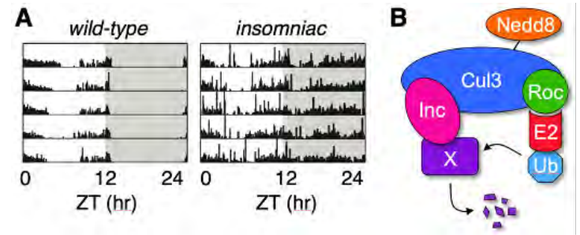

Figure 1. A) Five-day locomotor traces are shown for wild-type and insomniac mutant animals. Shading indicates night. **B)** Inc is an adaptor for the Cul3 ubiquitin ligase complex that ubiquitinates substrates for proteolysis (shown) or non-proteolytic regulation.

years of evolution.

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

A second area of our research focuses on the mechanisms underlying core attributes of sleep: locomotor inactivity and reduced responsiveness to sensory stimuli. While considerable attention has focused on circuits and molecules within the brain, how these elements ultimately suppress 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

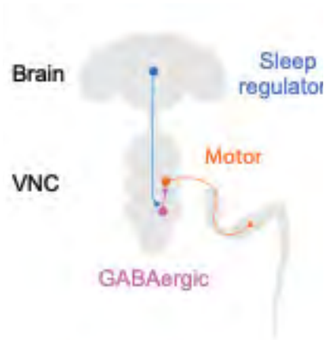

Figure 3. Model for suppression of motor neuron excitability during sleep by GABAergic VNC neurons that receive sleep-regulatory inputs from the brain.

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 GABAegic 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 motor 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-hydroxymethylcystosine (5hmrC) in Drosophila.

The 5hmrC modification is introduced to mRNA in vertebrates and Drosophila by the Tet (Ten-Eleven-Translocation) enzyme. Tet proteins have well-documented functions in development, maintaining vertebrate stem cells, 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 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 5hmrC transcriptome-wide in S2 Drosophila tissue culture cells and could show that Tet modifies ~ 1500 specific transcripts. Our working hypothesis is that Tet, mediated by its DNA-binding domain, localizes at actively transcribed target genes and controls the modification of their nascent transcripts. The 5hmrC mark is then recognized by reader protein(s) that direct the association of the bound mRNA with ribosomes ultimately controlling translational levels. To test this hypothesis, we performed ChIP-Seq experiments. Bioinformatic analysis identified 2242 protein binding peaks, and 1032 of the peaks map to promoter sites. 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-5hmrC antibody) on RNA isolated from wild type (wt) embryos as well as wt and Tet^{null} larval heads in order to map 5hmrC transcriptome-wide. In 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 5hmrC marks and mRNA levels, we compared the input RNA-seq from the hmeRIP experiments with the transcripts that showed the 5hmrC 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 5hmrC-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 5hmrC modified mRNAs do not change levels in Tet^{null} larval heads. Thus, the 5hmrC 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 5hmrC 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 5hmrC 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^{nul} 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.

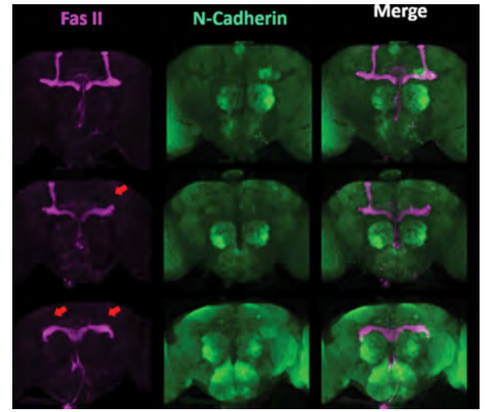

Figure legend: **Loss of mushroom body α lobe(s) in Tet^{4XXC} mutants.** Fasciclin II stains the mushroom bodies (red) N-cadherin is used as a cell marker (green). Top, wildtype brain, bottom, two different phenotypes observed in Tet^{4XXC} brains.

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YADAVALLI LAB

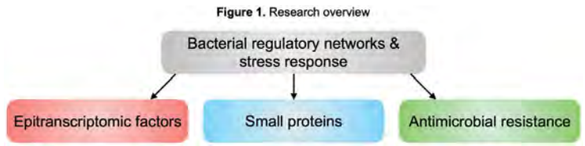
Bacterial stress response, biology of small proteins and antimicrobial resistance

Srujana Samhita Yadavalli
Genetics

Summary

Antimicrobial resistance is an alarming problem in our present and future. Stress response networks meant to protect bacteria against environmental challenges are increasingly being co-opted to promote antimicrobial resistance. Understanding the biochemical and regulatory pathways that underlie this resistance is of utmost importance to tackling the growing threat of untreatable multidrug-resistant bacterial infections. Our research is broadly focused on two distinct themes of bacterial stress response regulation, which have been under-appreciated in the past – (i) small protein regulators and (ii) epitranscriptomic regulators (Figure 1). Our research aims 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 resistance.

Figure 1. Research overview



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 2). 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

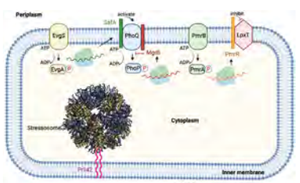


Figure 2. Small membrane proteins modulate signal transduction and stress responses in bacteria. Small proteins MgrB, SafA, PmrR, and Pr642 are all localized to the cell membrane (inner membrane in the case of Gram-negative bacteria). In *E. coli*, MgrB and SafA interact with the target PhoQ sensor kinase. In *S. enterica*, PmrR binds to LpxT, an enzyme involved in lipopolysaccharide modification. In *L. monocytogenes*, Pr642 anchors RsbR, a component of the multiprotein stress complex (Yadavalli & Yuan, 2022).

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 the 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 3). The control of septation by QueE may protect cells from antimicrobial peptide stress via stimulation of the PhoQ/PhoP signaling system. Currently, we are investigating the mechanistic details of cell division inhibition by this tRNA modification enzyme, QueE, and determining if its role in stress response confers a fitness advantage.

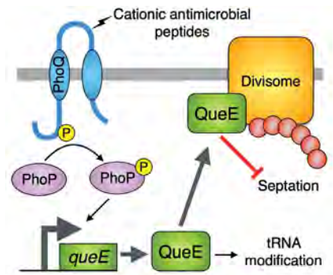


Figure 3. *E. coli* PhoQ/PhoP two-component system modulates cell division via QueE upon strong stimulation by antimicrobial peptides.

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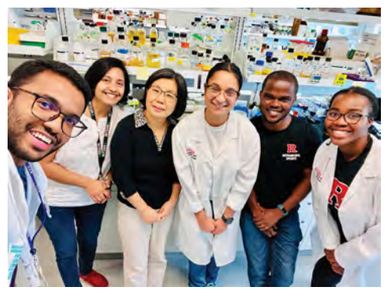

ZANDER LAB

Dissecting The Environmentally Responsive Plant Epigenome

Dr. Mark Zander
Plant Biology

Summary
Enhanced crop productivity will be essential to addressing the imminent food demands predicted in human population models. This is increasingly difficult to accomplish because the Climate Crisis is projected to not only shift the range of crop suitability but also expand the geographic range of numerous pathogens and herbivorous insects. These changes pose a significant threat to the yield and quality of food crops thereby elevating the risk of future food insecurity. A better understanding of the molecular mechanisms underlying plant immune responses and how these are impacted by the Climate Crisis is therefore imperative. Hallmark of a robust immune response is the pathogen-specific activation of massive defense gene regulatory networks (GRNs). The epigenome is a critical feature of these GRNs which refers *sensu lato* not only to all chemical modifications of DNA and histone proteins, but also to other gene regulatory aspects such as transcription factor (TF) DNA binding, chromatin accessibility, chromatin looping, nucleosome positioning and long noncoding RNAs. Specifically, the binding of master TFs at *cis*-regulatory elements (CREs) is a key epigenomic event that ensures an accurate spatiotemporal regulation of defense GRNs. Due to their regulatory significance, TFs serve as appealing targets for engineering stress-tolerant crops. Studies involving the overexpression of stress-responsive TFs in different plant species have demonstrated improved stress tolerance. However, this enhancement is often accompanied by undesirable side effects, including growth retardation under non-stressed conditions and imbalanced crosstalk between immune pathways. The Zander laboratory investigates the regularity importance of individual CREs and accompanied changes of their local chromatin environment within a TF cistrome (entire set of potential target CREs) and the functional interaction of TFs with their target CREs including their architecture. The goal is to significantly expand the known functional repertoire of master TFs to generate more stress-resilient plants.

Development of a high-throughput ChIP-seq platform for plant tissues
An integral part of the environmentally responsive plant epigenome is the intricate interplay between TFs and chromatin regulators that dynamically integrate environmental signals into the epigenomic landscape. A better understanding of plant-environment interactions requires experimental approaches and tools that can capture molecular readouts of these dynamic interactions. Genome-wide knowledge about DNA binding of TFs as well as about the occupancy of histone modifications is usually achieved with chromatin immunoprecipitation analysis coupled with next-generation sequencing (ChIP-seq), CUT&RUN, or CUT&tag. In addition to significant experiment-to-experiment variability, there are other notable limitations when using ChIP-seq in plants. These include the relatively high costs per sample (~$250) and, most importantly, the challenge of scaling up to a large number of samples, making it difficult to conduct experiments involving more than 20 samples. To overcome the financial and technical limitations of classical ChIP-seq, I developed **PHILO (P**lant **HI**gh-throughput **LO**w input**) ChIP-seq,** an inexpensive high-throughput ChIP-seq platform that can be carried out in 8-tube or 12-tube strips as well as in 96-well plates at a cost of ~ $35 for a single *Arabidopsis* TF or chromatin modification. The experimental setup of PHILO ChIP-seq is shown in Figure 1A and resembles a classical ChIP-seq protocol, executed in miniature format with a streamlined nuclei extraction procedure. The usage of expensive consumables such as antibodies, magnetic beads and library preparation supplies was reduced by up to 80%. The approximately eight times lower costs per sample together with the high-throughput feasibility enables the analysis of complex experimental setups.

Comprehensive analysis of the interplay between transcription factors and the epigenome
The functional relationship between transcription factors and the epigenome is highly complex depending on epigenome feature, type of TF and targeting mechanism of the respective chromatin modifying enzyme complexes. DNA methylation at *cis*-regulatory elements in humans and in *Arabidopsis,* for example, can prevent the binding of TFs. To investigate the interplay between TFs and the epigenome in more detail, we conducted 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 epigenome 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

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 2022-2023

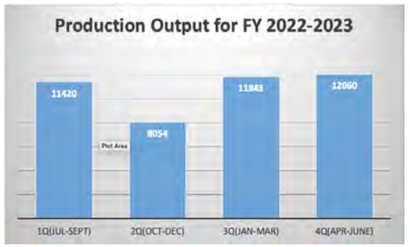
Over the past year, the scientists at the fermentation facility have completed projects for numerous biotech, pharmaceutical, and cosmetic industries as well as a couple of university and university affiliates. For this fiscal year, the total production has increased by approximately 10% from last year's output of microbe cultures of *E. coli*, *P. pastoris*, *Streptomyces* spp., and various strains of yeast and fungus. The revenue generated from all these works has 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.

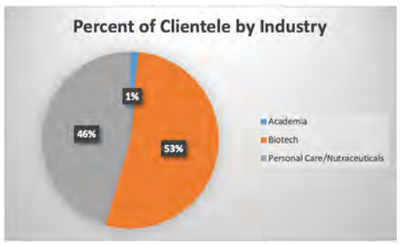
Dr. Arvin Lagda oversees and directs the overall operation of the facility, while the day-to-day projects are handled by a team of fermentation scientists led by Ms. Amanda Rodriguez (Production Manager) and supported by Dr. Sergey Druzhinin as Senior Scientist and Mr. Andrew Cloud and Mr. Nathan Hill as Laboratory Technicians. 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. The facility is also in current discussion with faculties from the Rutgers Chemical and Biochemical Engineering Department regarding the proposal to be a part of the Food and Drug Administration (FDA) Centers of Excellence in Biomanufacturing.

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 in 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 the 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 to the scholarship goals of Rutgers University through extensive collaboration with other academic departments through teaching/course integration, lectures, facility tours, and internship training programs for students.





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Waksman Genomics Core Facility

Waksman Genomics Core and Instrumentation Laboratory

Waksman Genomics Core and Molecular Biology Instrumentation Laboratory (WGCF) provides access to high-throughput next-generation sequencing and molecular biology instruments to the Waksman Institute's researchers as well as the 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 an 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 transcriptomes or up to 16 exomes in a single run. Whereas MiSeq with a 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 hardware 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 these are available for the benefit of Waksman Institute faculty and researchers.

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

Illumina NextSeq500 for NGS

Illumina MiSeq for NGS

Bio Rad CFX

Tecan Spark

Fragment Analyzer

StepOne Plus Real Time PCR

Dr. Arvin Lagda, Core Facilities/Shared Resources

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Waksman Institute Shared Imaging Facility

The Waksman Institute Shared Imaging Facility offers cutting edge optical imaging capabilities on four fluorescence microscopes, two image analysis workstations, and high capacity, secure data storage for Waksman researchers. Our microscopes are capable of super resolution imaging of fluorescently labeled cell components in three-dimensional space by optical sectioning and include several advanced features. Imaging can be done with most standard fluorophores in a variety of live or fixed samples. Our manager, Nanci Kane, provides the following services: consultation and demonstrations for instrument selection, comprehensive training, technical support, and troubleshooting. Upon completion of training, manager-approved researchers work independently on the core's microscopes.

This past year, the Imaging Facility acquired three new microscopes with a two million dollar grant from the State of New Jersey. Our current microscopes include Leica SP8 point scanning confocal, Leica Stellaris8 point scanning confocal, Andor Dragonfly spinning disk confocal, and Zeiss Elyra7 structured illumination. Some of the advanced features include deconvolution software to remove out of focus light and significantly improve resolution, large tile scans and multi-position experiments, time-lapse, environmental chambers, white light laser with adjustable excitation wavelengths, Fluorescence Lifetime Imaging Microscopy (FLIM), Förster Resonance Energy Transfer (FRET), Fluorescence Recovery After Photobleaching (FRAP), photo-ablation, optogenetics, Super Resolution Radial Fluctuations (SRRF-Stream), super resolution lattice and apotome Structured Illumination Microscopy (SIM^2), super resolution Single Molecule Localization Microscopy (SMLM), Total Internal Reflection Fluorescence (TIRF), wide field microscopy, as well as software tools for image analysis such as quantification and co-localization.

The Waksman Institute Shared Imaging Facility is open to researchers from Rutgers University as well as other institutions and companies in the area. The Facility has approximately 55 trained users from fifteen laboratories. Core Facility funding may be available to Rutgers researchers through the Rutgers Office of Research or to external customers through the New Jersey Commission on Science, Innovation and Technology (CSIT) voucher program. The future aim of the Facility is to continue to provide exceptional imaging capabilities and training to researchers.

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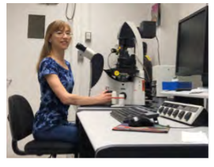

Waksman Greenhouse

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

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

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CHARLES AND JOHANNA BUSCH FELLOWS

Predoctoral Research

Hiep Tran
Steward Lab

Research Summary

Study Tet protein in regulating RNA hydroxymethylation and brain development

Chemically modified ribonucleotides in rRNA, including mRNA, have been known for decades. Recently, the mapping of 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 (5hmrC) in mRNA and that 5hmrC 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 a mutant strain in which the conserved C598 in the DNA-binding domain was changed to A (Tet^{AXXC}), and a second strain, in which H1886 to Y and D1888 to A mutations in the dioxygenase domain were induced (Tet^{YRA}). Tet^{AXXC} showed defects in axon guidance in 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. Further genetic studies found 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. Interestingly, mutations in the human TET3 gene have recently been found in individuals and families affected by neurodevelopmental disorders, indicating TET is also crucial for proper brain development in humans. RNA-seq in the Tet^{AXXC} mutant brain revealed that 1547 genes are up-regulated and 32 are down-regulated. Out of these genes, Glutamine synthetase 2 (Gs2), a key enzyme in glutamatergic signaling, is the most significantly reduced. Mutating or knocking down Gs2 recapitulated the Tet^{AXXC} axon guidance phenotype indicating misregulation of the glutamatergic signaling is responsible for the axon guidance defects in Tet mutant. Furthermore, Tet was found to regulate Gs2 in the insulin-producing cells (IPCs), which are neurosecretory cells in the *Drosophila* brain, and overexpressing Gs2 in these cells can rescue the Tet^{AXXC} mutant phenotype. Since the Tet^{AXXC} showed a very similar axon guidance phenotype as the previously described $Fmr1^{3}$ mutant, Gs2 was also used to rescue the $Fmr1^{3}$ mutant. Surprisingly, overexpressing Gs2 in the IPCs also rescues the axon guidance defects found in the $Fmr1^{3}$ mutant. These results demonstrate that Tet is required for proper axon guidance during early brain development and the regulation is via glutamatergic signaling by controlling Gs2 levels in the IPCs. The study also suggests a link between Tet and Fmr1 functions.

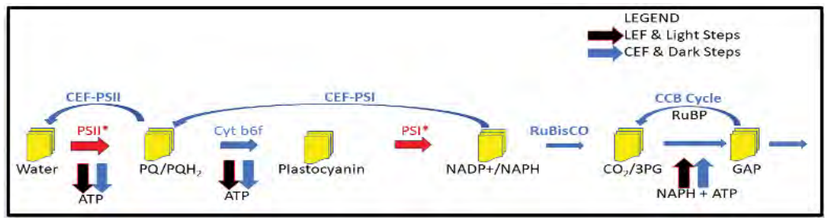
Christopher Turner
Dismukes Lab

Research Summary

The study of photosynthesis has never been more important. Solving the climate crisis, caused by man's disruption of the Earth's carbon cycle will be the challenge of my generation. The solutions will come from a better understanding of photosynthetic light capture and carbon fixation. Ultimately allowing better control of CO_2 emissions while also increasing crop yields. The Dismukes lab has been at the forefront of photosynthesis research for many decades. From the discovery of the tetra-manganese cluster responsible for water oxidation in Photosystem II (PSII-WOC), to the development of new instruments and techniques for the study of the photosynthetic electron transport chain (PETC) as well as the downstream carboxylation reactions. My research aims to better understand the limitations and controls in photosynthetic pathways leading to potential enhancements in productivity and efficiency.

Inorganic Mutants of the PSII-WOC: The PSII-WOC is an inorganic cluster of 4 Manganese, 1 Calcium, 5 Oxygen atoms, 4 water molecules arranged in a hetero-cubane structure inside the PSII dimer. When photons are absorbed, the high energy electrons from the P680 reaction center are sent downstream into the PETC. The PSII-WOC replaces these electrons, while at the same time, moving the "electron holes" onto the cluster. The accumulation of 4 holes drives a rapid "water splitting" reaction to replenish the 4 missing electrons all at once. Recent work from our group has shown that we can successfully replace the Mn^{2+} ions with Co^{2+} in the WOC cluster of ultrapure PSII microcrystals. These efforts are made possible by our collaboration with the Fromme Laboratory at ASU, who supplies the crystals along with their structural analysis of the protein. This will help us to understand why the PSII-WOC only contains Mn in nature and possibly result in more efficient PSII proteins. In addition to studying the Co-PSII-WOC further, I am also working to complement these inorganic mutants with other transition metal ions (ex. Sr-Ca, or Cd-Ca, etc.) that may enhance activity while lending critical insight to the function and assembly of the PSII complex.

Studying of the role and regulation of PSII cyclic electron flow (PSII-CEF): PSII-CEF is vitally important for the proper function of the PETC. Linear electron flow (LEF) from water via PSII delivers electrons to the $Cytb_6f$ complex via the plastoquinol (PQH_2) pool. Reoxidation of PQH_2 requires the presence of oxidized plastoquinone (PQ) to operate the Cytb6f Q-cycle and shuttle electrons downstream to PSI. PSII-CEF helps to prevent an over reduction of the plastoquinone pool under high light conditions by reoxidizing PQH2 by reversing electron flow back to the Mn cluster. Little is known about the mechanisms that regulate this process. My work aims to show the link between the "redox poise" of the PQ pool (i.e., PQH_2/PQ) and the control of PSII-CEF. I am analyzing the behavior of PSII (both *in vivo*, and in PSII crystals) using Fast Repetition Rate Fluorometry (FRRF) and flash oxygen experiments to show that higher redox states of PQ pool (high PQH_2/PQ) increase the rate of PSII-CEF, also known as "backwards transitions".



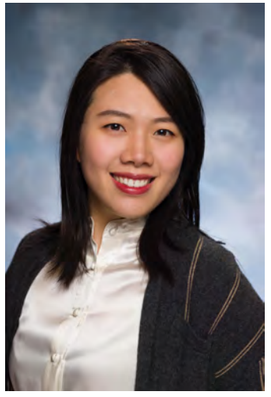
Elmira Kirichenko
Irvine Lab

Research Summary

Investigating the role of Jub dependent phase separation and phosphorylation of Wts.

Understanding how organ growth is regulated and what molecular mechanisms govern the regulatory network has been of fundamental interest to developmental biologists. 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 transcriptional 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 modification, 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 and 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. Recently, we have observed that addition of increasing amounts of Jub leads to the robust phosphorylation of N-terminal Wts. Using mass spectrometry, we have identified all the phosphorylation sites, upon mutation of which to alanine, the mobility shift of Wts reduces completely and stays unaffected by Jub introduction. Interestingly, every single phosphorylated serine and threonine had proline next to it, suggesting that the kinase responsible for the Jub-dependent Wts phosphorylation is a Proline-directed protein kinase. There are 34 known Proline-directed protein kinases in *Drosophila melanogaster*. We plan to identify this Jub-dependent kinase and investigate its role in the regulation of the Hippo signaling pathway.

In addition to phosphorylation, several members of the Hippo signaling pathway or Ajuba family LIM domain proteins were reported to regulate certain cellular processes by formation of biomolecular condensates. For example: phase separation of LIMD1 (Jub in Drosophila), regulated by phosphorylation, was found to assemble and organize mature focal adhesions in response to force (Wang et al., 2021); or upstream regulators of the Hippo Signaling pathway were found to form the functionally antagonizing biomolecular condensates (Wang et al., 2022). Under the conditions of high cytoskeletal tension Jub (LIMD1 in mammals) and Wts (LATS1 and LATS2 in mammals) were observed to localize to the cellular junctions in puncta-like structures both in Drosophila wing discs and mammalian cell culture (Rauskolb et al., 2022, Rauskolb et al., 2014, Ibar et al., 2018). However, the significance of the puncta-like structures is not determined. During the preliminary studies in Drosophila Schneider (S2) cells, we have observed phase separation of Jub alone, as it was previously reported with the closest mammalian homologue LIMD1 (Wang et al., 2021). In addition, association of Jub and Wts triggered the condensates formation in Wts raising the question whether the observed phase separation is part of the inhibitory mechanism of Wts by Jub. Occurrence of Jub-dependent phosphorylation and phase separation of Wts in S2 cells suggest that those two events possibly interdependent, where phosphorylation is regulated by phase separation or vice versa. The goal of the project is to identify the role and the functional significance of Jub-dependent phosphorylation and phase separation of Wts in the regulation of organ growth, and expand the understanding of Wts regulation in the Hippo signaling pathway.



Siwen Wu
McKim Lab

Research Summary

Investigating the interaction between HP1 and CPC in promoting spindle assembly and chromosome segregation during meiosis

Accurate chromosome segregation is crucial for maintaining genome integrity during female meiosis. Defects in this process can lead to infertility, miscarriages, and catastrophic birth defects. The chromosomal passenger complex (CPC) serves as a "master regulator" of spindle assembly and ensures accurate chromosome segregation during meiotic cell division. The CPC is composed of four subunits: inner centromere protein (INCENP), borealin, survivin, and Aurora B kinase. The CPC regulates meiotic events through its dynamic changes in localization at kinetochores and the central spindle. It recruits microtubules to the chromosomes for acentrosomal meiotic spindles and regulates kinetochore-microtubule attachments for accurate chromosome segregation upon the metaphase-to-anaphase transition in meiosis I. However, the mechanism responsible for the CPC recruitment to the chromosomes and the interaction between CPC and spindle microtubules is unknown. We found that the deletion of the middle region of Drosophila INCENP, which contains both the Heterochromatin protein-1 (HP1) binding domain and microtubule binding domains, leads to failures in spindle assembly, chromosome-microtubule attachment, and chromosome segregation. Meanwhile, another INCENP mutant deleting only the microtubule binding domains in *Drosophila* oocytes showed weak spindle defects, revealing a more complicated regulatory system in spindle assembly involved with HP1. Our previous study suggested that HP1 could be a potential binding site for CPC to target heterochromatin. Therefore, the overall goal of my project is to identify the role of HP1, and characterize the interactions between CPC, the microtubules and HP1 in meiosis.

To determine the requirement for HP1 in meiotic spindle organization and chromosomal biorientation, I used RNAi system to knock down HP1 throughout late stages of oocyte development in female *Drosophila*. The quantitative PCR and western blot results showed a more than 99.9% knockdown of the respective mRNAs and a ~95% knockdown of protein expression in these Drosophila oocytes. During metaphase, oocytes ensure equal genetic information segregation by having their paired homologous chromosomes move to opposite spindle poles, a process known as biorientation. However, I observed a 16.65% rate of biorientation defects in the Hp1 RNAi oocytes. The results demonstrate an elevated occurrence of mono-oriented chromosome segregation, where both probes align in the same direction. Additionally, a 13.65% rate of lagging chromosomes were observed. These findings indicate that HP1 is vital in regulating chromosome biorientation during meiosis. I then conducted immunofluorescence to determine the phenotype of the RNAi oocytes. We observed significant disrupted localization of the CPC on central spindles, centromere alignment defects, and frayed spindles or no spindles. These results suggest that HP1 is important for CPC localization, spindle organization and centromere alignment in late meiosis I.

INCENP features an HP1-binding region that is potentially binding to HP1, and an uncharacterized region (UR) of which the function remains unclear. To determine the mechanism of HP1-INCENP interaction in promoting spindle assembly and chromosome segregation, I have generated Incenp mutants which contain deletion of the HP1-binding and UR domains. I observed elevated occurrence of chromosome segregation errors on meiotic metaphase I oocytes from FISH experiments, indicating the role of HP1-INCENP interaction in regulating chromosome bi-orientation. Meanwhile, after losing the HP1-INCENP interaction, HP1 no longer localized to the central spindle and instead either diffused or formed clusters. CPC localization on central spindles also reduced, highlighting that HP1-INCENP interaction is required for building functional central spindle but not spindle formation. Additionally, we observed elevated errors in centromere alignment and increased spindle morphology defects. As a conclusion, these results indicate that HP1-INCENP interaction is not responsible for spindle formation, but regulating CPC localization, functional central spindle formation and centromere organization. Overall, elucidating the interactions between HP1 and CPC would enhance our understanding of chromosome segregation and spindle assembly, facilitating our understanding in meiosis and female infertility.

Sangeevan Vellappan
Yadavalli Lab

Research Summary

Magnesium is a crucial cofactor in essential cellular processes involving proteins and RNAs. MgrB, a small transmembrane protein, negatively regulates the sensor kinase PhoQ, which is important for gene regulation during magnesium starvation and other stress conditions. The absence of MgrB leads to hyperactivation of the PhoQ/PhoP two-component system, resulting in cell division inhibition and filamentation in *E. coli*, and acquired colistin resistance in clinical isolates of *Klebsiella pneumoniae*. MgtS, another small membrane protein, is induced under low Mg^{2+} stress and helps maintain magnesium homeostasis by protecting the MgtA magnesium transporter and inhibiting the PitA phosphate transporter. PmrR, the third small membrane protein expressed under low Mg^{2+} stress, is activated by the PmrAB system in *Salmonella enterica* and has been implicated in polymyxin B resistance through the PhoQ/P and PmrA/B two-component systems in *E. coli*. Recent advances in computational and experimental methods have led to the discovery of approximately 150 small proteins in *E. coli* grown in nutrient-rich media. Only a fraction of the small proteins are well-studied and shown to play crucial roles in essential cellular processes such as cell division, growth, transport modulation, and signaling under stress. We hypothesized that, like MgrB, MgtS, and PmrR, other small proteins with potential regulatory roles are yet to be identified as part of low Mg^{2+} stress response. To systematically identify the full set of small proteins induced under magnesium limitation in *E. coli*, we utilized the translation initiation profiling method – RETapamulin enhanced Ribo-seq (Ribo-RET). Using Ribo-RET, we identified a subset of 17 small proteins out of the >150 reported in *E. coli* to be upregulated, of which a staggering 14 had not been associated with low Mg^{2+} stress prior to this study. Our transcriptomic analyses using RNA-Seq and promoter-reporter assays show that many of these small proteins are transcriptionally upregulated under magnesium starvation, and the PhoQ/PhoP two-component system regulates at least eight candidates. Furthermore, our analysis uncovered that nine small proteins are regulated as part of operons. To characterize each of the stress-induced proteins, we predicted their localization (cytoplasmic/membrane) and found that 9 of the 17 proteins localize to the membrane. Ongoing investigations are focused on elucidating the loss-of-function and overexpression phenotypes of these small proteins, particularly their impact on growth and morphology under low magnesium stress. Future studies aim to identify potential binding partners of these small proteins and unravel their specific targets and functions under stress. Systematic characterization of these stress-induced small proteins will advance our understanding of their functions and physiological role in bacterial survival and adaptation, facilitating the development of novel antibiotics and therapeutics.

Yamei Zuo
Singson Lab

Research Summary

After finishing up a last few phenotypical analysis for *spe-13(syb4925)*, which is the *spe-13* null allele, I confirmed that SPE-13 has a sperm-specific function during fertilization. I then worked with SUNY Biotech to generate a CRISPR/Cas9 edited *spe-13::gfp* strain. This strain contains an endogenously tagged GFP at the end of C terminus of SPE-13.

I first examined the localization of SPE-13 in the live spermatids. I observed that SPE-13 localizes to the peripheral of the inner cell membrane, which is consistent with a pattern that has shown to be the molecules that localizes to in the membranous organelles (MOs) in the spermatids. Therefore, I used SPE-38 as MOs maker to test if SPE-13 also localizes to the MOs in the live spermatids. In the merged field with both SPE-13 and SPE-38, we saw strong colocalization of these two proteins. The Pierson Co-efficiency for these two proteins are averaging between 60-70%, suggesting that SPE-13 and SPE-38 are likely colocalized in the MOs.

In the past, our lab and other labs have observed that the sperm protein required for fertilization are often packed in the MOs in the spermatids. Upon activation, these proteins will redistribute to the cell membrane surface. In the in vivo activated spermatozoa, I observed that SPE-13 is distributed to the cell body membrane and pseudopod membrane, with bright puncta on the cell body membrane which is likely the SPE-13 in the fused MOs. This pattern has been consistent with some other "spe-9 class" proteins like SPE-38, SPE-36 and SPE-51. Since the interactions between the "*spe-9* class" proteins are not well understood, I decided to check if any of them are required for SPE-13's localization in the spermatids and in the spermatozoa.

Our lab has previous reported that loss of *spe-38* impacts the redistribution of SPE-41 to the membrane surface upon sperm activation. I first checked if loss of *spe-38* also affects SPE-13's localization. SPE-13's localization is not affected by the loss of *spe-38* in the spermatids. However, when *spe-38* is not present, SPE-13 failed to redistribute to the membrane surface. Later, similar results were observed for SPE-13's localization change in the spermatozoa when either SPE-9 or SPE-36 was absent. This suggests that SPE-9, SPE-36 and SPE-38 are all required for SPE-13's correct distribution to the membrane surface upon sperm activation.

Surprisingly, SPE-13::GFP signal is completely abolished in the spermatids and spermatozoa in the absence of SPE-45. To check if SPE-13 is produced in this mutant background, I examined SPE-13's localization in the primary and secondary spermatids as well as a special structure, residual body. I found that SPE-13 is present in the primary and secondary spermatids, suggesting SPE-13 is produced even when SPE-45 is absent. However, unlike the wild type that SPE-13 is present in the spermatids in the MOs, SPE-13 is completely left in the residual body in *spe-45* mutants, even though SPE-38 is still present in the MOs in the spermatids in this mutant. This suggests that SPE-45 is likely required to load SPE-13 onto the MOs when MOs are budding off from the Golgi/ER.

Postdoctoral Research

Deepanjali Verma
Dong Lab

Research Summary

Investigating the regulation of BSL1 function in asymmetric cell division during Arabidopsis stomatal development

Self-renewing Asymmetric Cell Division (ACD) plays a vital role in generating diverse cell types during the development of multicellular organisms. This process hinges on the polarization of proteins within the mother cell to yield daughter cells with distinct identities, a phenomenon known as cell-fate asymmetry. The Arabidopsis stomatal development serves as a model system for studying protein polarization during asymmetric cell division. In this intricate process, a Meristemoid Mother Cell (MMC) undergoes ACD, resulting in the production of two dissimilar daughter cells: a smaller meristemoid and a larger stomatal lineage ground cell (SLGC). The meristemoid retains the capacity for subsequent rounds of cell division before eventually differentiating into guard cells (GCs). On the other hand, the SLGC has a limited ability to divide and typically undergoes differentiation into a non-stomatal pavement cell (PC). The scaffold protein BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE (BASL) along with POLAR and BRX, serves as a foundation for assembling a polarity complex, which remains intact during the division of the MMC and is passed on to its Stomatal Lineage Ground Cell (SLGC) daughter. Throughout the progression of stomatal Asymmetric Cell Division (ACD), alterations in the composition of this polarity complex play a role in generating cell-fate asymmetry by regulating the activity of SPCH.

Within the MMC, the polarity complex includes the MAPKK kinase YODA (YDA) and the GSK3-like BIN2 kinases, acting as negative and positive regulators of SPCH, respectively. YDA inhibits SPCH by initiating a MAPK signaling cascade leading to SPCH degradation in the nucleus. In contrast, BIN2's association with the polarity complex inhibits YDA. Consequently, at the cell membrane, BIN2 effectively activates SPCH by preventing its degradation. Therefore, prior to ACD in the MMC, the interaction of BIN2 with the polarity complex allows SPCH activity to drive cell division. In the differentiated SLGC daughter cell, BIN2 relocates to the nucleus, alleviating its inhibitory effect on YDA. This, in turn, results in SPCH degradation.

The recent study by Guo et al. in 2021 addressed a previously existing gap in our understanding of the transition from cell division to differentiation by identifying a novel polarity protein called BSL1, which becomes a part of the polarity complex, while BIN2 relocates to the nucleus. BSL1, as a phosphatase, dephosphorylates YDA and initiates downstream kinase signaling, ultimately resulting in the degradation of SPCH and the induction of cell differentiation. However, the specific regulatory mechanisms governing the function of BSL1 remain unknown. In this study, our focus is on examining how BSL1 function is regulated. In pursuit of this goal, we successfully pinpointed a cell cycle regulatory kinase that phosphorylates BSL1 during in-vitro kinase assays. Various protein-protein interaction methodologies were employed to confirm the association between BSL1 and this kinase. Intriguingly, our experiments with kinase inhibitors indicated that the phosphorylation induced by this kinase could potentially promote BSL1 polarization. Nevertheless, establishing whether this regulation is direct or indirect remains a challenge. To delve deeper into this issue, I am currently engaged in a series of genetic and biochemical experiments.

Jonathan Dietz
Rongo Lab

Research Summary

The Role of the Metaxins in Mitochondrial Health and Homeostasis

Mitochondria are crucial for maintaining cellular health and homeostasis. Known for their role in producing ATP via oxidative phosphorylation, they are also the site of cofactor and metabolite biosynthesis, ion and lipid homeostasis, reactive oxygen species (ROS) production and signaling, and initiation of apoptosis. Perturbations to mitochondrial homeostasis and function manifest in severe clinical outcomes, such as inflammation, aging, cancers, metabolic diseases, cardiovascular disorders, and neurodegeneration. Mitochondria employ approximately 1000 proteins, which are predominantly synthesized from the nuclear genome. A highly sophisticated system imports this proteome and ensures the proteins are properly localized and matured at their appropriate compartments, including the MOM, the mitochondrial inner membrane (MIM), the intermembrane space (IMS), or the matrix delimited by the MIM.

The MOM is rich with β-barrel transmembrane proteins, which are channels/pores formed from a single peptide barrel of β-strands. Key β-barrel proteins in the MOM include TOM40, which mediates MOM protein import; SAM50, which mediates β-barrel assembly; and VDAC1, which mediates metabolite transport. These β-barrel proteins also mediate ion homeostasis, mitochondria-directed apoptosis, autophagy, and mitophagy. Import and assembly of nascent MOM β-barrel proteins require MTX-1 and MTX-2. MTX-1 and MTX-2, also referred to as the metaxins, are well characterized in *S. cerevisiae* and mammalian cell culture; however, their role and impact in multicellular eukaryotes lacks understanding. Interestingly, previous studies by our lab and others identified MTX-1, MTX-2, and one of their substrates, VDAC-1, as regulators of mitochondrial movement, a process called mitochondrial motility, in *C. elegans* neurons.

There are two overall goals of my project: (1) characterize the function of the metaxins in *C. elegans* and (2) determine the molecular mechanism governing mitochondrial movement regulation by the metaxins. To accomplish these goals, we are combining genetics, cell biology, biochemistry, and microscopy.

During our preliminary work, we found that strains lacking the metaxins have a reduced median lifespan suggesting that their role in mitochondrial homeostasis is critical for organismal health. These data are interesting because a recent study identified human variants of MTX-2 in patients with a progeroid disorder, which involves premature aging. To probe the impact MTX-1 and MTX-2 have on mitochondrial function, we assessed the mitochondrial membrane potential (MMP), an electrochemical gradient that is produce as a result of oxidative phosphorylation, and observed that the metaxins are required for the proper generation of the MMP and ultimate mitochondrial ATP production. Since the MMP was compromised in MTX-1 and MTX-2 mutants, we evaluated the activation of mitochondrial stress response pathways. Through this analysis, we discovered that the mitochondrial unfolded protein response (UPRmt) was active in mutants lacking MTX-2, but not MTX-1. The UPRmt activation in MTX-2-deficient strains resulted in resistance to heat stress and induction of mitohormesis, a phenomenon present in response to low levels of cellular stress that facilitates the sequestration of damaged cellular components and allows the organism to become resistant to further cellular insult.

Our next steps are to further our understanding of the molecular role for the metaxins in regulating mitochondrial motility in *C. elegans* neurons.

Hyuk Sung Yoon
Zander Lab

Research Summary

Dissecting the genetic architecture of glandular trichome formation in *hemp*

Hemp (*Cannabis sativa*) is an economically important crop used for medicinal, industrial, and recreational purposes. It is thought to be one of the first domesticated plants in early Neolithic times in East Asia and has since been cultivated in various parts of the world. The most prominent medicinal compounds of hemp are phytocannabinoids, which are predominantly produced in glandular trichomes (GTs). GTs are multicellular epidermal outgrowths that serve as natural factories for specialized metabolites and exist in a wide range of plants. They play a crucial role in protecting plants from various abiotic and biotic stresses, particularly herbivorous insects. The cellular organization of GTS can vary among plant species, from simple structures with just one glandular cell, as observed in tomato type I trichomes, to more complex structures, as seen in stalked GTs of hemp, which consist of a multicellular secretory disc secreting metabolites into a large extracellular storage cavity. The gene regulatory network underlying GT initiation in hemp is controlled by a largely unknown combination of internal and external stimuli, leading to the occurrence of glandular trichomes only on female flowers. It is known from various GT-producing species, that the plant defense hormone jasmonic acid (JA) plays an important role in the formation of GTs, however, its role in hemp is largely unknown.

The goal of my research is therefore to identify transcription factors (TFs) that govern the initiation of glandular trichomes and the production of phytocannabinoids with a focus on JA. Functional validation of transcription factor candidates requires the analysis of both overexpressing and knock-out lines. Since hemp is notoriously difficult to genetically transform, I am currently improving stable transformation protocols of hemp hypocotyls. In addition, I was also able to successfully express hemp TFs transiently in leaf and flower tissues which is a prerequisite of conducting ChIP-seq and MS-IP with candidate TFs.

Long-term JA treatment of hemp plants revealed that JA has an inhibitory effect on plant height and growth with flower buds being the exception. I found that bud size and development is stimulated by exogenous JA application in contrast to other plant tissues. This holds also true for the development of GTs, which mature more rapidly in the presence of JA. Interestingly, the number of GTs is not affected by JA indicating that the JA signaling pathway merges with the GT pathway after the GT initiation has already been established. A key component of the JA pathway are the JAZ repressor proteins which inhibit the activity of various TFs. In the presence of bioactive JA, JAZs form the JA co-receptor complex with COI1 which leads to their degradation and subsequent liberation of TFs. I am currently testing my set of candidate TFs for an interaction with hemp JAZ proteins using yeast-two-hybrid assays. Last, I also conducted ChIP-seq and RNA-seq in various hemp tissues to assess gene expression and genome-wide occupancies of the active histone modifications H3K4me3 and H3K9ac, as well as the repressive modification H3K27me3. In summary, my approaches collectively aim to discover key regulators that control the development of GTs, a unique plant structure with great agronomic and pharmaceutical potential.

BENEDICT MICHAEL FELLOW

Predoctoral Research

Naureen Hameed
Barber Lab

Research Summary

Neuromodulatory Signal Integration in a *Drosophila* Clock Output Region

Initially regarded as a disease solely linked to defective insulin secretion, our understanding of diabetes has evolved over time to recognize various axes of dysfunction, including dysregulated neuromodulation. However, there is currently a lack of neuromodulatory tools to effectively manage and potentially treat metabolic diseases such as diabetes. This is due to a dearth of information about neuromodulatory signaling pathways involved in downstream metabolic and physiological outcomes. My project leverages *Drosophila* as an effective model organism to identify clock-derived neural signaling pathways involved in modulating the proto-hypothalamic neuroendocrine axis, the *pars intercerebralis* (PI). I propose that clock neuromodulatory signals integrate in the PI via three distinct neurosecretory cell populations, influencing diverse feeding behaviors. To this end, I investigated the clock-derived signals responsible for modulating circadian feeding behavior by screening for defective feeding behavior when candidate clock neuropeptide receptors (NPRs) are knocked out in PI neuron populations. I used the fly-liquid interaction counter (FLIC) assay to obtain feeding-relevant data with high temporal resolution. I found that when a key clock neuropeptide pigment-dispersing factor's (PDF) receptor is knocked out in the insulin producing cells (IPCs) of the PI, flies display evening-specific overfeeding. I demonstrated that PDF+ small ventrolateral clock neurons (sLNvs) are presynaptic to these IPCs as well as the other two distinct PI neuron populations using the novel retrograde neuron circuit tracing tool, BAcTrace. We also found that the PI neuron populations are functionally connected to each other by using a calcium indicator-based stimulus response assay on acutely dissected brains. sLNv neurons are regarded as the master clock neurons that set the pace of downstream clock neural activity and rhythmic physiological behavior, including feeding. Furthermore, downstream clock neuron groups have been previously shown to functionally modulate PI neuron activity in a time-of-day dependent manner. Hence, circadian feeding behavior may be controlled through this clock hierarchy that has its output signaling in the PI. I aim to continue delving into this circuitry, seeking additional clock-related neuropeptides that influence various elements of rhythmic feeding patterns and untangle how the integration of this signaling by the various PI neuron populations consolidates a physiological output.

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 2022-2023

Advanced Plant Genetics
Advanced Inorganic Chemistry
Ethical Scientific Conduct
Ethical Scientific Conduct Refresher
Genetic Analysis I
Genetic Analysis II
Independent Studies in Chemistry
Inorganic Chemistry Molecular Biosciences
Laboratory Rotation in Chemistry
Microbial Biochemistry
Molecular Biology and Biochemistry
Molecular Biosciences Graduate Minicourse
Research in Microbial Biology
The Genetics and Cell Biology of Fertilization
Thesis Writing and Communication in Genetics

WAKSMAN STUDENT SCHOLARS PROGRAM

High School Outreach

Summary

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

For 30 years, faculty and scientists at the Waksman Institute have collaborated with high school teachers and their school administrators to address these issues. Our strategy has been to engage high school students and their teachers in authentic scientific research, 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 2022-2023 WSSP consisted of two interrelated parts: a Summer Institute (SI) and an Academic Year Program (AYP). In July 2022, 26 students and 5 teachers from 25 high schools attended a 9-day SI at the Waksman Institute. The SI consisted primarily of daily seminars and laboratory activities that focused on molecular biology and bioinformatics. The laboratory sessions introduced students and teachers to the content, background information, and laboratory procedures that were needed to carry out the research project at their schools during the academic year. Students and teachers used Internet resources to process and analyze their data. Some of the seminars that were presented dealt with bioethical issues and career opportunities in the fields of science, technology, engineering, and mathematics (STEM). Scientists met with participants to discuss recent research developments in the fields of plant ecology and Next Generation DNA sequencing.

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 2022-2023 academic year, teachers from 43 schools conducted the WSSP laboratory and bioinformatics sections of project in person. The WSSP provided the reagents and supplies for these schools to conduct the experiments.

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 990 high school students participated in the WSSP program during the 2022-2023 academic year.

The Research Question

The 2022-2023 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 1190 DNA sequences were generated by students conducting the laboratory portion of the WSSP at their schools. 652 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. During the summer of 2022 in-person two-week programs were held for 43 students in June 2022 and 44 students in the July 2022 program. This is an increase from previous years due to additional teaching laboratory space provided by the Waksman Institute. Students in these programs prepared over 500 DNA samples and analyzed these by PCR and restriction digests followed by gel electrophoresis. The sequences of 407 of these DNA samples were analyzed and 322 were submitted for publication on NCBI by 86 students.

Virtual Waksman Institute Summer Experience (vWISE)

During the pandemic (2020 and 2021) all WSSP and WISE programs were all held on-line. One advantage to conducting a on-line, virtual programs is that we were not limited by the number of students who could work safely in the laboratory. In addition, students from states across the US, along with several other nations were able to participate in these programs. Due of the success of these online programs, we offered a vWISE program in August 2022 for 81 students. The 2022 summer vWISE program was conducted over a two-week period, with two on-line discussion sessions each day. Students were each provided two DNA sequences and the vWISE students completed the analysis of 136 of these sequences and 109 were published on the NCBI database.

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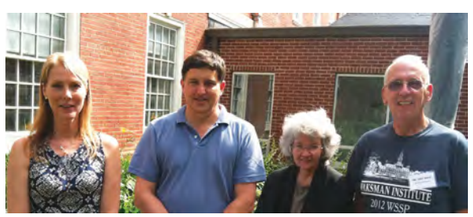

PRESENTATIONS & MEETING ABSTRACTS

Waksman Institute Hosted Seminars -
Pal Maliga: Pal Maliga/Corrine Best, Amersham 10800 Camera for Evaluation

Andrea Gallavotti, Dr. Kobayashi, Plant Biology, Rutgers University: Andrea Gallavotti, Waksman Institute, Rutgers, University Mechanisms and manipulations of maize inflorescence architecture

Annika Barber: Adam Hines, University of Queensland, Australia & University of Pennsylvania, Using Fruit Flies to understand a pre-synaptic mechanism of general anesthesia.

Annika Barber: Maria de la Fernandez, Barnard College, Connecting Patterns of Drosophila Pacemaker Neurons

Graduate Students in Waksman Institute: Gisela Therese Storz, NIH Distinguished Investigator,
Regulation by genes within genes

Juan Dong: June M. Kwak, Daegu Gyeongbuk Institute of Science & Technology (DGIST), Korea Cellular Precision in Plant Development

Juan Dong: Zhenbiao Yang, University of California – Riverside, Extracellular auxin: Perception and Coordination with intracellular auxin

First Annual Postdoc Speaker Daniel Dickinson, University of Texas – Austin, Cell Polarity: From molecules to embryos

Nicholas Stavropoulos: Neils Ringstad, New York University, Neural mechanisms of microbe sensing

Kenneth Irvine: Barry Thompson, The Australian National University, Physiological control of tissue growth via the Hippo pathway

Nicholas Stavropoulos: Mimi Shirasu-Hiza, Columbia University, You are what you eat: investigating intermittent fasting and lifespan extension in Drosophila

Kenneth Irvine: Seung K. Kim, Stanford University, New paradigms for insulin biology and pancreatic islet replacement: implications for researching and curing diabetes

Andrew Singson: Dawn Chen, University of Pennsylvania, Sexual selection and the female nervous system in Drosophila

Andrea Gallavotti: Bastiaan Bargmann, Virginia Tech, It's All in the Timing: Enhancing Regeneration Efficiency Using Morphogenic Factors

Andrew Singson: Diane Shakes, The College of William & Mary, Cellular insights from the genetic lawbreaker Auanema rhodensis
Bryce Nickels: Whitman Seminar, Bonnie L. Bassler, Princeton University, Quorum Sensing Across Domains: From Viruses to Bacteria to Eukaryotes

Andrea Gallavotti & Pal Maliga, Joachim Messing Memorial Symposium, Marja C.P. Timmermans, University of Tubingen, Germany, Drawing a straight line: pattern formation by small RNA morphogens

Waksman Student Scholars Programs
Waksman Student Scholars Program Summer Institute (WSSP-SI), July 5-July 15, 2022, 26 students, 5 teachers.

Waksman Institute Summer Experience (WISE June-22), June 20-July 1, 2022, 43 students.

Waksman Institute Summer Experience (WISE July-22), July 25-August 5, 2022, 44 students.

Virtual Waksman Institute Summer Experience (vWISE August-22), August 8-August 19, 2022, 81 students

Barber:
"A second-generation auxin-inducible gene expression system for temporal control of gene expression in Drosophila." Genetics Society of America Drosophila Meeting, Chicago IL.

"Sexually dimorphic roles for neuropeptides in Drosophila circadian behavior." Janelia Research Meeting - Neuropeptide Signaling: Bridging Cell Biology, Neurophysiology, and Behavior, April 2023.

Fukumura K, Kathirvel V, Barber AF. (2023). Unraveling circadian aging: The interplay of age and high-fat diet in circadian desynchrony. Poster presented at the AFAR Grantees Conference, Santa Barbara, California

Hameed N, Zhao C, Cirone E, Barber AF. (2023). Novel clock inputs to the Drosophila pars intercerebralis coordinate circadian locomotor activity. Poster presented at the Chronobiology Gordon Research Conference, Bates College, Maine.

Crespo-Flores SL, Fetchko M, Barber AF. (2023). Sexually dimorphic roles for Drosophila circadian clock neuropeptides in regulating rest-activity rhythms. Poster presented at the Chronobiology Gordon Research Conference, Bates College, Maine.

Dismukes:
June 2023, American Chemical Society National Meeting, Green Chemistry & Engineering Symposium, Long Beach, CA, Chemicals Made from Scratch: A Carbon-Negative, Renewably Powered Future without Biofuels

May 2023 , Gordon Research Conference, CO2 Assimilation in Plants from Genome to Biome, Ciocca, Italy: Direct Measurement of CO2 Carboxylation Kinetics across the Photosynthetic Tree of Life: Finding the Chokepoints and Validation by Microkinetic Modelling Investigating the impact of elevated CO2 on photosynthetic metabolism from the long-term adaption of plants to naturally high CO2 sources in the environment: Yellowstone NP

April 2023 Eastern Regional Photosynthesis Conference, Woods Hole, MA: How plants adapt to extreme environmental CO2 levels: What Yellowstone can teach us

Feb 2023, Dept of Chemistry, University Richmond, VA: Chemicals from Scratch

March 2022, American Chemical Society, Mid-Atlantic Regional Meeting, Rutgers University, NJ: Boosting CO2 electro-reduction efficiency above competing water reduction by design of catalysts
Changing the product of CO2 reduction on Ni2P electrocatalyst to ethylene glycol using a Lewis acid/base co-catalyst

Dong:
2023 Seminar at the Daegu Institute of Science and Technology, Daegu, South Korea
Title: Precisions in space and time: Cell polarity and asymmetric cell division in plants

2023 Seminar at the Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX
Title: Precisions in space and time: Cell polarity and asymmetric cell division in plants

2023 Seminar at the Department of Molecular, Cell, and Developmental Biology, UCLA, Los Angeles, CA
Title: Precisions in space and time: Cell polarity and asymmetric cell division in plants

2022 Invited Talk at the 13th Biennial Conference, the Chinese Biological Investigators Society, Las Vegas, NV
Title: Precisions in space and time: Cell polarity and asymmetric cell division

2022 Seminar (Virtual) at the College of Life Sciences, Lanzhou University, Lanzhou, China
Title: Precision in time and space: Signaling control in stomatal development

2022 Seminar (Virtual) at the School of Life Sciences and Biotechnology, Shanghai Jiaotong University, China
Title: Precision in time and space: Signaling control in stomatal development

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

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

2022 The 2022 Joint MAS-ASPB and UMD Plant Symposium, University of Maryland, MD
Title: Switching on or off: signaling control in stomatal development.

Ebright:
"Gain of Function Research." Emerging Threats and Spending Oversight Subcommittee, US Senate Homeland Security Committee, Washington, D.C., 2022 (testimony; remote).

Structural basis of transcription termination." Department of Chemistry, Princeton University, Princeton, New Jersey, 2022.

"Structural basis of Rho-dependent transcription termination," Meeting on Post-Initiation Activities of RNA Polymerases, Mountain Lake, Virginia, 2022.

"Structural basis of transcription termination," Gordon Conference on Microbial Transcription, Southern New Hampshire University, New Hampshire, 2023.

Bird, H., Yin, Z., Kaelber, J., Kooshkbaghi, M., Tenenbaum, D, Kinney, J., Nickels, B., and Ebright, R.H.

(2022) Lambdoid phage Q protein: mechanism of a transcription antiterminator and insights into intrinsic termination, Molecular Genetics of Bacteria and Phages Meeting, Madison WI, August 1-5, 2022,

Marshall, C., Molodtsov, V., Ebright, R.H., and Santangelo, T. (2023) Polarity in Archaea: a kinetic competition between transcription termination and translation. International conference on transcription mechanism and regulation by archaeal RNA polymerases and eukaryotic RNA polymerases I, III, IV, and V, Jaen, Spain, May 30-June 2, 2023.

Fermentation:
Lagda, AC "Biomanufacturing Summit" Rutgers University, New Brunswick, NJ. July 7-8, 2022

Lagda, AC" Association of Biomolecular Resource Facility NERLSCD Annual Meeting" University of Rochester, Rochester, NY. September 12-14, 2023

Lagda, AC "New Jersey Academic Drug Discovery Consortium on Infectious Diseases" NJ Center for Science, Technology and Mathematics, Union, NJ. December 16, 2022

Lagda, AC. "2023 Plant-Based Council Meeting Annual Conference", Washington DC. March 27-29, 2023
Lagda, AC "2023 INTERPHEX" New York City, New York. April 25-27, 2023

Lagda, AC; Rodriguez A ,, "New York Society of Cosmetic Chemists Suppliers Day" New York City, New York. May 2-3, 2023

Lagda, AC. "New Jersey Research Cores Partnering Conference", Princeton University Materials Institute, Princeton, New Jersey. June 22, 2023

Gallavotti:
Gallavotti, A. Genetic control of maize meristem function. 5th European Maize Meeting, Bologna, Italy. June 14, 2023.

Gallavotti, A. Mechanisms and manipulation of maize inflorescence architecture. Iowa State University, Ames, IA. April 11, 2023.

Gallavotti, A. Mechanisms and manipulation of maize inflorescence architecture. Institute of Plant and Microbial Biology, University of Zurich, Zurich, Switzerland. April 5, 2023.

Gallavotti, A. Mechanisms and manipulation of maize inflorescence architecture. Department of Plant Molecular Biology, University of Lausanne, Lausanne, Switzerland. March 31, 2023.

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Chen, Z., Galli, M., Gallavotti, A. "Dissecting cis-regulatory control of ZmWUS1 expression through the binding of type-B response regulator proteins". 65th Annual Maize Genetics Meeting. St. Louis, MO, USA, March 16-19, 2023.

Chaudhry, A., Galli, M., Gregory, J., Chen, Z., Schmitz, R.J., Gallavotti, A. "Genetic dissection of maize shoot and inflorescence architecture". 65th Annual Maize Genetics Meeting. St. Louis, MO, USA, March 16-19, 2023.
Gregory, J., Liu, X., Chen, Z., Gallavotti, A. "Master meristem manipulators: regulation of meristem size by the REL2 corepressor family". 65th Annual Maize Genetics Meeting. St. Louis, MO, USA, March 16-19, 2023.

Chen, Z., Debernardi, J., Dubcovsky, J. Gallavotti, A. "The combination of morphogenic regulators BABY BOOM and GRF-GIF improves maize transformation efficiency". 65th Annual Maize Genetics Meeting. St. Louis, MO, USA, March 16-19, 2023.

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Bang, S., Zhang, Y., Gregory, J., Chen, Z., Gallavotti, A., Schmitz, R.J. "Understanding the role of ZmWUS1 and cis-regulaotry elements in maize inflorescence development at single-cell resolution". 65th Annual Maize Genetics Meeting. St. Louis, MO, USA, March 16-19, 2023.

Gallavotti, A., Chiles, A., Chen, Z., Taylor-Teeples, M., Macias, J., Galli, M., Moss, B. Nemhauser, J. "Monitoring the effects of auxin signaling variation on maize inflorescence architecture". Auxin 2022 Meeting. Cavtat,

Croatia, October 2-7, 2022.

Irvine:
"April 20, 2023 Seminar at Dept. Cell, Developmental, and Regenerative Biology, Icahn School of Medicine at Mount Sinai, NY NY

Maliga:
Pal Maliga, Engineering Organelle Genomes, Discussion Leader, Chloroplast Biotechnology Gordon Research Conference, Ventura, CA, March 26-31, 2023

Malihe Mirzaee, "Expression of recombinant proteins in seed plastids". Chloroplast Biotechnology Gordon Research Conference, Ventura, CA, March 26-31, 2023

Pal Maliga, "PPR10 RNA binding-protein for protein expression in tobacco seed plastids". 2023 In Vitro Biology Meeting, June 10-14, 2023, Norfolk, VA.

Pal Maliga, "Progress in engineering the chloroplast and mitochondrial genomes and prospects for new agronomic applications". Centre for Agricultural Research, Martonvasar, Hungary, June 22, 2023

Corinne Best and Pal Maliga. "Design of gRNAs for CRISPR/Cas9 mediated engineering of the plastid genome." Chloroplast Biotechnology Gordon Research Conference, Ventura, CA, March 26-31, 2023

Aki Matsuoka, Julia Ferranti and Pal Maliga. "Engineering VirD2 for T-DNA delivery to chloroplasts." Chloroplast Biotechnology Gordon Research Conference, Ventura, CA, March 26-31, 2023

Malihe Mirzaee, Alyssa Leung, Alifiya Quresh, Ana Candia, Aki Matsuoka, Kerry Lutz, and Pal Maliga. "Expression of recombinant proteins in seed plastids." Chloroplast Biotechnology Gordon Research Conference, Ventura, CA, March 26-31, 2023

Rahim Khan, Pal Maliga and Kerry Lutz "Development of a Routine Protocol for Chloroplast Transformation from Leaf Tissue in the Model Plant Arabidopsis thaliana". American Society of Plant Biology Northeastern Section 2023 Meeting, Old Westbury, NY, April 22, 2023

Singson:
Frontiers in Reproduction Course Lecture, the Marine Biological Laboratories, Woods Hole MA.
Lab section: C. elegans reproductive tract cell biology.

Stavropoulos:
"A heteromeric nicotinic acetylcholine receptor promotes sleep by relaying GABAergic signals within a locus of motor and sensory integration" (poster)
Gordon Research Conference on Chronobiology, June 2023.

"Mechanisms that construct sleep circuits and relay their signals"
Mt. Sinai Sleep Grand Rounds, May 2023.

"A heteromeric nicotinic acetylcholine receptor promotes sleep by relaying GABAergic signals within a locus of motor and sensory integration" (poster)
Dros 23 Conference, March 2023.

"Mechanisms underlying the control of sleep: Building sleep circuits and relaying their signals"
Dalhousie University, Department of Biochemistry and Molecular Biology, (virtual visit), November 2022.

"Mechanisms underlying the control of sleep: Building sleep circuits and relaying their signals"
University of California, San Diego, Department of Pharmacology, October 2022.

"Mechanisms underlying the control of sleep: Building sleep circuits and relaying their signals"
University College London, Queen Square Institute of Neurology, October 2022.

"Mechanisms underlying the control of sleep: Building sleep circuits and relaying their signals"
Imperial College London, Department of Life Sciences, October 2022.

"Developing insomnia: from brain development to sleep regulation"
University of Pennsylvania, Chronobiology & Sleep Institute, September 2022.

Waksman Institute Shared Imaging Facility:
Kane, NS. "Waksman Institute Shared Imaging Facility" poster and presentation. Waksman Institute Retreat, Rutgers University, Piscataway, NJ. October 6, 2022.
Kane, NS. "Waksman Institute Shared Imaging Facility" presentation. Super Worm Group, Rutgers University, Piscataway, NJ. November 30, 2022.

Kane, NS. "Waksman Institute Shared Imaging Facility" poster. Genetics Department Retreat, Rutgers University, Piscataway, NJ. May 18, 2023.
Kane, NS. "Waksman Institute Shared Imaging Facility" poster. NJ Research Cores Partnering Conference, Princ-

eton University, Princeton, NJ. June 22, 2023.

Yadavalli:
2022 – Adeleye SA and Yadavalli SS. Dual Functions of a Biosynthetic Enzyme (QueE) in tRNA Modification and Bacterial Stress Response. Microbial Stress Response Gordon Research Conference (GRC), Mount Holyoke College, South Hadley MA (talk)

2022 – Vellappan S, Favate J, Jagadeesan P, Shah P, Yadavalli SS. Identification and characterization of stress-induced small proteins in E. coli. Symposium titled 'The Unexplored Biology of Small Proteins' at the American Society of Microbiology (ASM) Microbe conference, Washington DC (talk)

2023 – Low magnesium stress-induced small proteins in E. coli. Annual microbiology symposium, Rutgers University, New Brunswick NJ

2023 – Epitranscriptomic enzymes and small proteins: Emerging regulators of stress response. Genetics, Cell Biology and Anatomy seminar series, University of Nebraska, Omaha NE (Virtual)

2023 – Epitranscriptomic enzymes and small proteins: Emerging regulators of stress response. Biological sciences seminar series, Vanderbilt University, Nashville TN

2023 – Epitranscriptomic enzymes and small proteins: Emerging regulators of stress response. Microbiology seminar series, The Ohio State University, Columbus OH

2022 – Dual Functions of a Biosynthetic Enzyme (QueE) in tRNA Modification and Bacterial Stress Response. Biology seminar series, La Salle University, Philadelphia PA

2022 – Small Proteins and Epitranscriptomic Factors: Emerging mechanisms in bacterial gene regulation. Biology seminar series, Rutgers-Camden NJ

Zander:
Zander M. (Invited Speaker) Dissecting the environmentally responsive plant epigenome. October 2022, Fall '22 Molecular Plant Sciences Seminar series, Michigan State University, US

Zander M. (Invited Speaker) Dissecting the environmentally responsive plant epigenome. December 2022, CSH Asia Meeting - Integrative Epigenetics in Plants, Japan

Zander M. Jasmonate signaling through the lens of epigenomics. June 2023, International Conference on Arabidopsis Research 2023, Japan

Yoon H.S., Ammari M., Zander M. Charting the epigenome of female Cannabis sativa inflorescences. January 2023, Plant Animal & Genome Conferences (PAG), San Diego, US

PATENTS & PUBLICATIONS

Patents

Dismukes:
Dismukes, G.C., A. Laursen and K. Calvinho, Title*:* Lewis/Bronsted Acid/Base And Nickel Phosphide Binary Catalyst-System (Co-Catalysts) For Direct Electrochemical Co_2 Reduction To Hydrocarbons, in USPTO, R.T.I.-F.R.F.N. 070439.01571, Editor. 2022.

Dismukes, G.C. and M. Dhiman, Notice of Inventions Disclosure "Electroreduction of carbon dioxide (CO2) to multi-carbon products on molybednum diphosphide (MoP2) electrocatalyst" in Rutgers University. 2023.

Dismukes, G.C. and C.W. Cady, Notice of InventioDisclosure "Catalysts for the selective partial oxidation of natural gas to methanol and hydrocarbons to alcohols", in Rutgers University. 2023.

Ebright:
Ebright, R., Ebright, Y., and Lin, C.-T. (2022) Antibacterial agents: dual-targeted RNA polymerase inhibitors. US Patent US11447502.

Ebright, R., Ebright, Y., Mandal, S., Wilde, R., and Li, S. (2022) Antibacterial agents: N(alpha)-aroyl-N-aryl-phenylalaninamides. European Patent EP3102193.

Ebright, R., Ebright, Y., Freundlich, J., Gallardo-Macia, R., and Li, S.-G. (2023) Antibacterial agents: arylalkylcarboxamido phloroglucinols. US Patent US11572337.

Patent Application

Ebright, R. and Ebright, Y. Antibacterial agents: dual-targeted RNA polymerase inhibitors: conjugates of benzoxazino- and spiro-rifamycins with Na-aroyl-N-aryl-phenylalaninamides. WO2023023378, PCT/US22/40964 (filed 08/19/22).

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Barber:
Zhang Y, Li Y, Barber AF, Noya SB, Williams JA, Li F, Daniel SG, Bittinger K, Fang J, Sehgal A. (2023) The microbiome stabilizes circadian rhythms in the gut. Proc Natl Acad Sci USA. 120:e2217532120.

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Dismukes:
Zournas, A., K. Mani and G.C. Dismukes, Cyclic electron flow around photosystem II in silico: How it works and functions in vivo. Photosynthesis Research, 2023. 156(1): p. 129-145.

Zhang, Y., G. Ananyev, A. Matsuoka, G.C. Dismukes and P. Maliga, Cyanobacterial photosystem II reaction center design in tobacco chloroplasts increases biomass in low light. Plant Physiol, 2023. 191(4): p. 2229-2244.

Gu, H.F., F. Zhang, S. Hwang, A.B. Laursen, X. Liu, S.Y. Park, M.J. Yang, R.C. Bramante, H. Hijazi, L. Kasaei, L.C. Feldman, Y.W. Yeh, P.E. Batson, B.W. Larson, M.J. Li, Y.F. Li, K. Wyatt, J.L. Young, K. Teeluck, K. Zhu, E. Garfunkel and G.C. Dismukes, Interfacial Connections between Organic Perovskite/n(+) Silicon/Catalyst that Allow Integration of Solar Cell and Catalyst for Hydrogen Evolution from Water. Advanced Functional Materials, 2023. In review.

Gates, C., G. Ananyev, S. Roy-Chowdhury, P. Fromme and G.C. Dismukes, Regulation of light energy conversion between linear and cyclic electron flow within photosystem II controlled by the plastoquinone/quinol redox poise. Photosynth Res, 2023. 156(1): p. 113-128.

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Severinov:
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2023 WAKSMAN INSTITUTE ANNUAL RETREAT
September 21, 2023
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New Brunswick, NJ 08901

Presentations

Moonia Ammari (Zander lab): “High-throughput elucidation of the jasmonic acid-responsive chromatin landscape

Saai Suryanarayanan (Singson lab): “The role of SPE-21, a palmitoyltransferase in sperm activation”

Deepanjali Verma (Dong lab): “Regulation of BSL1 function during asymmetric cell divisions in Arabidopsis stomatal development”

Ekaterina Semenova (Severinov lab): “tRNA anticodon cleavage by target-activated CRISPR-Cas13a effector”

Richard H. Ebright (Ebright lab): “Structural basis of archaeal factor-dependent transcription termination”
Malihe Mirzaee (Maliga lab): “Expression of recombinant proteins in seed plastids”

Sangeevan Vellappan (Yadavalli lab): “Identification and characterization of low magnesium-stress induced small proteins in E. coli”

Siwen Wu (McKim lab): “HP1 interacts with the Chromosome Passenger complex to promote spindle assembly and chromosome segregation during meiosis”

Bipin Tripathi (Irvine lab): “Dachsous cytoplasmic domain is required for growth and planar cell polarity”

Christopher Turner (Dismukes lab): "Unfolding the Flashes: Interpreting the behavior of photosynthetic electron transport using variable fluorescence"

Keisuke Fukumura (Barber lab): “The coincidence of aging and nutritional stress has complex effects on molecular and behavioral rhythmicity in Drosophila”

Zongliang Chen (Gallavotti lab): “Genetic dissection of cis-regulatory control of ZmWUS1 expression by type-B response regulators”

Tatiana Popovitchenko (Rongo lab): “Foraging through hypoxia: the role of Protein Kinase G in the hypoxia stress response”

Nicholas Stavropoulos (Stavropoulos lab): “Molecular cloning of dracula”

Nanci Kane & Arvin Lagda: “A brief overview of the Waksman Imaging and Fermentation core facilities”

POSTER SESSION

Sergio Crespo-Flores, Michael Fetchko, and Annika F. Barber
"Sexually dimorphic roles for *Drosophila* circadian clock neuropeptides in regulating rest-activity rhythms"

Naureen Hameed, Chenyue Zhao, Evan Cirone, and Annika F. Barber
"Neuromodulatory signal integration in a clock output region"

Vinithra Kathirvel, Keisuke Fukumura, and Annika F. Barber
"Evaluating the role of nutritional stress and aging on circadian rhythmicity in *Drosophila*"

Akanksha Mathivanan, Sham Gupta, Maryem Abdelgelil, Michael Fetchko, and Annika F. Barber
"Traumatic brain injury induces sleep changes and increased AP1 activation in *Drosophila* glial cells"

Charles Dismukes
"Improving Photosynthesis both Natural and Artificial"

Elizabeth Chan, Deepanjali Verma, and Juan Dong
"Investigating the role of KKVI domain in BSL1 function"

Aobo Huang and Juan Dong
"Application of proximity labeling in plant cell polarity study"

Taylor Rossiter, Aobo Huang, and Juan Dong
"Characterization of Polarity Proteins in Plant Cells"

Sanqiang Zhang and Juan Dong
"PRC1-mediated regulation of stomatal development in *Arabidopsis thaliana*"

Shuya Yang, Ian C. Nova, Abhishek Mazumder, Andrew H. Laszlo, Ian M. Derrington, Jens H. Gundlach, and Richard H. Ebright
"Translocation of RNA polymerase relative to DNA in transcription elongation, pausing, and termination: single-molecule picometer-resolution nanopore tweezers (SPRNT)"

Jason Gregory, Jason Punskovsky, Xue Liu, Zongliang Chen, and Andrea Gallavotti
"Master Meristem Manipulators: Regulation of meristem size by the REL2 corepressor family"

Amina Chaudhry, Zongliang Chen, Jason Gregory, Mary Galli, and Andrea Gallavotti
"Genetic dissection of shoot and inflorescence architecture in maize"

Jason Punskovsky, Jason Gregory, and Andrea Gallavotti
"Genetic interaction analysis of maize meristem regulators"

Elmira Kirichenko, Kush Mansuria, Derrick Michell, Mayank Chauhan, Sarah Choi, and Kenneth Irvine
"Investigating the role of Jub-dependent phase separation of Warts in the Hippo signaling pathway"

Deimante Mikalauskaite, Cordelia Rauskolb, Tom Lehan, Srividya Venkatramanan, and Kenneth Irvine
"Transcriptional co-repressor Atrophin regulates Hippo pathway target genes in Drosophila"

Corinne Best, Malihe Mirzaee, and Pal Maliga
"gRNA design for CRISPR/Cas9 mediated engineering of the plastid genome"

Shaunak Kinare, Aki Matsuoka, and Pal Maliga
"Impact of VirD2 level on Agrobacterium-mediated plant transformation efficiency"

Aki Matsuoka, Angela Kitanski, and Pal Maliga
"Measuring plastid-targeted VirD2 endonuclease activity"

Malihe Mirzaee, Alyssa Leung, Alifiya Quresh, Aki Matsuoka, Ana Candia, Kerry Lutz, and Pal Maliga
"Expression of recombinant proteins in seed plastids"

Margaret Howland, Helen Nguyen, Justin Mathew, Nikunj Patel, Vandana Apte, Mercedes R. Gyuricza and Kim S. McKim
"Exploring the C(2)M Cohesin Complex: Structure, Dynamics, and Ability to Facilitate Assembly of the Synaptonemal Complex"

Diya Surray, Madeline Terry, Mark Aziz, Kim S. McKim
"Discovering Meiosis Mediating Genes Using RNA Interference"

Keara Greer, Siwen Wu, Manisha Persaud, and Kim S. McKim
"Borealin and the Chromosome Passenger Complex in meiosis"

Sho Ogino, Tatiana Popovitchenko, and Christopher Rongo
"Neuropeptide signaling regulates oxygen deprivation-induced behavior in *C. elegans*"

Jonathan Dietz, Nanci Kane, Eunchan Park, Erin Rose, Nathalie Salazar-Vasquez, and Christopher Rongo
"The role of the metaxins in mitochondrial health and homeostasis"

Joelle Smart, Eunchan Park, Natalia Morsci, Sierra Swords, Barth Grant, Monica Driscoll, and Christopher Rongo
"Mitophagy Activity in Aging *C. elegans* Tissues"

Erin Rose, Jonathan Dietz, and Christopher Rongo
"The Effects of Mitochondrial Function on Neurodegeneration"

Katherine Maniates, Kendall Flanagan, and Andrew Singson
"A forward genetic screen reveals that an ortholog of PGM3 may be required for fertilization in the oocytes of *C. elegans*"

Yamei Zuo and Andrew Singson
"SPE-13 is a sperm membrane protein involved in the fertilization synapse complex and functions during fertilization in C. elegans"

Raad Altawell, Joseph Lopez, Emily Van Beek, Faith Verderose, Shuhao Li, Qiuling Li, and Nicholas Stavropoulos
"The G-protein beta subunit Gβ13F is required for neurogenesis and sleep and is a putative substrate of the Cul3-Insomniac ubiquitin ligase complex"

Faith Verderose, Qiuling Li, Alexie Lessing, Kayla Lim, and Nicholas Stavropoulos
"mud mutants and re-evaluating the role of the Drosophila mushroom body in sleep regulation"

Samuel A. Adeleye and Srujana S. Yadavalli
"Queuosine biosynthetic enzyme (QueE) moonlights as a cell division regulator"

Maryam Mohammed and Srujana S. Yadavalli
"Role of QueE enzyme in metal stress response and growth phenotypes in *E. coli*"

Hyuk Sung Yoon and Mark Zander
"Molecular dissection of jasmonate response in *Cannabis* sativa"

Nanci Kane and Arvin Cesar Lagda
"Waksman Institute Shared Imaging Facility"