ABOUT US

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

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

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

Giving

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

Joachim Messing (September 10, 1946 - September 13, 2019)

Joachim (Jo) Messing, the Selman A. Waksman Professor of Molecular Genetics, University Distinguished Professor, and long-time director of the Waksman Institute at Rutgers University, has died at age 73. Messing made pioneering and foundational contributions that underpin the modern field of molecular genetics, genomics, and evolutionary biology. He invented “shotgun” sequencing of DNA, an approach that vastly empowered the advance of the genomic era of biology. The tools he created and disseminated were coming into full bloom. They provided the funding and shared the technology and knowledge that Waksman built the first building of the Institute that bears his name and of which Messing was an outpouring of tributes from colleagues and students.

Since the sudden death of Jo Messing there has been an outpouring of tributes from colleagues and students.

I am currently postdoctoral in Dr. Messing’s laboratory. Thursday we got just celebrate his birthday in the lab on Friday night, so shocked! After his return from the trip to China, he was excited to let us know that in China there are totally? Distinctly Round Bran and Long Bran elected by SOP China research on maize genetics (I personally think that’s a sort of equivalent to AAGS Fellows in US, and three out of four maize sciences trained by Dr. Messing. He is satisfied by such an achievement. He always encouraged us to work harder as those excellent lab alumni did.)

Dr. Messing shared the same birthday cake with me, since our birthdays are so close. Mine is Sep 1st while his is Sep 10th. This year, we just celebrated our birthdays together last Thursday. And he was so healthy, so robust and so excited. After his return from the trip to China, he was excited to let us know that in China there are totally? Distinctly Round Bran and Long Bran elected by SOP China research on maize genetics (I personally think that’s a sort of equivalent to AAGS Fellows in US, and three out of four maize sciences trained by Dr. Messing. He is satisfied by such an achievement. He always encouraged us to work harder as those excellent lab alumni did.)

Dr. Messing gave an inspiring title for this manuscript entitled “Towards...” What an inspiring title!

Dr. Messing never gave us a hard time because our research went slow since he was an outpouring of tributes from colleagues and students.

Dr. Messing was the recipient of many awards and honors. He was elected a Fellow of the American Association for the Advancement of Science in 2002. He led the team awarded World Technology Award in Biochemistry in 2003 and the USDA Secretary’s Honors Award in 2004. He was named one of the German National Academy of Sciences Leopoldina (2007). He was awarded the Wolf Prize in Agriculture (2013) and a member of the US National Academy of Sciences (2015). He was also a Fellow of the American Academy of Microbiology (2015) and the American Academy of Arts and Sciences (2016).

Messing’s life was one of many achievements and a few ironies. Amongst the latter was his attendance, at age 23, at summer science conference in Lindau in 1970. A treasured remembrance from that conference is a now-faded photograph of Jo standing behind Selman A. Waksman, the winner of the 1952 Nobel Prize in Physiology or Medicine and a long-time Rutgers faculty member. It was with funds from the prize and other sources that Waksman built the first building of the Institute that bears his name and of which Messing was the fourth director.

Since the sudden death of Jo Messing there has been an outpouring of tributes from colleagues and students.

I am currently postdoctoral in Dr. Messing’s laboratory. Thursday we got just celebrate his birthday in the lab on Friday night, so shocked! After his return from the trip to China, he was excited to let us know that in China there are totally? Distinctly Round Bran and Long Bran elected by SOP China research on maize genetics (I personally think that’s a sort of equivalent to AAGS Fellows in US, and three out of four maize sciences trained by Dr. Messing. He is satisfied by such an achievement. He always encouraged us to work harder as those excellent lab alumni did.)
out is something I cannot forget from him. Him seeing my capabilities as just a graduate student then means a lot and this probably explains my continued interest in plant improvement and genetics. I am grateful for the learning experience I had with him as his graduate student and I would not have it any other way if I get to see my PhD. - J. Rivera

Within the few short years I have been with Waksman, I have already known Dr. Messing as a very kindhearted man. He knew all of us by name, and would always offer a warm smile and hello, if passing in the hall. An exceptional combination of a person to have his pure brilliance in science and his importance to the Institute, be so humble and kind. It was a privilege to have worked, in some small way, for him. He will be missed. - J. Baucher

Dr. Messing was such a kind man and I will always remember going to a party at his house and feeling very welcomed by him.- M. Blanco

My first knowledge of Jo Messing was when he had submitted a grant proposal to the USDA Genetic Mechanisms program in 1978-79. The panel was extremely enthusiastic, to say the least, about the proposal that involved his M13 use in DNA sequencing. With this introduction to Jo, I later worked together with others to get him on the University of Minnesota faculty. His lab worked in part on a high methionine strain of corn discovered in my lab. Thus, we had a close connection and always found time at meetings to sit down and talk science. I cherished these talks.

Another fun time with Jo was when we went on a fishing trip to the Boundary Waters of Minnesota. Jo and I got our lines tangled. He was in the middle seat of the canoe, trying to get us lined up, when I landed my line. Jo thought that I had hooked something but I had not. Jo was so impressed that I could be so calm during all this time. I never told him that the fish was hooked accidentally near the canoe by chance. - B. Phillips

Beyond his success in science and his quest for the greater good, he was a very kind man. - M. Bythell

The footprint, "Dedicated to Rita and Simon. When I was newly hired I really struggled to recover and fight type M13 I planted for a distribution request by constructing an M13 mp mutant that I had saved in a collection of old vials. He was so excited when it finally worked and he grinned and said, "It was also plate number three when I made mp1!" He was very concerned that his research helped perpetuate and bring honor to Rutgers University. It was obvious to all but he said he still loved his job at Waksman and also, "I envy you Jennie, working in the lab." Jo once proped you to do more than you thought possible than Dr. Messing. He was an inspiring mentor and most importantly, had great faith in his students.- J.A. Quer
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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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Cover Images: Spirodela polyrhiza strain 9509, Paul Fourounjian, Messing Lab; Hemisphere of the developing Drosophila brain, stained for expression of Dachshund (yellow, marks lamina neurons), E-cadherin (magenta, marks neuroepithelial cells), and Deadpan (green, marks neuroblasts), Venu Redd, Irvine Lab; Lemma gibba anther stain, Paul Fourounjian, Messing Lab
Mission Statement
The Waksman Institute’s mission is to conduct research in microbial, developmental, and plant molecular genetics. The Institute also is a catalyst for general university initiatives, life science infrastructure, undergraduate, graduate, and outreach education, and a public service function for the state.

Background
The principal mission of the Waksman Institute is research. Although the initial emphasis of the institute at its founding was microbiology, its focus soon turned towards molecular genetics, and was later broadened to include also multicellular organisms. Our founding director said at the opening of the Institute: “This Institute will devote its efforts to the study of the smallest forms of life, the microbes, wherever they are found and no matter what their activities may be,” he also appreciated the dynamics of all scientific endeavors by saying: “Let this Institute serve as a center where scientists from all parts of the world may gather to work, to learn, and to teach. These Halls are dedicated to the free pursuit of scientific knowledge for the benefit of all mankind.” This freedom in scientific research had enabled the members of the Institute to push the frontiers of scientific knowledge today to new levels from better nutrition to drug-resistance of infectious diseases, from cancer to birth defects.

Central to the Institute’s advances in molecular genetics is the introduction of interdisciplinary programs with chemistry, biology, and computational sciences. Indeed, the institute’s research mission has evolved from a diversity of disciplines centered on antibiotics to a unified discipline of molecular genetics with a more diverse set of biological problems. The institute today employs faculty teams that concentrate on certain classes of organisms amenable to genetic analysis such as unicellular organisms (e.g., Escherichia coli, yeast, and algae), animal systems (e.g., Drosophila and C. elegans), and plants (e.g., Arabidopsis, maize, sorghum, tobacco, and duckweeds). To apply advances in scientific knowledge to the benefit of mankind, the Institute continues to seek practical and commercially viable applications of its discoveries. Historically, in fact, the institute owes its existence to the symbiotic relationship that exists between academic research institutions and the private sector. In 1939 Dr. Selman Waksman, the institute’s founder and namesake, entered into an agreement with Merck & Company of Rahway, New Jersey, to study the production of antimicrobial agents by soil bacteria. Within three years, streptomycin, the first effective antibiotic against tuberculosis, was discovered, patented, and licensed to the pharmaceutical industry by Rutgers University.

Organization
The Waksman Institute is a research unit of the New Brunswick campus of Rutgers University, The State University of New Jersey. It receives a budget from the state to support the recruitment and appointment of faculty, whose salary is split with decanal units of the campus, where they hold their tenure. This facilitates faculty appointments in different disciplines and enriches the interdisciplinary research unique to the institute. The decanal units simultaneously receive an enhanced instructional and service program, in addition to their traditional departmental tasks consistent with the mission of a state university.

With the merger of Rutgers and UMDNJ on July 1, 2013, chancellors have been appointed and the institute reports to the Chancellor of New Brunswick since then. The faculty of the institute will continue to participate in the various graduate programs, thereby remaining fully integrated into the state university system.

Administration
The Institutes administration consists of only 6 people under Robert Rossi as Executive Director of Finance and Administration. I share my administrative assistant with the faculty, helping them with the pre-award grant application process. Erin Sorge works closely with Marge Piechota, who directs the business office and manages the post-award phase of grants. Because of the size of outside awards, procurement is the highest activity of the administration, followed by personnel action including visa needs for all foreign students and scientists. Finally, receiving of orders at the loading dock is monitored and building maintenance requires work orders.
Unique for New Jersey is a cell and cell products fermentation facility. Built in 1954 and renovated in 1984, the Cell and Cell Products Fermentation Facility, located within the Waksman Institute at Rutgers University is a state-of-the-art facility that provides fermentation services to a multitude of clients including academic institutions, international pharmaceutical corporations, virtual enterprises and not-for-profit, specific niche services to produce biologics and similar products including purification, bulk production, scale-up and R&D. We operate independent of state support, using a fee for service arrangement with our clients. Our bio-reactors include two 1,000-liter, three 125-liter, and four 30-liter systems. These systems and our downstream equipment have been recertified to NIST standards and work is conducted under cGIL/GMP guidelines. Our staff follows SOPs and adheres to good record keeping.

The second largest facility is the Waksman Genomics Core Facility (WGCF), offering multiple platforms and several other tools to assist with genetic and genomic research. The WGCF employs the latest technologies to generate high-quality sequencing data with fast turnaround and competitive prices. In its commitment to enhance the productivity of research, the Waksman Institute revamped its Genomics Core Facility with the purchase of the Sequel Sequencer of Pacific Biosystems with funds from the chancellor’s office. This new equipment provides long sequence reads, which is critical for analyzing full-length cDNAs and the assembly of whole genomes from shotgun sequencing reads.

Our infrastructure also includes a cell biology core facility with multiple imaging and microscopy platforms. There are two laser-scanning confocal-microscopes - a Leica SP5 II and a Leica SP8 - both of which are spectral confocal microscopes equipped for imaging multiple wavelengths, including UV, and capable of collecting high resolution optical sections where out-of-focus light is eliminated. Both microscopes have sensitive GaAsP detectors, and the SP5 uses the latest high-speed resonance scanner for rapid image acquisition for real-time imaging. Computing software is available for the analysis and deconvolution of three- and four-dimensional image data, as well as for FRAP, FLIP, and FRET measurements. The core also possesses a Zeiss Axioplan-2, which is a fully motorized immunofluorescence system capable of the automated collection of 3D and 4D image stacks using multiple wavelengths for routine work.

Both the Genomics and Cell Biology Core are very computing intensive units. The Waksman computing staff is responsible for maintaining the high availability of these resources 24/7 with minimal downtime. It has dedicated space on the fourth floor in the building’s Old Wing. Randy Newman has two people with dedicated responsibilities, Daja O’Bryant for all desktop equipment and Brian Schubert for server updates and services.

The Institute’s computational resources are provided by a state-of-the-art data center which hosts 30+ servers, a high-performance computing cluster with nearly 400 logical compute cores, and over 1,000 TB 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 10G Ethernet and 8G 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 is a member of the Rutgers High Performance Cooperative Cluster (HPCC). This shared computing resource is available to select departments across Rutgers and its users have access to its large pool of high memory compute nodes complete with NVIDIA Tesla GPUs, FDR Infiniband, 10G Ethernet, and a high-performance distributed Lustre filesystem. This cluster is ideally suited for many computationally intensive research tasks.

By utilizing Rutgers’ Internet 2 connection, Waksman users have a high speed, high bandwidth direct connection to 450+ universities and 32 affiliate members of the Internet 2 consortium. The Institute provides its users with traditional office software and common molecular biology tools, but also offers multi-functional sequence analysis application suites: Lasergene DNAStar and Vector NTI.

The fourth facility is the Institute’s Farm. It includes a greenhouse with seed storage and sorting head house. The close-by fenced-in field space is serviced with the necessary farm equipment. The Farm Supervisor, Joshua Gager, was leaving us after two seasons and we were joined now by Themios (Tim) Chionis as his successor.

Other service functions include small instrument repairs and glass washing units in the old and new wing, respectively.

Personnel and faculty affiliations

In the academic year 2018/2019, the Institute consisted of fifteen resident, two non-resident, and six emeriti faculty members. The Institute accommodates six assistant research professors, ten visiting student/scholar researchers, fifteen research associates, twenty-one postdoctoral researchers, eighteen technical assistants, eighteen graduate students, and six emeriti. The Waksman Institute’s total resident population is currently 114, which does not include forty-six undergraduate students that did independent research during the last year. There has been a continuous decline in research personnel from 135 in 2016 to 114 last year. In particular, there was a drop in graduate students from 32 to 18, whereas the number of postdocs stayed the same. A major factor was certainly increased costs in graduate education, the closure of two laboratories due to retirements, and the loss of HHMI funding.

There were five institute faculty members in the Department of Molecular Biology and Biochemistry, five in the Department of Genetics, three in the Department of Plant Biology and Pathology, two in the Department of Chemistry, one in the Department of Ecology, Evolution, and Natural Resources, and one in the Department of Biochemistry and Microbiology (Dr. Dismukes has two departmental affiliations). Of the fifteen resident and two non-resident members, two were Associate Professors, nine Professors, four Distinguished Professors, one Board of Governors Professor, and one Distinguished University Professor. I am also the first holder of the Selman Waksman Chair in Molecular Genetics. The Institute currently has six professor emeriti, who are all well, and periodically join us here for events. Three professors are members of the National Academy of Sciences (US), three of the American Academy of Arts and Sciences, one of the National Academy of Inventors, one of the German National Academy of Sciences, one of the Hungarian Academy of Sciences, five are Fellows of the American Academy of Microbiology, and eight Fellows of AAAS.

Last academic year, Chuck Dismukes was on sabbatical and Richard (Rick) Paddock retired as Professor of Molecular Biology and Biochemistry. He continued for one more year with a leave of absence except for student committees. I wrote a brief summary of his career already last year.

Lectures

Because there are so many lectures in the life sciences on our and the Cook campus, the institute conducts mainly ad hoc seminars of visitors of our faculty that are listed at the end of the Report. Especially, we were pleased to have our former member Xuemei Chen from the University of California at Riverside on May 1st, 2019, to give the lecture “Small RNA Networks in Plants”. We also list the program of our annual retreat from September 7th, 2018. In addition, the institute sponsored The Microbiology Symposium, in New Brunswick, NJ, in February 2019 at Trayes Hall, Douglass College.

Recruitment & Funding

We had two openings last year for faculty recruitment, which were supported by Chancellor Molloy, Dean March, and Dean Goodman. One was for the replacement of Rick Paddock in SAS for the animal genetics group at the institute. The departmental affiliation was left open to the candidate, which could have been in Molecular Biology and Biochemistry, Genetics, or Cell Biology and Neuroscience. The other one was the replacement of Hugo Dooner in the Department of Plant Biology of SEBS for the plant genetics group at the institute. Recruitment was enhanced with the addition of new laboratory space to the new wing to be completed this fall. Chris Rongo chaired the search in SAS and Pal Maliga the one in SEBS. The SAS search was successful, and Annika Barber from the University of Pennsylvania is joining us January 1st, 2020. She uses Drosophila to study circadian rhythm and sleep, expending our expertise in the neuroscience. Her tenure-track appointment will be in the Department of Molecular Biology and Biochemistry. Because of her choosing, she will move to the second floor in the old wing to switch places with Drew Vershon, given us the opportunity to highlight the commitment for our outreach program to High Schools by moving Drew’s operation onto the first floor of the new Addition. As Annika comes with the new year, there will be time to renovate Drew’s space for her, allowing us to concentrate fruit fly genetics on the second floor of the old wing. The SEBS search will be renewed for the coming year but be opened to all ranks. In addition to Annika Barber, Chancellor...
Molloy and Dean March provided us with additional resources to recruit Sam Yadavalli for the Microbial Genetics group as a special case, which will increase our membership by one more slot than in the past. She comes from the Nickels’ laboratory and studies the regulation of gene expression in bacteria under different growth conditions. She will move into Rick Padgett’s space.

Clearly, the outside support achieved through competition is the most notable highlight. Congratulations to all faculty that received either new grants or renewals. On average, two-thirds of all Institute annual resources are based on external grants and contracts. Over a 11-year period, total external funding of about $9.5M has fluctuated significantly. We expected a significant drop because of the most recent retirement of Hugo Dooner and Rick Padgett and the loss of a second Howard Hughes Investigator appointment. We recently recruited two new junior faculty and anticipate this trend to reverse.

Awards/Honors

I am pleased to report several awards/honors of our faculty this year. Maureen Barr got promoted to Distinguished Professor. Andrea Gallavotti received the early career award from the Maize Genetics Executive Committee. Chuck Dismukes received the NASA CO2 Utilization Challenge Prize and the Rutgers Goldman Innovation Prize. Ken Irvine became the Director of the Rutgers Graduate Program in Cell and Developmental Biology. I received an Honorary Professorship of Zhejiang University, Hangzhou, China and I was elected and inducted to the National Academy of Inventors last April at the Space Center in Houston. Congratulations to these accomplishments of our members!
Summary

Research: Cilia, extracellular vesicles, and animal behavior

My research program studies fundamental questions in cilia cell biology and to model human polycystic kidney disease and other ciliopathies. Cilia have the capacity to both receive and send information, the latter in the form of extracellular vesicles (EVs). EVs are nano-communication devices that cells shed to influence the behavior of other cells, tissues, or even organisms. EVs may be beneficial or pathological, depending on their cargo content. Very little is known about EV cargo sorting, formation, or function, largely because their tiny size escapes detection by light microscopy. We have developed the only in vivo system to study EV biogenesis and bioactivity in a living animal, C. elegans, and are poised to make important fundamental discoveries that may have profound impact on human health and disease.

In the brain of the worm, the cephalic male (CEM) and inner labial (IL2) neurons are the only known ciliated EV-releasing neurons. These EV-releasing cilia display non-canonical variations of the 9+0 doublet microtubule axoneme. During sexual maturation, CEM doublets splay to 18 A-tubule and B-tubule singlets while the IL2 transition zone remodels from 9+0 to 4-7+0. We previously showed that the tubulin code via tubulin glutamylation and tubulin isotype controls this unique ciliary ultrastructure. Ciliary EV shedding and release is also controlled by the tubulin code writers (a glutamylase TTLL-11 and α-tubulin TBA-6), erasers (deglutamylase CCPP-1), and readers (the IFT machinery and kinesin-3 KLP-6). Using super-resolution imaging, we recently discovered that cilia shed EVs at two distinct sites, that ciliary EV cargo sorting is a highly selective process, and that cilia use mechanosensation and calcium to modulate EV shedding and potentially bioactivity.

We have two ongoing NIH-funded research projects (DK59418 and DK116606). My lab is also supported by an NIH diversify supplement (to grad student K. Tiger), a NJ Commission on Spinal Cord Injury fellowship (to postdoc J.S. Akella), and an award from the NIH-funded Kansas PKD Center (to research assistant professor J. Wang).

IRVINE LAB

Developmental Biology

Summary

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

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

One major area of research has involved investigations of the Hippo signaling network, which has emerged over the past decade as one of the most important growth regulatory pathways in animals. We study its regulation, molecular mechanisms of signal transduction, and its roles in different developmental and physiological contexts. We discovered regulation of Hippo signaling by the Dachsous and Fat cadherins over a decade ago, and have continued to define key steps in this branch of Hippo pathway regulation. Most recently, we identified and characterized the early girl gene as a novel component of the Fat-Hippo signaling pathway, which acts through regulation of Dachs protein levels.

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

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

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

We have also investigated how tissue patterning and mechanics influence morphogenesis. As one simple model, we have combined genetic analysis, live imaging, and computation image analysis to investigate cellular and molecular mechanism that govern wing shape in Drosophila. One unexpected outcome of these studies was the discovery that orientation of cell divisions is not required for normal wing shape.

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MCKIM LAB
Molecular Genetics of Meiotic Recombination and Chromosome Segregation

Summary
Meiosis I, or the reductional division, is when pairs of homologous chromosomes segregate from each other. Chiasmata, the result of meiotic crossing over, facilitate this process by binding the homologous chromosomes together until they can orient correctly on the bipolar meiosis I spindle. This occurs when the homologous centromeres are oriented towards opposite poles. This is known as bi-orientation, and during meiotic metaphase I it establishes correct homologous chromosome segregation at anaphase I. The goal of our work is to understand how each pair of homologous chromosomes makes attachments to the spindle while successfully orienting in opposite directions prior to segregation at meiosis I. In humans, errors in chromosome segregation in the oocyte lead to aneuploidy and are the leading cause of miscarriage, infertility and birth defects. Indeed, the fidelity of meiosis is fundamentally important to all sexually reproducing organisms. We are studying the mechanisms of bi-orientation and the features of the oocyte spindle that make it susceptible to chromosome segregation errors.

1) Investigate the structure and function of the meiotic centromere and kinetochore

The conserved KMN network is required for KT-MT attachments in vivo and is composed of three groups of proteins: SPC105/KNL1, the MIS12 complex and the NDC80 complex. NDC80 is required for end-on MT attachments, which are important for moving the chromosomes towards the poles at anaphase. We have identified several activities that depend on SPC105R, kinetochore assembly (NDC80 recruitment), lateral microtubule attachments, and sister centromere fusion. The latter is important for coorientation, the mechanism that promotes attachment of sister kinetochores in meiosis I to microtubules from the same pole.

To study the function of SPC105R, we use tissue-specific RNAi to deplete the protein in oocytes. To develop a system to study the functions of SPC105R in oocytes with mutants, we generated an Spc105R RNAi-resistant transgene. This transgene rescues all Spc105R RNAi phenotypes. Using this construct, we are generating RNAi-resistant transgenes with deletions of each SPC105R domain proposed to recruit proteins with specific functions (Figure 1). We are particularly interested in separation-of-function mutants that identify the region(s) required for lateral attachments and sister centromere fusion. Surprisingly, the large central domain and the N-terminal MT-binding domains are not essential for meiosis. However, a transgene expressing only the C-terminal domain appears to be sufficient to localize to the centromeres, recruit other kinetochore proteins, and most surprising, recruits cohesin protection proteins such as MEI-S332/Shugoshin. These studies are continuing with the generation and characterization of additional mutations.

Dr. Kim McKim
Genetics

Figure 1: Structure and function of kinetochore protein SPC105R. A) Schematic of Spc105R protein structure. Sequence motifs are above the line. Studies in mitotic cells have suggested the N-terminal domain interacts with microtubules and phosphatase PPI. The central domain includes regions that recruit checkpoint proteins. The C-terminal domain may interact with centromere components. B) Localization of RNAi-resistant SPC105R (red) with centromere protein CENP-A/CID (white). C) An oocyte expressing a Spc105R transgene lacking the central “PEED” domain. The spindle is normal and SPC105R (red) localizes to the centromeres. D) An oocyte expressing a Spc105R transgene expressing only the C-terminal domain. The spindle is abnormal, but SPC105R (red) and NDC80 (white) localize to the centromeres, showing the C-terminal domain is sufficient for kinetochore assembly. In all images, the microtubules are in green, the DNA in blue, and the scale bar is 5 um.
Prior to meiosis, the DNA replicates. Cohesin is the protein complex that holds these sister chromatids together until meiosis I and II. Cohesin regulation is complex because it must be protected from destruction prior to these stages, and then released in stages. First the chromosomes arms during meiosis I, and then the centromeres and the pericentromeric regions in meiosis II. Regulate sister centromere fusion. The analysis of kinetochore proteins SPC105R, KNL1 and Protein Phosphatase 1 (PP1-87B) has shown that two independent mechanisms maintain sister centromere cohesion in Drosophilia oocytes. Maintenance of sister centromere cohesion by SPC105R involves protection against Separase, the enzyme that destroys cohesin proteins. In contrast, maintenance of sister centromere cohesion by PP1-87B does not involve Separase. Instead, PP1-87B maintains sister centromere cohesion by inhibiting stable KT-MT attachments. In a recent paper describing this work, we propose two mechanisms regulate co-orientation in Drosophila oocytes. First, SPC105R protects cohesins at sister centromeres to maintain fusion. Second, separation of sister centromeres in meiosis II occurs without degrading cohesins, depends on end-on microtubule attachments, and during meiosis I is prevented by PP1-87B mediated destabilization of these attachments.

SPC105R maintains sister centromere cohesion during meiosis I by recruiting Protein Phosphatase 2A (PP2A), which modifies cohesins so they cannot be destroyed. What regulates stepwise removal of cohesion in the pericentromeric regions and the chromosome arms is not known. This analysis is complicated by the fact that Drosophila have two PP2A subunits, WDB and WRD, that are partially redundant. In oocytes lacking SPC105R, WDB does not localize to the centromeres. With RNAi oocytes, however, a mild defect in cohesion, displaying occasionally showing separated centromeres and precocious anaphase. When both WDB and WRD are depleted by RNAi, we observed a complete failure to maintain sister chromatin and arm cohesion in metaphase I oocytes (Figure 2). We are currently investigating how PP2A is recruited to the pericentromeric regions and chromosome arms. Based on published work in mitotic cells, it is possible that, if Dalmatian, the Drosophila homolog of cohesin regulator SORORIN, recruits PP2A to the chromosomes in addition to MEI-5332/Shugoshin. Our preliminary data shows that Dalmatian is localized to the meiotic chromosomes during early prophase. It may protect cohesion during the long prophase arrest experienced by oocytes, and during meiosis I may be redundant with MEI-5332/Shugoshin.

When Aurora B was inhibited in PP2A RNAi oocytes, the spindle was maintained, demonstrating that PP2A antagonizes the Aurora B spindle maintenance function. We have further shown that the PP2A negatively regulates KLP10A, a kinesin 13 that depolymerizes microtubules. Thus, we have found that PP2A opposes CPC functions, probably by dephosphorylating Aurora B spindle targets.

To identify the phosphatases that regulate Aurora B activity, we used a small molecule inhibitor of Aurora B, Binculein (2 RN2). When oocytes are treated with this drug, they lose their microtubule spindles. Thus, continuous Aurora B activity is required to preserve the spindle during meiosis I and spindle dynamics may be regulated by a phosphatase. When Aurora B was inhibited in PP2A RNAi oocytes, the spindle was maintained, demonstrating that PP2A antagonizes the Aurora B spindle maintenance function. We have further shown that the CPC negatively regulates KLP10A, a kinesin 13 that depolymerizes microtubules. Thus, we have found that PP2A opposes CPC functions, probably by dephosphorylating Aurora B spindle targets.

4) Investigate how the central spindle interacts with kinetochores and promotes bi-orientation

Based on previous studies of kinetochore proteins, we have proposed that homologous chromosome bi-orientation depends on interactions between the kinetochores and an array of antiparallel microtubules present in the center of the spindle. Because these microtubules do not end at the chromosome, the kinetochores move laterally along them. Our goal is to identify the central spindle and kinetochore components that mediate these lateral attachments. We previous-ly showed that in the absence of NDC80, SPC105R is recruited to the kinetochores and is sufficient for lateral microtubule attachments. We therefore use Ndc80 RNAi to generate oocytes with only lateral microtubule attachments. Using FISH, we found that pairs of homologous centromeres make lateral attachments to the same bundles of antiparallel microtubules and bi-orient, supporting the model that lateral interactions between the kinetochores and the central spindle promote biorientation of homologs.

Based on studies in mitotic cells, we are investigating the model that lateral attachments depend on the phenomenon of kinetochore expansion, where the amount of a subset of kinetochore proteins is dynamic and can increase to promote interactions with the central spindle microtubules. We have preliminary evidence that this occurs in Drosophila oocytes. When oocytes are treated with colchicine to depolymerize microtubules, MPS1 and ROD, two proteins required for biorientation, accumulate and expand at the kinetochores. We are currently using SPC105R, mps1 and rod RNAi or mutants to test a model that SPC105R recruits proteins like MPS1 and ROD required for kinetochore expansion, and these interactions promote KT-MT lateral interactions. The biggest challenge and long-term goal is to determine how kinetochores, when interacting laterally with central spindle microtubules, promotes biorientation of homologous chromosomes.

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Figures:

- Figure 2: Precocious anaphase in PP2A knockdown oocytes. A) Wild type oocyte. B) wdb RNAi oocyte. C) wdb RNAi oocyte held in buffer prior to fixation, showing a susceptibility to precocious anaphase, defined as the separation of chromosomestowards the poles of the spindle. D) wdb with double RNAi oocyte showing precise anaphase. In all images, INCENP is in red (except D), the microtubules are in green, the DNA in blue, and the scale bar is 5 um.
As an aid to our TGFβ work, we have developed useful CRISPR tools for Drosophila. Genome editing using CRISPR is an animal invention and does not exist in yeast or plants. Evolutionary analysis of the TGFβ pathway indicates that two complete TGFβ pathways exist in the most primitive animals, sponges and trichoplax. Each primitive animal has an member of the I-Smad family, which is conserved in all phyla and is a downstream target of TGFβ signaling. As expected, C. elegans: 1) an F2 screen for small animals (a mutant phenotype exhibited by many genes in the pathway), 2) suppressors of lon-2, an upstream gene of the pathway, and 3) suppressors of lon-1, a downstream gene in the pathway. These genes have identified all the major conserved signaling components of the pathway known. Given the successes of these screens, several additional mutants are being examined, which have led to new insights into TGFβ signaling.

From our genetic screen, we have focused recently on one locus, sma-10. It encodes a transmembrane protein that consists of leucine and immunoglobulin repeats, with a short cytoplasmic tail (19 aa), and is highly conserved with the vertebrate LRIGs. However, LRIGs from other species often contain a non-conserved cytoplasmic tail. Genetic epistasis experiments place sma-10 between the ligand and the receptor, supporting the molecular data suggesting it is a transmembrane protein. It is required for signaling in the body size pathway, but male tails of these mutants are normal, suggesting that sma-10 may be a tissue-specific factor. sma-10 is highly conserved in Drosophila, and in vertebrates, further supporting an essential role in TGFβ signaling. Members of this family of proteins are not only related by sequence but are also functionally conserved as we showed the Drosophila homolog, lambik, rescues the small body size mutation in C. elegans.

Given that SMA-10 is a transmembrane protein, we reasoned it could physically interact with either the ligand or the receptors to facilitate signaling. Using biochemical-binding experiments, we have shown that SMA-10 physically interacts with the TGFβ ligand and the receptors after they are internalized. This places sma-10 in a unique class of important regulators and studies are underway to determine how SMA-10 affects trafficking of the TGFβ receptors. As a foundation for further SMA-10 studies, we examined the normal trafficking of TGFβ receptors in the nematode. We find that the two TGFβ receptors, type I and type II, traffic differently from each other. The type I receptor, SMA-6, is recycled through the retromer, which is a novel and unexpected finding. SMA-6 physically interacts directly with the receptor, supporting the molecular data suggesting it is a transmembrane protein. It is required for signaling in the body size pathway, but male tails of these mutants are normal, suggesting that sma-10 may be a tissue-specific factor. sma-10 is highly conserved in Drosophila, and in vertebrates, further supporting an essential role in TGFβ signaling. Members of this family of proteins are not only related by sequence but are also functionally conserved as we showed the Drosophila homolog, lambik, rescues the small body size mutation in C. elegans.
Defects in TGFβ receptors affect subcellular localization of the receptors in Marfan-like syndromes

In an expansion of our endocytosis work, we have begun to look at naturally-occurring mutations in TGFβ receptors of patients. Interestingly, we observed that these mutations are located near important motifs involved with receptor trafficking. These mutations contribute to various cancers or to Marfan-like syndromes, depending on the particular patient. The hypothesis we formulated was whether some of these diseases are due to improper trafficking of the TGFβ receptors. If true it would challenge some of the common paradigms of these diseases. To test this idea, we introduced several of these mutations into the C. elegans type I and type II receptors and find that the subcellular localization of the receptor is altered, supporting our hypothesis. Further, we find that these mutated receptors still function, but are mis-trafficked, often leading to a stronger signal. This data sheds light on the possible molecular defects in TGFβ signaling that contribute to Marfan-like syndromes. This work has just been published.

Closing remarks

During the last year, I have shut down my research lab, with my last graduate student finishing in the spring of 2019. Simply put, it was a fantastic scientific journey for me for almost 30 years. I have had the privilege of having a superb group of students and postdocs in the lab over the years, who had gone on to successful careers. In addition, I have enjoyed the incredible colleagues that Rutgers University has attracted over the years. When I first interviewed, a plan for tremendous future growth in the research enterprise was presented to me. While seemingly risky, much of that plan became reality and the Waksman Institute and other departments on campus have helped propel Rutgers University into the upper ranks of US universities. It’s been fun to have been part of those activities and I know there is still a lot of growth to come.

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RONGO LAB
Synapse Formation, Hypoxic Stress, and the Central Nervous System

Summary

Our nervous system is the primary organ by which we sense, interpret, remember, and respond to the outside world and to our own internal physiology. This elaborate system of neurons functions as a communication network, with vast arrays of chemical and electrical synapses between individual neuronal cells (Fig. 1). The nervous system also interfaces with other tissues of the body, either directly (e.g., neuromuscular junctions at skeletal muscles) or indirectly (e.g., the release of hormones, biogenic amine neurotransmitters, and neuropeptides into the blood stream), to regulate physiology and behavior, as well as maintain overall body homeostasis. Unlike many bodily tissues, the nervous system is largely incapable of replacing damaged cells once development is complete, making it susceptible to traumatic injury and age-associated decline. The high energy demands of electrochemical signaling, combined with the inability to store energy in the form of glycogen reserves, makes neurons highly dependent on oxygen, oxidative phosphorylation, and mitochondria. The nervous system has evolved multiple mechanisms to maximize mitochondrial function and prevent damage from acute oxygen starvation. Indeed, the underlying etiology of many neurological disorders and diseases, including ischemic stroke, Parkinson’s Disease, and Alzheimer’s Disease, are due to defects in one or more of these key neurophysiological processes. A more complete understanding of these processes will facilitate better diagnosis and treatment of multiple neurological disorders.

We focus on understanding three areas of neurophysiology (Fig. 1). First, we are interested in understanding how the transport and dynamics of mitochondria are mediated along axons and dendrites, as well as at synapses. Second, we are interested in understanding how neurons, synapses, and neuronal mitochondria respond to hypoxic stress (e.g., ischemic stroke). Finally, we are interested in understanding the function of the Ubiquitin Proteasome System (UPS) and its role in cellular aging, including the function of the UPS in neurons, as well as how neurons can regulate the UPS and proteostasis in distal tissues.

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

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

Environment can impact nervous system function, and neurons can respond to accommodate a changing environment. Specifically, oxygen influences behavior in many organisms, and low oxygen levels (hypoxia) can have devastating consequences for neuron survival due to excitotoxicity from overactivated neurotransmitter receptors and impaired mitochondrial function. In multicellular organisms, cells respond to hypoxia through the Hypoxia Response Pathway (Fig. 2). Normal levels of oxygen are sensed by a prolyl hydroxylase (PHD) enzyme, which uses that oxygen to covalently modify key proline residues on the transcription factor HIF alpha. This modification results in the ubiquitination and degradation of HIF alpha. Under hypoxia, PHD enzymes are inactive, resulting in the stabilization of HIF alpha. HIF alpha dimerizes with HIF beta, enters the nucleus, and regulates gene expression so as to minimize the impact of hypoxia on underlying development and physiology.

We have shown that hypoxia blocks the membrane recycling of glutamate-gated ion channels to synapses, thereby blocking the response of neurons to low oxygen levels (hypoxia and anoxia). Environment can impact nervous system function, and neurons can respond to accommodate a changing environment. Specifically, oxygen influences behavior in many organisms, and low oxygen levels (hypoxia) can have devastating consequences for neuron survival due to excitotoxicity from overactivated neurotransmitter receptors and impaired mitochondrial function. In multicellular organisms, cells respond to hypoxia through the Hypoxia Response Pathway (Fig. 2). Normal levels of oxygen are sensed by a prolyl hydroxylase (PHD) enzyme, which uses that oxygen to covalently modify key proline residues on the transcription factor HIF alpha. This modification results in the ubiquitination and degradation of HIF alpha. Under hypoxia, PHD enzymes are inactive, resulting in the stabilization of HIF alpha. HIF alpha dimerizes with HIF beta, enters the nucleus, and regulates gene expression so as to minimize the impact of hypoxia on underlying development and physiology.

We have shown that hypoxia blocks the membrane recycling of glutamate-gated ion channels to synapses, thereby depressing glutamatergic signaling. Surprisingly, C. elegans HIF alpha, encoded by the hif-1 gene, does not mediate this effect. Instead, a specific isoform of the prolyl hydroxylase (encoded by the egl-9 gene in C. elegans) recruits LIN-10, a known PDZ scaffolding protein, to endosomes, where together the two proteins promote glutamate receptor recycling. This is a novel way by which animals can sense and respond behaviorally to oxygen levels, and it suggests that the protective mechanisms are more diverse than originally appreciated.

A complete understanding of the hypoxia response pathway (i.e., EGL-9 and HIF-1) is important for understanding ischemic stroke. In addition, this pathway has become a target of interest for new chemotherapeutics, as HIF-1 is activated and plays a key role in cancer progression and metastasis. Therefore, we have broadened our studies of this pathway, and we are now conducting RNA-seq and ChIP-seq experiments to identify both HIF-1-dependent and HIF-1-independent targets of hypoxia-induced gene regulation. We have also identified over 400 unique metabolites that are regulated by this pathway and correlate with the changes in gene expression.

Regulators Of Mitochondrial Transport and Dynamics In Neurons.

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

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

We also generated a C. elegans transgenics strain that expresses MitoKeima, new reporter for mitochondria undergoing mitophagy. MitoKeima has a differential, pH-dependent fluorescence excitation spectra that allows one to discriminate healthy mitochondria in the neutral pH of the cytosol from damaged mitochondria in the low pH environments of autophagosomes, autolysosomes, and lysosomes (Fig. 3). Mitophagy can be triggered by mitochondrial stress or even by starvation (Fig. 4). Using this and other mitochondrial reporters, we are now examining how mitochondrial dysfunction contributes to a tau-based genetic model of Alzheimer’s Disease.

Figure 2. The Hypoxia Response Pathway Senses Low Oxygen And Mediates Compensatory Responses

Figure 3. Detecting Mitophagy Using pH-Dependent Changes in Excitation Spectrum of Fluorescent Protein MitoKeima. As a quality control measure, damaged mitochondria undergo fission to generate smaller mitochondria. These mitochondria contain mitophagy receptors that recruit autophagy factors, resulting in the nucleation of a phagophore. Phagophores then enclose the mitochondria, resulting in autolysosomes, where the mitochondria are eventually digested and removed. To differentiate healthy mitochondria from mitochondria undergoing mitophagy, we employed a transgenic reporter called MitoKeima. MitoKeima emits 620 nm wavelength light. However, it is differentially excited depending on the pH. Inside healthy mitochondria, where the pH is around 7.6, MitoKeima is excited by 440 nm light. Inside mitochondria in autolysosomes, where the pH is around 5.4, MitoKeima is excited by 586 nm light. Using different filter sets, we can use this differential excitation to observe these two kinds of mitochondria separately.
Dopamine Signaling Activates The UPS In Distal Epithelial Tissues.

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

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

SINGSON LAB
Reproductive Biology, Cell-Cell Interactions

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

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

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

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

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

We continue to identify and characterize genes required for sperm function at fertilization taking advantage of the most up to date molecular tools. We have recently identified candidates for the spe-9 class genes spe-13, spe-36, spe-45, and spe-51 with next generation whole genome sequencing. SPE-45 is a single pass transmembrane molecule with a single immunoglobulin domain (IG) that has a conserved function from worms to humans. SPE-36 and SPE-51 appear to be the first secreted sperm molecules required for fertilization. SPE-51 has features that suggest it could be a long sought after sperm-egg fusogen.

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 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.
lar systems. We have recently proposed the concept of a fertilization synapse. This framework takes into account the molecular and cellular complexity required for reproductive success (See Figure 1).

Sperm activation

Post meiotic sperm differentiation (spermiogenesis) is required for a haploid spermatozoon to build cellular structures required for motility and interactions with the egg. We recently cloned two new genes (spe-24/zipt-7.1 and spe-43) that are required for C. elegans spermiogenesis. The spe-24/zipt-7.1 encoded protein is a zinc transporter and demonstrates zinc as an important second messenger for sperm activation in vivo. The spe-43 gene is a novel transmembrane protein that is alternately spliced. Further characterization of this gene will help us better understand how sperm become competent to move towards and fertilize the egg.

Egg functions

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

The oocyte-to-embryo transition

The last class of mutants that we study defines genes required in the egg to trigger development after fertilization. The egg-3, egg-4 and egg-5 genes encode inactive protein tyrosine phosphatases or “antiphosphatase” required for egg activation after sperm entry. Recently, through forward genetic screens, we have identified at temperature sensitive allele of both genes leads to complete sterility and the production of oocytes that can never be fertilized by wild-type sperm. Screening strategy that will help us identify additional components of the egg-3 pathway.

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

Recent Reproductive Span

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

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Stem cells, Hematopoiesis, Oogenesis, and epitranscriptomic modification of mRNA in Drosophila

Summary

Zfrp8, a new gene functioning in hematopoietic and ovarian stem cells in Drosophila, controls the assembly of specific ribonucleolar complexes.

Drosophila hematopoiesis represents an excellent model for blood cell development in humans since the signaling pathways regulating fly blood development involve orthologs of proteins functioning in vertebrate hematopoiesis.

We have identified Zfrp8 (PDCD2) in vertebrates) from its grossly enlarged lymph gland (site of hematopoiesis in flies) phenotype. We have established that Zfrp8 is essential in both hematopoietic and ovary stem cells, as mutant stem cells stop dividing and are ultimately lost. The Drosophila and human proteins are 38% identical and expression of human PDCD2 in flies rescues the Zfrp8 mutant phenotype, underlining the structural and functional conservation of the proteins.

Because of the essential function of Zfrp8 in fly hematopoiesis we collaborate with Dr. Arnold Rabson who has created a conditional PDCD2 knockout(KO)mouse. He and his laboratory have confirmed that the gene is essential in mouse embryonic stem cells and in mouse embryos at the blastocyst stage, before implantation. They have shown that PDCD2 KO mouse embryo fibroblasts fail to grow and ultimately die similar to the phenotype observed in Drosophila ovaries.

Zfrp8 forms a complex with FMRI and Tet, a methylcytosine dioxygenase

In yeast two-hybrid screens using Zfrp8 or PDCD2 as baits we identified NUFI (Nuclear fragile X mental retardation-interacting protein) and we have been able to show that Zfrp8 forms a complex with NUFI and FMRI (Fragile-X Mental Retardation Protein). The Fmr1 (Fragile-X Mental Retardation 1) gene is essential in humans and Drosophila for the maintenance of neural stem cells and Fmr1 loss results in neurological and reproductive developmental defects in humans and in flies.

We also identified Tet as a direct interactor of Zfrp8/PDCD2 in flies and human cells. Tet encodes a methylcytosine dioxygenase that transforms 5 methyl cytosine(5mC) into 5 hydroxy-methylcytosine (5hmC). There are 3 TET proteins in vertebrates. They have well-documented functions in the maintenance of vertebrate stem cells and are associated with neuronal problems in mice. Despite their emerging central roles in stem cells and gene regulation in mammals, Tet has not been characterized in flies.

Fly Tet encodes two distinct proteins that are similar in organization to the vertebrate proteins. Both contain the enzyme’s catalytic domain, but only the larger protein contains also the conserved DNA binding domain. Both Tet null is 100% pupal lethal.

In flies 5mC and consequently 5hmC is absent in DNA. Previously, in collaboration with Dr. Fukai’s laboratory at the Free University of Brussels, we have mapped 5hmC transcriptome-wide in S2 Drosophila tissue culture cells and could show that Tet modifies specific transcripts. Our hypothesis is that Tet, a DNA binding protein, may become localized to actively transcribed sites on the DNA and that it then controls the modification of the nascent RNAs. We have performed Chip-Seq experiments on DNA isolated from 0-12 h embryos, and identified 771 protein binding peaks distributed on 654 genes. ~ 40% of the peaks map to promoter sites and the majority of these Tet peaks co-localize with chromatin modification marks associated with the transcription start site of actively transcribed genes in embryonic stages.

Recently we have mapped 5hmC transcriptome-wide in mRNA from 0-12 hour embryos, and normal and Tet-knockout larval heads (containing mainly brain tissue). In larval heads we identified 3750 peaks in mRNAs encoded by 1825 genes and in embryos we obtained 1815 peaks on RNAs encoded by 1404 genes, almost 40% of which overlap with the tar-
gets observed in larvae. We are now in the process of comparing these results with those obtained from mutant larval heads.

In parallel we have induced a CRISPR mutant in which one of the completely conserved Cs in the DNA binding domain of Tet is replaced by an A (C598A). Homozygotes for this Tet\(^{AXXC}\) allele are semi-lethal and show reduction in climbing velocity. In brains of surviving Tet\(^{AXXC}\) flies, the mushroom bodies, that control behavior, are affected (Fig. 1).

Using our fly line that expresses GFP-tagged Tet protein under the endogenous promoter we have identified a Tet complex by immunoprecipitation. In single and double knock-down experiments we are systematically testing mutants affecting these proteins for a common function with Tet and also for potential genetic interaction. The screen is designed so that we can test genes that either function together with Tet in RNA processing, or targets, that are regulated by Tet.

Previously we reported that 5hmC modified RNAs are found in ribosome fractions, suggesting a correlation between 5hmC abundance and active mRNA translation. We thus explored whether the 5hmC modification has a direct effect on translation, by examining ribosome occupancy across the transcriptome through sequencing of ribosome protected fragments by ribosome profiling (Ribo-seq) analysis. By comparing Ribo-seq results in wild type and Tet\(^{null}\) brain preparations we found that a significant number of transcripts show diminished levels of ribosome occupancy in homozygous Tet\(^{null}\) mutants. Furthermore, in brains of surviving TetAXXC flies, the mushroom bodies, that control behavior, are affected (Fig. 1).

Figure legend: Loss of mushroom body α lobe(s) in Tet\(^{AXXC}\) 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\(^{AXXC}\) brains.

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

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 antibacterial agents and broad-spectrum antibacterial agents.

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

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

We are using x-ray crystallography to determine high-resolution structures of transcription initiation complexes, fluorescence resonance energy transfer (FRET) to define distances between pairs of site-specifically incorporated fluorescent probes, photo-crosslinking to define polypeptides near site-specifically incorporated photo-crosslinking 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 photo-crosslinkers at specific sites within large multisubunit nucleoprotein complexes, and we are developing automated docking algorithms to integrate structural, biophysical, biochemical, and genetic data in order to construct models for structures of complexes.

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

1. RNA polymerase (RNAP) binds to promoter DNA, to yield an RNAP-promoter closed complex.
2. RNAP unwinds ~14 base pairs of promoter DNA surrounding the transcription start site, rendering accessible the genetic information in the template strand of DNA, and yielding an RNAP-promoter open complex.
3. RNAP begins synthesis of RNA as an RNAP-promoter initial transcribing complex. During initial transcription, RNAP uses a “stepping” mechanism, in which RNAP translocates relative to DNA in transcription elongation; by stepping in transcription elongation); (2) binding of the incoming nucleotide; (3) formation of initial transcription; by stepping in transcription elongation; (2) binding of the incoming nucleotide; (3) formation of transcription initiation factor \(\sigma\). The bacterial transcription initiation complex contains six polypeptides (five in RNAP, one in \(\sigma\) and promoter DNA, and has a molecular mass of 0.5 MDa.

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

During transcription elongation, RNAP uses a “stepping” mechanism, in which RNAP translocates relative to DNA in each nucleotide-addition step. Each nucleotide-addition cycle during initial transcription and transcription elongation can be subdivided into four sub-steps: (1) translocation of the RNAP active center relative to DNA (by scrunching in initial transcription; by stepping in transcription elongation); (2) binding of the incoming nucleotide; (3) formation of
We are using FRET and photocrosslinking methods to define distances and contacts within trapped intermediates in transcription elongation complexes without incoming nucleotides and for transcription elongation complexes with incoming nucleotides. Based on these crystal structures, we have shown that each nucleotide addition cycle is coupled to an RNAP active-center conformational change, 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 photocrosslinked complex. According to this proposal, the closing and opening of the RNAP active center is mediated by the folding and the unfolding of an RNAP active-center structural element, the "trigger loop.

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

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

**Regulation of Transcription: Regulation of Transcription Initiation**

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

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

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

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

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

Recently we have extended our studies of transcriptional regulation to encompass regulation at the level of transcription antipauing 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 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 DNA 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, paused deficient, termination-deficient TEC.

Q proteins from different lambdoid bacteriophages comprise three different protein families (the Ql 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 analogous (with identical functions but unrelated structures and origins), rather than homologous (with identical, interchangeable functions and related structures and origins).

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

We are systematically determining high resolution single-particle cryo-EM structures of Qlambda-, Q21-, and Q82-dependent transcription antitermination complexes. Results for Q21 reveal that Q21 forms a torus—a "nozzle"—that extends and narrows the RNA-exit channel of RNA polymerase, that the nascent RNA is threaded through the Q nozzle, and that the threading of the nascent RNA through the Q nozzle precludes the formation of pause and terminator RNA hairpins.

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

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

**Inhibitors of Transcription: Antibacterial Drug Discovery**

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

The rifamycins are examples of antibacterial agents that target bacterial RNA polymerase. The rifamycins are of particular importance in treatment of tuberculosis; the rifamycins are first-line antibiotics against tuberculosis and are among the only antibiotics 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 target sites within bacterial RNA polymerase 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 pathogens.
We seek to address the following objectives: to develop new classes of antituberculosis agents and broad-spectrum antibacterial agents, to develop antibacterial agents effective against pathogens resistant to current antibiotics, to develop antibacterial agents effective against pathogens of high relevance to public health, and to develop antibacterial agents effective against pathogens of high relevance to biodefense.

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NICKELS LAB
Regulation of Gene Expression in Bacteria

Summary
Proper control of gene expression is essential for organismal development, cellular response to environmental signals, and the prevention of disease states. Transcription is the first step in gene expression and thus is highly regulated. Transcription in all cells is performed by multi-subunit RNA polymerases (RNAPs) that are conserved in sequence, structure and function from bacteria to humans. Our lab utilizes a range of approaches including molecular biology, genetics, biochemistry and high-throughput screening to obtain a detailed understanding of the mechanism and regulation of transcription. To facilitate our studies, we use bacterial RNAP as a model for understanding gene expression paradigms in all organisms. Transcriptomes are dynamic and responsive to alterations in environmental conditions or growth state. According to the classical model, transcription is regulated primarily through the action of DNA-binding proteins that activate or repress transcription initiation, with a few long-studied exceptions. However, it is now abundantly apparent that cells employ a highly diverse range of mechanisms to control gene expression during all three phases of transcription: initiation, elongation and termination. An overarching goal of our studies is to understand the diversity of regulatory mechanisms that link changes to cellular state to changes in RNAP activity.

Mechanism and impact of gene expression control by “nanoRNAs”.

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

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

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

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

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

During each phase of transcription, RNAP makes extensive interactions with nucleic acids and is responsive to sequence context. In addition, as each phase of transcription is a multi-step process, different steps during initiation, elongation, and termination can be rate limiting for different transcripts, and thereby serve as potential targets for regulation. Thus, predicting how a given transcription unit (i.e. promoter and transcribed region) will respond to alterations in conditions, and identifying the sequence determinants that dictate the response, represents an immense challenge. While structural studies have revealed some RNAP-nucleic acid interactions that modulate transcription, a full understanding of the relationship between nucleic acid sequence and functional output remains a fundamental gap in our knowledge. Thus, my lab seeks to leverage the capabilities of high-throughput sequencing technologies to address this knowledge gap. In this regard, we have developed experimental platforms for massively multiplexed transcriptomics, massively multiplexed protein-DNA crosslinking, and massively multiplexed DNA footprinting (termed “MASTER,” “MASTER-XL,” and “MASTER-FP,” where “MASTER” denotes massively systematic transcript gnd readout, “XL” denotes crosslinking, and “FP” denotes footprinting).

MASTER, MASTER-XL, and MASTER-FP first involve the construction of a template library that contains up to at least 4⁴ (~1,000,000) barcoded sequences. For MASTER experiments RNA transcripts are produced from the template library in vitro or in vivo and analyzed by high-throughput sequencing to determine the sequence of transcript ends and the relative transcript yields. For MASTER-XL experiments unnatural amino acid-mediated protein-DNA photo-cross-linking (or other forms of protein-DNA cross-linking) is performed and high-throughput sequencing is used to define the location of specific regions of RNAP on each member of the template library. For MASTER-FP experiments chemical reagents that detect changes in DNA accessibility (e.g. KMnO₄, chloroacetaldehyde, DMS, hydroxyl radicals) are used to perform protein-DNA footprinting and high-throughput sequencing is used to detect RNAP-dependent changes in DNA accessibility on each member of the template library.

In published work, we have used MASTER and MASTER-XL to define the sequence determinants and mechanism of transcription start site selection for E. coli RNAP. In current work, we are using MASTER, MASTER-XL, and MASTER-FP to analyze transcription elongation and termination for bacterial RNAP and to define the sequence determinants and mechanisms of transcription start site selection in eukaryotes. In principle, these approaches can be readily adapted to perform a comprehensive mechanistic dissection of any process involving nucleic acid interactions. Thus, although our current studies are focused on transcription, the technical innovations derived from our studies are likely to have wide-ranging applications across many areas of biology.

Defining the role of endoribonuclease toxins in bacterial pathogenesis.

We are interested in applying our high-throughput sequencing-based methods for analysis of transcription to other areas of RNA biology. In this regard we have developed a method, termed MORE RNA-seq (where MORE denotes mapping by overexpression of an RNase in Escherichia coli), to define the cleavage consensus sequences of endoribonuclease toxins from the bacterial pathogen Mycobacterium tuberculosis.

Toxin/antitoxin (TA) systems are widespread in pathogens and have been implicated in virulence, survival during stress, and in promoting formation of a dormant state that is refractory to antibiotic treatment. In M. tuberculosis, there are greater than 80 TA systems. Furthermore, the majority of the toxins associated with these TA systems are homologues of sequence-specific endoribonucleasees. Therefore, to understand the physiological role of these M. tuberculosis toxins, there is a need to define the cleavage specificity of each toxin. In current work performed in collaboration with Nancy Woychik (Rutgers) we are using MORE RNA-seq as a tool to determine the cleavage recognition sequences of the endoribonuclease toxins in M. tuberculosis, which, in turn, will provide a critical first step towards identification of the targets and physiological roles of these toxins.
Summary
Our laboratory studies bacteria, their interactions with mobile genetic elements such as phages, plasmids and transposons, and with each other. The following research projects were actively pursued during the last year.

Studies of CRISPR-Cas bacterial adaptive immunity
CRISPR-Cas (Clusters of Regularly Interspersed Palindromic Repeats/CRISPR associated sequences) loci provide bacteria with adaptive immunity to phages and plasmids. We concentrate on CRISPR-Cas systems from *Escherichia coli*, *Thermus thermophilus*, and human pathogen *Clostridium difficile*. To study the fundamental aspects of CRISPR-Cas function, evolution, and ecology, we developed highly efficient experimental model systems to study CRISPR-Cas interference with mobile genetic elements have been created in our laboratory. They are complemented by efficient systems to study CRISPR adaptation – the process of acquisition of new spacers from foreign DNA that next provide immunity to subsequent infections. Recent work, carried out in collaboration with Bryce Nickels laboratory, uncovers mechanisms that couple the interference and adaptation arms of CRISPR response and allow bacteria to rapidly adapt to viruses that escape CRISPR immunity.

Powerful *in vitro* methods, including fluorescent beacon assays inspired by our work with RNA polymerases, are being used to determine how genomic editor Cas9 programmed with various RNA guides differentiates between target and non-target DNA. This research helps avoid off-target activity of genomic editors that limits their practical use.

New CRISPR-Cas systems are being identified through bioinformatics searches in collaboration with Eugene Koonin laboratory from NIH and are validated experimentally. Some of these systems have superior properties compared to the widely used Cas9 and may find use in genome editing applications.

We use the diversity of CRISPR spacers to monitor local adaptation of bacterial populations to viruses. By comparing repertoires of spacers in geographically separated bacterial communities we find evidence of global transfer of bacteria around the world. Similar approaches are used for epidemiological monitoring of strains of *C. difficile*, an important nosocomial pathogen.

Structure-activity analyses of peptide antibiotics
Ribosomally-synthesized post-translationally modified peptides (RIPPs) form a broad and diverse class of molecules with highly unusual structures and potentially useful properties, such as antibiotic activity. We use powerful bioinformatics pipelines to predict new RIPPs. We next determine their structures, characterize the enzymes involved in their synthesis, and determine the modes of their antibacterial action. Structure-activity analysis of new RIPPs leads to development of molecules that are not found in nature but possess superior properties and may be used to treat bacterial infections. Current work concentrates on three distinct classes of RIPPs: peptide-nucleotides related to microcin C, oxazole-thiazole peptides related to microcin B, and lasso-peptides related to microcin J. Significant development of the past year were the establishment, in collaboration with Professor Tony Maxwell from John Innes Center, UK, of the three-dimensional structure of molecular machine that installs oxazole and thiazole modifications in microcin B and discovery of a new class of oxazole-thiazole peptides that inhibit bacterial ribosome (a collaboration with Yury Polikanov from UIC and Jamie Cate from UC Berkeley).

Structure-functional analysis of novel transcription enzymes
Through genome mining we identified several bacteriophage encoded RNA polymerases very distantly related to cellular RNA polymerases. The structure of one phage enzyme, multisubunit RNA polymerase encoded by a giant *B. subtilis* AR9 phage, has been determined to high resolution by combining X-ray crystallography and cryo electron microscopy methods (a collaboration with Pett Leiman from UTMB). Another non-canonical RNA polymerase we are now studying is encoded by a csAa phage, the most abundant virus of human gut.

SEVERINOV LAB
Mechanisms of Transcription in Microorganisms

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Transcriptional Regulation in Yeast

Dr. Andrew Vershon
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Summary

Gene expression profiling experiments have revealed the presence of a large number of non-coding RNAs (ncRNAs) in a wide range of organisms. It is now clear that many ncRNAs also have important roles in gene regulation. For example, RNAi-mediated regulation controls gene expression in *C. elegans*, *Arabidopsis*, humans, and many other organisms. However, there also appear to be a large number of ncRNAs that are not involved in RNAi-mediated regulation. For example, there are over 900 ncRNAs expressed in the yeast *Saccharomyces cerevisiae*. However, yeast lacks the enzymes that are required for RNAi, and therefore, it must utilize different mechanisms for ncRNA-mediated regulation. Our laboratory is investigating the role and mechanisms of gene regulation by non-coding RNAs in yeast.

The yeast alpha2 and a1 proteins, members of the homeodomain family of DNA-binding proteins, bind in combination to specific DNA sites to repress haploid-specific genes in the diploid cell type. To determine which genes are regulated by the a1/alpha2 repressor complex, we searched for potential target sites of the complex in the yeast genome. In addition to finding binding sites in the promoter regions of genes that are repressed by the a1/alpha2 complex, we identified binding sites downstream of two genes that require a1/alpha2 for expression in diploid cells. We have shown that these sites regulate transcription of haploid-specific, antisense ncRNAs that interfere with expression of their respective genes. Repression by these antisense transcripts only works in a cis configuration to the genes that they are regulating, suggesting that that mechanism of repression is not through the formation of double stranded RNA. Chromatin immunoprecipitation (ChIP) assays showed that the repression of the sense transcripts does not interfere with transcription factors binding to the promoter. We have identified regions within the open reading frame of both genes that are required for antisense-mediated repression. Inversion of this region in one of the genes causes a loss of repression, indicating that there is a context dependent orientation of this element that is required for repression. Our results suggest that these genes are being repressed through a novel mechanism.

There are over 900 known non-coding transcripts in yeast, and it is likely that some of these non-coding transcripts have a role in gene regulation. To identify other cases of this form of regulation, we have used SOLID RNA deep sequencing technology to examine strand-specific expression in different yeast cell types and growth conditions. In preliminary examination of this data we have found over 100 examples of genes that show differential expression of the antisense transcripts under different conditions. We are currently testing if these protein-coding genes are regulated by their respective antisense transcripts. We have also identified several genes that appear to be regulated by the expression of overlapping ncRNAs in the sense direction. These RNAs appear to prevent transcription factors and RNA polymerase from binding to the promoters of the protein coding genes. These sense ncRNAs are therefore repressing transcription through a different *cis*-acting mechanism than the antisense transcripts.

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DISMUKES LAB

Biological and Chemical Approaches to Renewable Energy Research

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

Summary

The Dismukes research group conducts fundamental and applied research in the areas of renewable energy production via biological and chemical approaches. Our strategy is to apply the principles of enzymatic catalysis and metabolic regulation to design biomimetic catalysts, reaction networks and microorganisms exhibiting improved performance that operate using electrical or solar energy power sources. Our laboratories are located in the Waksman Institute of Microbiology and the Wright-Rieman Chemical Laboratory at Rutgers University. In the 2018-July 2019 period the group was comprised of 39 researchers (listed below).

Research Projects:

1) Diversity of Photosynthetic Water Oxidizing Enzymes.

This research investigates “photosynthetic outliers” from the field or culture collections, seeking non-classical metabolisms. We have characterized the earliest branching cyanobacterium on the tree of life (1), the fastest growing “Usain Bolt” of photosynthesis (2), metabolisms of hypercarbonate and hypersaline tolerant strains (3), and created transgenic strains to test numerous metabolic hypotheses (4). The resulting advances in knowledge: molecular basis of light energy conversion to biomass components, non-classical metabolic pathways for biosynthesis, and novel reaction-centered based photoprotection mechanism in algae called PSII-cyclic electron flow. To achieve this, we built new instruments/methods for detection of dissolved O2, H2, Chl, NAD(P)H, pH, and intracellular metabolite fluxes by LC-tandem-MS (5). Supported by DOE-BES. Collaborations: Arizona SU, TU Delft.

2) Photoautotrophic Carbon Fluxomics.

Metabolic pathways for model organisms can be found in textbooks. However, these are widely modified across the tree of life and novel pathways for making carbon products abound in nature. Our goal is to use flux balance analysis and isotopically nonstationary metabolic flux analysis (INST-MFA, figure) to quantitatively understand carbon flux distributions and pathway used by the cyanobacterium *Synechococcus* sp. PCC 7002 and other phototrophs during photosynthesis. This enables discovery of new roles for existing metabolic pathways and completely new pathways not previously known. NSF-MCB, invited Moore Foundation. Collaboration TU Delft.
3) Optimizing Photosynthetic Light Conversion by Protein Subunit Engineering.

From the two differentially expressed cyanobacterial isoforms of the D1-PSII reaction enter subunit, we learned that nature designs the PSII reaction center to operate better at high light intensity by having faster charge recombination, or for optimal operation at low light intensity by having slower charge recombination. In this project, we applied this principle of cyanobacterial PSII functioning to the higher plant *Nicotiana tabacum* and demonstrated that greater biomass yield is achievable by genetic engineering of its D1-PSII to incorporate the cyanobacterial phenotype for high light tolerance (graphic 2). Supported by DOE-BES and GCEP.

4) Photo-Assembly & Inorganic Mutants of Photosystem II and Water Oxidizing Complex.

This project aims to understand the steps in biogenesis of the oxygenic reaction center (PSII) and the chemical functions of the inorganic components comprising its catalytic site (WOC). We do so by substitution of the native inorganic cofactors (Mn$^{2+}$, Ca$^{2+}$, Cl$^{-}$, CO$_3$H$^{-}$, H$_2$O) and examination of the consequences using multiple novel tools designed by our lab staff. Our biogenesis work has examined the assembly of the WOC during the greening (etiolation) process in barley (collaboration with Umea U.) and future work on arabidopsis thaliana (collaboration Ruhr U.) Supported by DOE-BES.

5) Bioinspired Electrocatalysts for Water Splitting and CO$_2$ Reduction.

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 (Photosystem II) and CO$_2$, conversion (acetogens and methanogens) to chemicals and food. Bioinformatics and data science tools are used to determine the chemically relevant attributes for catalysis by the CO$_2$ reducing enzymes. The synthesized bioinspired catalysts are made from earth abundant elements, exceed or equal the activity and stability of the best commercial catalysts used today, and use renewable feedstocks like water and CO$_2$. The image depicts our electrolytic process for converting water and CO$_2$ into C$_1$ to C$_5$ products selectively. Supported by the DOE-EERE-HydroGEN, NASA, NREL, Rutgers Goldman Prize and Rutgers TechAdvance. Collaborations: UPenn, CU Boulder, NREL.


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 chemical products of 11%. Support NSF-CBET, DOE-EERE-HydroGEN. Collaborator: NREL.

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SUMMARY
Cell polarity, in both animals and plants, is of paramount importance for many developmental and physiological processes. The establishment and maintenance of cell polarity is required for asymmetric cell division (ACD), an indispensable mechanism 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 also have the capability to polarize non-transmembrane proteins and utilize such polarized protein distribution to regulate asymmetric cell division (Figure 1).

By using BASL as an anchor for genetic and physical interactor screens, and by using 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.

Figure 1. BASL localization and stomatal asymmetric cell fate

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

BASL scaffolds BPP phosphatases to regulate stomatal asymmetric cell division
Our previous work showed that BASL is phosphorylated and activated by MAPK 3 and 6 (MPK3/6) and becomes polarized to the cell cortex, where it recruits the MAPKK Kinase YODA and MPK3/6 to inhibit stomatal differentiation in one of the two daughter cells. Recent work showed that, prior to a stomatal ACD, the polarity complex employs POLAR to recruit the GSK3-like kinase BIN2 that releases the suppression of YODA on stomatal differentiation, therefore stomatal ACD is promoted. Therefore, the stomatal polarity complex by scaffolding different signaling molecules could promote the division potential before an ACD and suppress the division potential after an ACD. However, how the transition of these two seemingly opposing procedures can be achieved by the same polarity complex remained a major challenge towards understanding stomatal ACD. Here, by using immunoprecipitation combined with mass spectrometry (IP-MS), we identify a family of protein Ser/Thr phosphatases, BPPs (BASL phosphatase partners), as BSAL-interacting proteins. Genetic analysis places BPPs upstream of the YODA MAP kinase cascade and downstream of the plasma membrane receptors. In addition, the founding member BPP-1 colocalizes with BASL in a polarized manner at the plasma periphery. Interestingly, the recruitment of the BPP phosphatases in the polarity module confers a negative role to BIN2 complex but a positive role to the YODA MAPK module. Thus, our study reveals a crucial function of the BPP phosphatases in bridging the two opposing protein functional modules to control the balance of cell division potential and cell fate determination in plant ACDs.

Protein Phosphatase 2A promotes stomatal development by stabilizing SPEECHLESS in Arabidopsis
Stomatal guard cells control gas exchange that allows plant photosynthesis but limits water loss from plants to the environment. In Arabidopsis, stomatal development is mainly controlled by a signaling pathway comprised of peptide ligands, membrane receptors, a mitogen-activated protein kinase (MAPK) cascade, and a set of transcriptional factors. The initiation of the stomatal lineage requires the activity of the bHLH transcriptional factor SPEECHLESS (SPCH) with its partners. Multiple kinases were found to regulate SPCH protein stability and function through phosphorylation, yet no antagonistic protein phosphatase activities have been identified. Here, we establish the conserved PP2A phosphatases as positive regulators of Arabidopsis stomatal development. We show that mutations in genes encoding PP2A subunits result in lowered stomatal production in Arabidopsis. Genetic analyses place the PP2A function downstream of the MAPK cascade, but upstream of SPCH. Pharmacological treatments support a role for PP2A in promoting SPCH protein stability. We further show that SPCH directly binds to the PP2A-A subunits in vitro. In plants, non-phosphorylatable SPCH proteins are less affected by PP2A activity levels. Thus, our research identifies PP2A as the missing phosphatases that function antagonistically with the known kinases to maintain the phosphorylation balance of the master regulator SPCH in stomatal development.

Our lab will continue to use Arabidopsis as a model system, by studying a few newly identified factors, to investigate how proteins become polarly localized, how polarity proteins are involved in establishment of cellular asymmetry, and how cell polarity is instructive of cell fate and differentiation in plants. New regulators participating in the regulation of stomatal production

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Spontaneously arisen mutations provide the raw material for natural selection and evolution in all organisms. Yet, spontaneous mutations in maize pollen are frequent in some lines and arise mainly from retrotranspositions by mobile endogenous plant retrotransposons, which are related to mammalian retroviruses. Most interestingly, the frequency of occurrence is so low that special “mutation accumulation” lines are used to study them. In the course of conventional genetic experiments, we found that spontaneous mutations in corn genes arise relatively frequently in the pollen of some, but not all, lines. Pollen grains represent the male gametes in corn plants; in contrast, the female gametes on the ears carried no detectable gene mutations. We estimated the mutation frequency to be several per gene per million pollen grains. Since an average corn plant produces around 10 million pollen grains, this means that, in some corn lines, a single plant will produce in one season mutations in every gene of the genome. In some cases, these mutations can be caused by mutagenic retrotransposons differed from line to line. Our findings sheds light on the source, frequency, and nature of spontaneous mutations in maize. They also may help to explain the genetic instability that has been associated with some corn lines. It will be of interest to determine whether useful genetic variation can be generated by activating spontaneous mutations in maize. They also may help to explain the genetic instability that has been associated with some corn lines. It will be of interest to determine whether useful genetic variation can be generated by activating spontaneous mutations in maize.
We identified a large number of TFs that contain specific repressor motifs that allow the interaction with REL2-type corepressors and repress the transcription of their target genes. According to the repressor motif embedded in their sequences, these transcriptional regulators interact with REL2 using distinct mechanisms. We are currently characterizing a series of pathways regulating spikelet and flower development that require REL2-mediated repression by a combination of genomic, genetic and molecular approaches. One of this pathway is involved in the domestication of maize ears from its wild progenitor teosinte.

Identification of cis-regulatory modules in plant genomes

Transcription factors 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 alternative to ChIP-seq, to map TF binding events. We observe that many TFs often bind within close proximity to one another forming putative cis-regulatory modules (CRMs; also commonly referred to as enhancers). 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. This approach is providing 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.

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MALIGA LAB
Plastid Molecular Genetics

Introduction

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

Plastid transformation in Arabidopsis thaliana

Plastid transformation has been inefficient in Arabidopsis thaliana due to a natural tolerance of Arabidopsis to spectinomycin, the selective agent employed to enrich transformed plastid genomes. Tolerance to spectinomycin has been linked to a duplication of the ACCase biosynthetic pathway in chloroplasts. We have shown that plastid transformation is 100-fold more efficient in Arabidopsis lines defective in the plastid-targeted ACC2 nuclear gene (Yu et al. Plant Physiol. 175: 186-193, 2017). This information has been obtained in the the Col-0 ecotype that is recalcitrant to plant regeneration. We now report ACC2 defective lines in the RLD andWs ecotypes, which readily regenerate plants from cultured cells. ACC2 knockout lines were obtained using CRISPR/Cas9 genome editing tools. The spectinomycin hypersensitive phenotype is characterized by the lack of shoot apex when germinated on a selective medium. This phenotype has been confirmed in both accessions, indicating that deletion of the ACC2 gene is generally applicable to obtain spectinomycin hypersensitive plants in all species in which duplication of the ACCase pathway has been conserved. Testing of transformation competence in the knockout lines was carried out with new, Arabidopsis-specific plastid vectors that are suitable for the delivery of reporter genes. The vectors target insertions in the trnV-rps12/7 intergenic region, and carry a highly-expressed green fluorescent protein (GFP) gene. Plastid transformation efficiency could be evaluated in six weeks post-bombardment due to high-level expression of GFP. The efficiency of plastid transformation was as high in the RLD and Ws knockout lines as in the Col-0 and Sav-0 ecotypes, about one transplastomic event in two bombarded samples, confirming that spectinomycin tolerance has been the cause of inefficient plastid transformation in the past. The experiments were carried out in collaboration with Prof. Kerry A. Lute, Farmingdale State College, Farmingdale, NY.

Post-transcriptional regulation tunes protein output of chloroplast transgenes

Predictable protein output is required in synthetic biology applications to achieve expression of genes from polycistronic mRNAs in a desired stoichiometry. Because the operon is transcribed from a single promoter, establishing stoichiometry can be accomplished only by post-transcriptional regulation. We report here on expression tools that enable the choice of protein output in tobacco chloroplasts from the same mRNA over a 60-fold range, comprising 0.4%-25% of total soluble protein (TSP) (Figure 1). The system is based on having the PPR10 protein binding site upstream of the ORF and protecting the mRNA from exonucleolytic degradation. Expression of the green fluorescent protein (GFP) from a wild-type binding site yields protein levels at about 25% or 15% TSP, depending on whether the site derives from the tobacco or maize atpF gene. A mutant binding site upstream of the ORF reduces GFP levels to about 2% TSP. Incorporation of a URNA leads to further reduction of GFP to about 0.4% TSP due to rapid degradation of the mRNA from the processed end. The experiments reported here provide important insights into the mechanisms of PPR10-mRNA interaction in vivo and provide a new versatile tool for plastid synthetic biology. This research was supported by a joint grant with Prof. Alice Barkan, Oregon State University, Eugene, OR.
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Figure 1. GFP accumulation in tobacco chloroplasts visualized by illumination with UV light. Shown are tobacco plants expressing GFP from the maize and tobacco wild-type PPR10 binding sites (atpH Zm and atpH Nt, respectively) and the mutant maize GG and AA binding sites.

### Introduction

The Messing lab works in plant genetics and genomics. Publications during the last academic year reported in several categories including gene cloning by Shotgun DNA Sequencing, Bioenergy, Protein Quality, RNAi, Genome Evolution, and Epigenetics.

### Gene cloning by shotgun DNA sequencing

I invented shotgun DNA sequencing about 45 years ago and it has been used to sequence many genomes, the first one in 1981 (CAMV). Significant advances in computation and robotics since then have contributed to drastic decreases in the cost of sequencing even large or the number of genomes. Given the feasibility to manage large amounts of data, has prompted us to revisit a classical collection of maize mutants, the Neuffer-Sheridan collection, that interfere with normal seed development and therefore are called defective kernel (dek) mutants.

These mutants were induced with EMS and should produce single nucleotide polymorphisms (SNPs). To find SNPs in a large genome like maize (2.3 GB), we wanted to employ shotgun DNA sequencing as opposed to selected sequencing of mapped intervals. To reduce the complexity of sequence information, we used either exome or transcriptome sequencing. Because the mutations were generated in a mixed background, they were first backcrossed to two maize inbreds that had been sequenced so that they could be used as allelic references. A computational pipeline was therefore developed that would use the sequencing results of bulked segregants of specific dek mutants to generate lists with ranked scores of sequences linked to the phenotype. Because one could compare two independent introgressions and Neuffer and Sheridan’s chromosomal assignments of individual mutants from genetic analysis, the pipeline was successful in identifying single candidate genes in the maize genome. Furthermore, other mutant alleles of the same gene could be used to validate the accuracy of the pipeline.

### Bioenergy

One of the plant genome sequencing efforts that we have participated in is the sorghum genome, which is a close relative of maize. Whereas maize has undergone allotetraploidization from two progenitors about 4.8 mya, they share a common progenitor with sorghum that split 11.9 mya. Sorghum is therefore diploid and has retained a number of useful traits like better drought tolerance and disease resistance. It is planted on marginal land with tropical climate. Another interesting feature is a variety known as sweet sorghum (Rio) in contrast to grain sorghum (Btx406). Sweet sorghum like sugarcane, also a close relative of maize and sorghum, accumulates higher levels of soluble sugars in its stem. However, sugarcane has a more complex genome and given the sorghum diversity, sorghum is amenable to a genetic/genomic approach to dissect carbon allocation during photosynthesis.

One factor that contributes to the sugar content in the stem is flowering time. When flowering is delayed, then sugar destined to seeds has to be stored in the stem. Therefore, a sorghum conversion line (R9188) was compared with the sweet and grain sorghum parents. Although the conversion line has also high stem sugar levels, it flowers earlier. Furthermore, the introgressed regions from Rio could be mapped based on transcriptome analysis. The different sorghum genotypes could then be used to sample tissues from different internodes to determine the metabolome at different stages of growth. Although Rio has the highest level of sucrose concentration, the conversion line has medium levels, where Btx406 is significant lower. Moreover, lower levels of sucrose correlate with a decrease in sugar phosphates for precursors of starch and cell wall biosynthesis, illustrating a switch in carbon allocation in a genotype-dependent manner. Analysis of introgressed chromosomal regions are enriched for genes regulating trehalose 6-phosphate phosphatase (TPP), which appears to be a branchpoint in carbon allocation. Therefore, regulators of TPP could provide an important entry point to optimize Biofuel properties in plants.
To map these regulators and clone them, a high-throughput and cheap scoring system of high sugar phenotypes was needed to implement a genetic analysis of a segregating population of grain and sweet sorghum. Therefore, the assay that was used was adapted to microtiter plate format that could be processed on a robotic pipetting system.

**Protein quality, RNAi, and genome evolution**

Crop seeds are a source for three major food components, oil, starch, and protein. Maize is mainly a source for starch and protein. The latter is fortified with soybeans and synthetic methionine to supplement essential amino acids in animal feed. We have therefore investigated how accumulation of starch and protein is regulated. This time, we took a different approach. Due to polyploidy in crops like maize, one could argue that gene duplications render conventional methods for detecting seed phenotypes as described above ineffective. Genes regulating both starch biosynthesis and protein storage could be redundant so that a knockout of one copy does not produce a phenotype. In 2003, we demonstrated how RNA interference (RNAi) can be used as a dominant genetic marker in maize. Therefore, we searched the maize seed transcriptome database of duplicated transcription factors. Out of 112 predicted transcription factors belonging to the NAC superfamily, two of them were highly expressed during early seed development, labeled as NAC128 and NAC130 that arose in homoeologous chromosomal position from the allotetraploidization event described above.

Because the coding sequences of NAC128 and NAC130 are so conserved, it was easy to generate an RNAi transgenic event targeted to transcripts from both genes at the same time in a dominant fashion. Indeed, a strong kernel phenotype of reduced seed size segregated in the expected ratio. Subsequent analysis showed a reduction in both starch and protein. Transcriptome analysis of mutant seeds provided us with putative transcriptionally activated genes in both starch biosynthesis and protein accumulation. These results were employed to identify target promoters including the binding site for the two transcription factors. In fact, a common sequence motif was detected then in several genes that appeared to be regulated by these NAC-type transcription factors. Interestingly, a CRISPR knockout of a single copy had no phenotype, confirming the genetic redundancy of this regulatory system. Moreover, rice a more distant relative of maize and sorghum, showed a syntenic alignment to one copy with a segmental duplication comprising a second one, overcoming the lack of polyploidy in rice.

**Epigenetics**

Another source of Biofuel could be duckweeds, which are aquatic plants. They float on ponds and low-streaming waters and are an exceptional example for natural adaptation. They can double in biomass in as short as two days by clonal reproduction, mainly driven by runoffs of overfertilized lawns. Previously, we sequenced the genome of the duckweed *Spirodela polyrhiza*, which is the smallest so far among monocotyledonous plants. A special feature of the rapid growth is the suppression of the juvenile to adult transition phase, known as neoteny.

To understand the different regulatory circuits between land and aquatic plants and the adaptation of duckweeds to their changing environments, we investigated aspects of post-transcriptional regulation of gene expression by analyzing their repertoire of small RNAs and their targets in the presence of different environmental stimuli. As a result, we could determine mRNA turnover of specific functions and establish a new catalog of miRNAs and their targets. In particular, miR156 and miR172 expression reflected the neotenous growth of *Spirodela*. An interesting observation was the low level of 24nt siRNA, which are involved in the epigenetic regulation of repeat sequences of land plants. This class of small RNAs are critical in silencing retrotransposition during meiosis, which usually does not take place in duckweeds due to their clonal propagation.

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CORE FACILITIES

Cell and Cell Products Fermentation Facility

OVERVIEW

The Cell and Cell Products Fermentation Facility (CCPFF) is a nonprofit state of the art facility designed to help the Waksman Institute researchers, university faculty as well as other scientific/research/biotechnology industry develop new fermentation technologies and provide bench top and pilot scale fermentation projects for research and biologies 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. 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 diafiltration 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 2018-2019

Over the past year, the scientists at the fermentation facility have completed projects for 1-2 small biotech start-ups, 2 suppliers in the biotech, > 5 pharmaceutical and cosmetic industries as well as a couple of university and university affiliates. For this fiscal year alone, the total production output is approximately 30,000 liters of microbe cultures of E. coli, P. pastoris, Streptomyces spp. and various strains of yeast and fungus. The revenue generated from these work have provided valuable support to the research goals of the Institute as a whole and will continue to do so in the years to come. Moreover, as part of the CCPFF’s contribution to the Institute’s scholarship mission, Dr. Arvin Lagda conducted a seminar on the basics of Fermentation and Industrial Microbiology at the Rutgers Dept. of Microbiology and Biochemistry last Spring 2019 attended by students and faculties. This year also marks as the first year for Dr. Arvin Lagda (full-time) and Mr. Joseph Troyanovich (part-time) as member of the team and the Waksman Institute of Microbiology family.

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

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Waksman Genomics Core Facility

Waksman Genomics Core Facility (WGCF) provides high-throughput next generation sequencing services to the Rutgers research community and to the broader scientific community. Waksman institute is one of the earliest adopters of sequencing technology with extensive experience in NGS sequencing. Since 2008, core offers single read, paired-end, and multiplex sequencing using various Next Generation Sequencers. WGCF harbors three sequencers covering broad range of NGS requirements. One of the main driving force behind having multiple instruments is to provide latest sequencing service across the entire spectrum ranging from high throughput sequencing to small sequencing need. The facility is staffed with three full-time professionals to assist with the library preparation, conducting of sequencing and analysis of data.

The Single Molecule Real-Time (SMRT) sequencer, the Sequel System uses Single Molecule Real-Time (SMRT) technology to produce long reads, uniform coverage, and high consensus accuracy. The Sequel long 10-15kb reads will greatly enhance whole genome, full-length transcript, or long amplicons sequencing projects. Additionally, its SMRT sequencing technology can also be used to directly detect DNA base modification. NextSeq500, Illumina’s desktop sequencing instrument that provides roughly 120 Gb data from its 2x150 bp configuration. NextSeq 500’s push-button operation provides a thirty-hour turnaround time for an array of popular sequencing applications such as single human genome, 20 transcriptome or up to 16 exomes in a single run. Whereas, MiSeq with relatively long read-length and low throughput, is best suitable for small genome sequencing and targeted sequencing. Accordingly, the WGCF expanded its library preparation and sequencing services to extensively cover Illumina platform.

With these instruments, WGCF is capable of producing 150 Gb of data per day from over a billion reads. Data from DNA sequencing services are typically reported to customers within two weeks. Funding for the operation of the core facility comes from a combination of user fees and cost sharing from Waksman Institute keeping the instrument use cost low. This creates an exceptional opportunity for a researcher to perform experiments with state-of-the art instruments. User fees are used to cover part of instrument service contracts cost and consumable reagents expense. WGCF accepts raw samples as well as prepared libraries, giving researcher’s flexibility to try their own protocols. In addition to sequencing, WGCF also offers Real-Time PCR on Thermo Fisher’s StepOnePlus system, DNA shearing services using Covaris, as well as DNA qualification services using fluorometer Qubit, NanoDrop and Agilent bioanalyzer.

In Addition to the wet-lab services, Waksman Genomics Core Facility also provides powerful, accessible and intuitive analysis tools through its bioinformatics consulting and data analysis services. This allows researcher to bypass learning curve and focus on the result that are both statistically and biologically meaningful. WGCF collaborate with researchers in designing experiments and analyzing complex data sets by applying various data-analytical and theoretical methods. The facility successfully developed several comprehensive pipelines, which enable researchers to process large-scale data. Facility expanding suite of software applications includes DNA mapping against reference genomes, de-novo assembly, ChIP-seq and RNA-seq data analysis.

Our mission is to keep the core facility as comprehensive and accessible as possible in order to increase research productivity. The equipment and services provided by the genomics core are aimed for reducing the wait time and providing the degree of expertise necessary for an individual user to design and execute genomics experiments. WGCF is used by a diverse group of researchers within as well as outside Waksman. WGCF complete sequencing business has been roughly 60% DNA, 30% RNA, 5% ChIP, and 5% other. Our projects include bacteria, plants, as well as mammals, reflecting the diversity in research at the Waksman Institute.

Common Applications and Platforms at Waksman Genomics Core

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Waksman Confocal Imaging Facility

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

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

The Waksman Confocal Imaging Core Facility has approximately 60 trained users, primarily Waksman researchers, from fifteen laboratories and is used an average of 68 hours per week. The Confocal Manager provides training, troubleshooting, and consultation on the use of our confocal microscopes. The future aim of the facility is to continue to provide exceptional imaging capabilities to Waksman researchers.

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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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SUPPORT SERVICES

TRAINING FUTURE LEADERS
Successful fertilization requires the sperm and egg to recognize and bind to each other in order to fuse and form a zygote. Little is known about the molecular basis of fertilization. In mammals, Izumo, an immunoglobulin superfamily protein on the sperm, and Juno, a GPI-anchored protein on the egg is the only known receptor pair that is necessary for fertilization (Bianchi, 2014). In C. elegans, the spe-9 class of genes are required for sperm function during interactions with the oocytes. All the spe-9 class genes identified to date encode transmembrane proteins on the sperm surface. The non-redundant roles of these molecules suggest that they function with one another to form a complex. Thus, we have proposed the fertilization synapse model where complex protein-protein interactions at between the gamete surfaces form during fertilization, similar to what is at the neuronal or immune synapse (Krauchunas, 2016). Over the last year, I have characterized a new member of the spe-9 class, spe-51. spe-51 mutant hermaphrodites and males are sterile due to a defect in the sperm. Mutant spermatids can activate in vitro in pronase. After mating, these mutant sperm can migrate to the spermatheca, and can out-compete hermaphrodite self-sperm. These data suggest that spe-51 is not required for sperm activation but for sperm function. Protein-domain prediction suggests that the protein contains an immunoglobulin (Ig)-like fold, and a possible transmembrane domain. Surprisingly, ectopically expressed SPE-51 in the body-wall muscle was taken up by coelomocytes, suggesting the protein is secreted rather than membrane-bound. Thus, SPE-51 is a secreted molecule that functions at the fertilization synapse. Ongoing work focuses on interactions of SPE-51 with other proteins on the sperm membrane as well as localization of SPE-51.

In C. elegans, EGG-1 and EGG-2 are the only known egg surface proteins that are required for fertilization and they don’t bind with known sperm surface proteins, suggesting that other molecules are involved. Using a reverse genetic approach, I have been testing candidate genes that may function during fertilization or oocyte-to-embryo transition. I filtered all the oocyte-enriched genes by the presence of predicted transmembrane domains or GPI-anchors. Of these, I sorted out paralogous pairs with potentially redundant functions which would otherwise have been neglected in forward genetic screens. Further literature search then generated a short list of paired genes. I then test the functions of these genes by single or double RNAi. One pair of genes, gfat-1 and gfat-2, which encode enzymes that are required for synthesis of egg shell components, have shown a lack of oocyte-to-embryo transition when knocked down. It remains unclear how gfat-1 and gfat-2 regulate oocyte-to-embryo transition through eggshell synthesis. I will further test their functions by investigating localizations of key regulators during egg activation.

Research summary

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Research Summary
Identification and Characterization of Genes that Regulate Mitochondrial Dynamics and Transport in Caenorhabditis elegans

Mitochondria are essential organelles for all eukaryotic cells. They participate in processes as diverse as the generation of cellular energy, sugar and fatty acid breakdown, steroid and lipid synthesis, calcium homeostasis, and programmed cell death. Given their critical and diverse roles in cellular physiology, mitochondrial dysfunction contributes to the etiology of multiple diseases including many neurodegenerative diseases. This association occurs because neurons in particular have high-energy demands but do not store glycolytic reserves and instead rely on mitochondrial oxidative phosphorylation to meet these demands. Additionally, mitochondria can contribute to degeneration by producing reactive oxygen species that can lead to both cell damage and apoptosis.

As highly dynamic organelles, mitochondria exist in states of fusion and fission and move within cells via the cytoskeletal network; such dynamics are critical for mitochondrial function. While significant advances in studying mitochondrial dynamics have been made using single celled organisms and cell lines, attention is now shifting to understanding mitochondria in multicellular organisms in vivo. In addition, mitochondria interact with various motor and adaptor proteins for mitochondrial transport within the cell. In neurons for example, this is particularly important for meeting the energy needs of distal synapses in neurons. The role of mitochondrial dynamics and transport in multicellular development, cell-cell signaling, disease, stress, and aging remains an important topic of research.

Understanding the molecular and cellular mechanisms underlying mitochondrial dynamics and transport should help us further understand diseases that are characterized by mitochondrial dysfunction. To this end, we conducted a forward genetic screen using the model organism Caenorhabditis elegans to find genes that modulate or mediate mitochondrial dynamics and transport in neurons. To date, we have identified ten mutants displaying either elongated mitochondria (fission mutants) or displaying defects in transporting mitochondria out of the cell body and into the axons (transport mutants).

With the help of conventional mapping strategies, candidate gene screens, and Whole Genome Sequencing, we have identified potential causative genes for two of our mutants that show mitochondrial transport defects. Additionally, we’ve found new alleles of a conserved gene that regulates mitochondrial fission. We are in the process of further validating and confirming these candidate genes, as well as understanding the role it plays in mitochondrial transport in neurons and the interactions with other known genes.

Hiep Tran
Steward Lab

Research Summary
Hydroxymethylating RNA and function during Drosophila brain development

Chemically modified ribonucleotides in rRNA, including mRNA, have been known for decades. Recently, the mapping these modifications by next generation sequencing as well as the discovery of enzymes that deposit (“writer”), eliminate (“eraser”), and bind (“reader”) to the modifications revealed the biological functions of the modifications. We have previously shown that Drosophila Tet is responsible for hydroxymethylating ribocytosine (5hmC) in mRNA and that 5hmC is enriched in Drosophila brain mRNAs. To further study the modification, I am addressing two questions. When and how does Tet modify mRNA? And what is the functional requirement of the modification during brain development?

Tet protein has two domains: a zinc finger CXXC-type DNA-binding domain and a 2OG-Fe(II) dioxygenase domain. We hypothesize that Tet binds to DNA through its CXXC domain and co-transcriptionally modifies nascent mRNA by its dioxygenase domain. By a gene replacement method using CRISPR/Cas9 and homologous directed repair (HDR), I have generated mutant lines in which the absolutely conserved C598 in the DNA-binding domain was changed to A (TetAXXC line), and a second line, in which H1886 to Y and D1888 to A mutations in the dioxygenase domain were induced (TetYRA line). TetAXXC shows defects in the development of the mushroom body, the Drosophila brain structure essential for learning and memory while, the TetYRA exhibits severe locomotion defects, indicating that the two protein forms may have specific functions. I am now testing if these mutations affect the 5hmC level in brain mRNAs. The lab has identified Tet-target mRNAs by mapping 5hmC modifications transcriptome wide. I am verifying the requirement of these target mRNAs during brain development. My future direction will be to identify Tet cofactors which are required both for DNA binding and for setting the 5hmC in brain mRNAs.

Nathaly M. Salazar-Vasquez
Rongo Lab

CHARLES AND JOHANNA BUSCH FELLOWS
Predoctoral Research
Research Summary

Transcription is the first step of gene expression and a primary target of regulation in all cells. During transcription initiation, RNA polymerase (RNAP) recognizes and binds to specific DNA sequences (promoters) initially as double-stranded DNA, unwinds ~13 bp to form an “RNAP-promoter open complex,” selects a transcription start site, and synthesizes an RNA of ~11 nt before releasing promoter contacts and forming a transcription elongation complex (promoter escape). Despite decades of intense effort, most of the structural rearrangements that occur between the initial binding of RNAP to promoter DNA and promoter escape have not been defined. Due to the limitations of current structural methods, nearly all of the available structural information for transcription initiation complexes comes from results obtained in vitro on only a few promoter sequences. Thus, our understanding of the mechanism of transcription initiation and the relationship between promoter sequence and RNAP-promoter complex formation remains incomplete.

To overcome limitations of current methods for studies of structural changes that occur during transcription initiation I am developing methods for systematic, comprehensive unnatural-amino-acid mediated protein-DNA photo-crosslinking in vivo, in E. coli. Using these methods, I will define the protein-DNA interactions that occur during transcription initiation in vivo for E. coli RNAP on a diverse library of promoter sequences. Specifically, I will determine how variations in promoter sequence affect the nature of the structural remodeling that occurs during transcription initiation and I will determine whether alterations in RNAP-promoter structure provide a mechanistic explanation for why ppGpp activates certain promoters and inhibits others.

Over the past year I have designed and built a library to incorporate the photoactivatable unnatural amino acid p-benzoyl-L-phenylalanine (Bpa) into the RNAP β, β', and σ subunits, I have generated a library containing every E. coli promoter sequence, and I have demonstrated that we can map crosslinks from several Bpa positions to a single promoter in vivo. I am now poised to combine the Bpa-containing libraries with promoter sequence libraries to comprehensively map the in vivo structural changes that occur on natural E. coli promoters in the presence and absence of the transcription regulator ppGpp.

In principle, the methods developed here for studies of transcription will be applicable to the study of other protein-DNA complexes that form in vivo, including complexes that form in DNA replication, DNA repair, chromosome organization, and chromosome remodeling.
Research Summary

**NEEDLE1 (NDL1) ENCODES A MITOCHONDRIA LOCALIZED PROTEASE REQUIRED FOR THERMOTOLERANCE OF MAIZE GROWTH**

Maize (Zea mays L.) is one of the most important commercial crops in the world as well as an important model organism for basic research in plant biology. The shoot architecture of maize is primarily determined by apical and axillary meristems. Plant axillary meristems (AMs) are groups of stem cells initiated at the axils of leaves during post-embryonic development. AMs are ultimately responsible for the production of branches, lateral organs and stems, thus they directly affect crop yield.

The previous identification of several mutants showing defects in inflorescence development has shed light on the essential role of the plant hormone auxin in regulating maize AM initiation. Here we characterized and cloned a new mutant regulating maize inflorescence architecture named *needle1* (*ndl1*). *ndl1* is a temperature sensitive mutant with variable phenotypic expressivity, showing several defects in development, the most notable of which is the formation of tassels with reduced number of branches and spikelets. Interestingly, *ndl1* mutants showed strong genetic interactions with several auxin-related mutants, as well as a lower concentration of auxin in inflorescence meristems. By positional cloning and transgenic complementation, I demonstrated that *NDL1* encodes a mitochondrial metalloprotease belonging to the FTSH (FILAMENTOUS TEMPERATURE-SENSITIVE) protease family. Further analysis indicates that together with the hyperaccumulation of reactive oxygen species (ROS), *ndl1* mutant inflorescences show the up-regulation of many genes involved in stress responses and mitochondrial retrograde regulation (MRR). These findings uncovered an essential pathway that coordinates meristem redox status with the hormonal control of reproductive organogenesis, and regulates overall maize growth.

**Stomata are epidermal pores required for plant gas exchange and transpiration. The development of *Arabidopsis* stomata involves a process precisely controlled by asymmetric cell division (ACD). The plant-specific protein BASL (BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE) was identified as an intrinsic polarity factor that regulates stomatal ACD. When tagged with GFP, BASL initially appeared in the nucleus and later polarized at the cell cortex. However, the polarization mechanism of BASL, a peripheral membrane protein, remained largely unknown. In our lab, previous studies on protein dynamics suggested BASL may reach to its destination through the endomembrane system and this process may rely on its physical partners. By yeast two-hybrid screening and co-IP mass-spectrometry, the BIP2 (BASL INTERACTING PROTEIN FAMILY 2) family was isolated as BASL’s physical partner. I confirmed the physical interaction between BASL and BIP2 members by pair-wise yeast two-hybrid and Bimolecular fluorescence complementation (BiFC) in tobacco epidermal cells. By performing CRISPR/Cas9 mutagenesis, I created high order *bip2* mutants and found that simultaneously knocking out four family members led to stomatal ACD defects and the loss of BASL polarization. By examining the subcellular localization of the BIP2 proteins in *Arabidopsis thaliana* and *Nicotiana benthamiana* leaf epidermal cells, I found that individual BIP2 protein may preferentially occupy differential endomembrane compartments but also overlap on certain organelle populations. In addition, protein domain prediction indicated a conserved phospholipid binding domain existing in all of the BIP2 members. My results showed that deletion of this lipid binding domain interrupted the typical endomembrane localization of one of the BIP2 members. Overall, my findings provided experimental evidence supporting BIP2s as novel plant membrane trafficking regulators that coordinate plant cell polarity and phospholipid signaling in stomatal ACD.**
**Research Summary**

Cell division happens when the chromosomes are bi-oriented and segregated by a bipolar spindle. In somatic cells and most of the cell types, a bipolar spindle is assembled through the centrosome where it plays a major role as a microtubule organizing center. Interestingly, oocytes do not follow this conventional pathway for spindle assembly. Oocytes assemble bipolar spindles in the absence of centrosomes, the chromosomes in oocytes direct the process instead. When oocytes enter prometaphase, the microtubules cluster around the chromosomes and sort into a bipolar spindle after nuclear envelope breakdown. The chromosomal passenger complex (CPC) is shown to be important in regulating spindle formation and orchestrating chromosome bi-orientation in oocytes; however, it remains unclear about the detailed mechanisms and how the chromosomes are involved. Here, we used the model organism, *Drosophila melanogaster*, to address this fundamental question. We found that heterochromatin protein 1 (HP1) has an essential role in assisting the CPC to promote both spindle assembly and homologous chromosome bi-orientation in *Drosophila* oocytes. The CPC is comprised of two targeting proteins, Survivin and Borealin, a scaffold protein, INCENP, and Aurora Kinase. The interaction between HP1 and Borealin is crucial for the initial recruitment of the CPC to the chromosomes. This initial recruitment is sufficient to build kinetochores and form the kinetochore microtubules. We also found that HP1 relocates onto the spindle similar to the CPC. Additionally, we have shown that the CPC regulates homologous chromosome bi-orientation through the central spindle, the central overlapped region on the spindle, rather than the centromeres. A functional central spindle relies both on CPC localization to the spindle and the HP1-INCENP interaction. Together, we demonstrate that, in oocytes, HP1 plays an important role in regulating the CPC chromosome localization and its central spindle function to further affect both spindle assembly and homolog bi-orientation.

**BENEDICT MICHAEL FELLOW**

**Predoctoral Research**

Research Summary

Non-green plastids are desirable for the expression of recombinant proteins in edible plant parts to enhance the nutritional value of tubers or fruits, or to deliver pharmaceuticals. However, plastid transgenes are expressed at extremely low levels in the amyloplasts of storage organs such as tubers. Here, we report a regulatory system comprising a variant of the maize RNA-binding protein PPR10 and a cognate binding site upstream of a plastid transgene that encodes green fluorescent protein (GFP). The binding site is not recognized by the resident potato PPR10 protein, restricting GFP protein accumulation to low levels in leaves. When the PPR10 variant is expressed from the tuber-specific patatin promoter, GFP accumulates up to 1.3% of the total soluble protein, a 60-fold increase compared with previous studies (0.02%). This regulatory system enables an increase in transgene expression in non-photosynthetic plastids without interfering with chloroplast gene expression in leaves.
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 candidates 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 2018-2019

Advanced Inorganic Chemistry
Advanced Plant Genetics
Biophysical Chemistry
Core Seminars in Plant Biology
Developmental Genetics
Fundamentals of Molecular Biosciences
Genetic Analysis II
Honors Introduction to Molecular Biology and Biochemistry Research
Human Genetics
Introduction to Molecular Biology and Biochemistry Research
Introduction to Research in Genetics and Molecular Biology
Microbial Biochemistry
Microbiology
Molecular Biology and Biochemistry
Molecular Biology and Biochemistry Honors Thesis Seminar
Molecular Biology and Biochemistry Research and Writing
Molecular Biology of Gene Regulation & Development
Molecular Biosciences
Plant Molecular Biology
Thesis Writing and Communication in Genetics

WAKSMAN STUDENT SCHOLARS PROGRAM

High School Outreach

Summary

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

For 26 years, faculty and scientists at the Waksman Institute have collaborated with high school teachers and their school administrators in an effort to address these issues. Our strategy has been to engage high school students and their teachers in authentic scientific research, in an effort to bridge the gap between how scientific research is conducted versus how science is taught.

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

The 2018-2019 WSSP consisted of two interrelated parts: a Summer Institute (SI) and an Academic Year Program (AYP). In July 2018, 32 students and 9 teachers from 30 high schools attended a 12-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 plant ecology 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.

After the SI, teachers and students returned to their schools and recruited additional students who contributed to the research project during the academic year. Some teachers incorporated the project into existing research courses or advanced placement biology courses at their schools. Others conducted the project as after school clubs. These courses and clubs provided additional students beyond those who attended the SI with opportunities to conduct and contribute to the research project. The Biology Equipment Lending Library (BELL), which was previously established with generous support from the Toshiba America Foundation and the Johnson & Johnson Foundation, enabled the WSSP to loan expensive equipment to the schools so that many students could conduct the research project during the AYP.

Seven after school workshops were conducted at the Waksman Institute during the school year. In these sessions, students presented their results from their research efforts and discussed the problems they encountered while working on the project at their schools. These sessions were also used to review the background material, to update all the participants in changes in laboratory protocols and share new findings in molecular biology.

Near the end of the academic year, each school team presented their research findings at a poster session held on June 3, 2019 at the Rutgers University campus, Piscataway, NJ to which scientists, school administrators, and parents were
invited. Each poster was carefully reviewed by scientists from Rutgers, each student team received feedback on their poster, and certificates were awarded to all contributing students and teachers.

In addition to the activities based at the Waksman Institute, the WSSP also supported the program at sites beyond the central New Jersey region. Dr. Forrest Spencer at John Hopkins University, in Baltimore MD helped run the AYP for 4 high schools in Maryland. A two-week summer Institute for 4 teachers and 16 students was conducted at the Lawrence Livermore National Laboratory, Livermore, CA, and 4 teachers and 40 students conducted investigations during a summer session in Waipahu, HI. As a result of these extended outreach activities and the growth in the number of schools participating locally in New Jersey, a total of 1520 students from 54 different high schools in NJ, MD, PA, CA and HI participated in, and contributed to, the WSSP this past year. This is an increase in student participation in the program of almost 25% over the previous year.

The Research Question

The 2018-2019 research project focused on identifying the genes and proteins of the duckweed, *Landoltia punctata*. Duckweeds are fresh water aquatic plants that is used in bioremediation and can be potentially used for biofuel. Several research laboratories at Rutgers are currently investigating these plants.

To conduct the project, a plasmid cDNA library from *Landoltia punctata* was prepared by the WSSP staff. During the summer and continuing throughout the academic year, the students grew bacterial cultures of individual clones from the cDNA library, performed minipreps of the plasmid DNA, cut the DNA with restriction endonucleases, performed polymerase chain reactions, analyzed DNA electrophoretically, had inserts from their clones sequenced, and analyzed these sequences with the aid of a computer program called the DNA Sequence Analysis Program (DSAP) that was developed by the project director and faculty. From the 2018-2019 SI and AYP, over 2600 plasmid clones were purified and 2052 were sequenced. To date, 1604 DNA sequences have been analyzed by the students. 890 DNA sequences have been or will be submitted for publication on the National Center for Biotechnology Information (NCBI) DNA sequence database citing the students’ names as contributing authors.

WISE

The WSSP project involves both students and teachers conducting research at their high schools during the academic year. Since involvement in the program requires the participation of the teacher and support of the schools, many students from schools that are not involved in the program cannot conduct the research project. To accommodate these students, we offered two 10-day summer programs called Waksman Institute Summer Experience (WISE) in which students perform the same research project as conducted in the WSSP. In June and August 2018, 34 and 36 students, respectively, participated in WISE and each of the students successfully purified and analyzed a novel DNA sequence and published their findings on the NCBI database. Due to the success of WISE and the demand from students wanting to participate in the program we plan to offer two WISE summer institutes during the 2019 summer.

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**Dr. Janet Mead, Laboratory Director**

John Brick, Laboratory Assistant

April Rickle, Undergraduate Work Study Student

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**SHARING OUR DISCOVERIES**

**Waksman Annual Retreat**

**Presentations & Meeting Abstracts**

**Patents & Publications**
**Presentations**

- Zhenru Zhou - Irvine lab: Oriented cell divisions are not required for Drosophila wing shape
- Chao Bian - Dong lab: Novel functions of PP2A phosphatases in Arabidopsis stomatal development
- Xue Mei - Singson lab: Izumo-like immunoglobulin superfamily proteins in fertilization from worms to humans
- Qiguo Yu - Maliga lab: Construction of Chloroplast Transformation-Competent Arabidopsis Plant Lines Using CRISPR-Cas9
- Kyle Skalenko - Nickels lab: Sequence determinants of nanoRNA-mediated priming of transcription initiation
- Paul Fourounjian - Messing lab: Expression and activity survey of miRNAs in greater duckweed, Spirodea polyrhiza
- Richard Ebright - Ebright lab: Antibacterial nucleoside-analog inhibitor of bacterial RNA polymerase; pseudouridimycin
- Mehul Vora - Rongo lab: Identifying transcriptional targets of the hypoxia response pathway
- Qiuju Liu - Gallavotti lab: NEEDLE1 (NDL1) is a mitochondria localized protease required for thermotolerance of maize growth
- Badri Nath Singh - Steward lab: Function of Tet in Drosophila neural development by RNA-hydroxymethylation
- Dibyendu Kumar - Genomics Core Facility: Waksman Genomics Core: 10 years and beyond
- Lin-Ing Wang - McKim lab: Regulation of Chromosome-directed spindle assembly in oocytes
- Yuan Zhang - Dismukes lab: Metabolic engineering of Nannochloropsis Oceanica CCMP1779 for biofuel production
- Konstantin Severinov - Severinov lab: Detection of in vivo intermediates of CRISPR adaptations

**POSTER SESSION**

- All authors listed on individual posters if not included below

**Barr Lab**
- Kumar Tiger, Juan Wang, Maureen Barr: Determining the Function of the Globin Gene glb-28 in Sensory Neurons

**Dismukes Lab**
- Tie Shen and G. Charles Dismukes: Cyanobacterial pyruvate ferredoxin oxidoreductase regulates photosynthetic growth rate: implications for increasing carbon fixation in photosynthetic organisms
- Apostolos Zouras and G. Charles Dismukes: Applications of Fast Repetition Rate Fluorometry (FRRF) in photosynthesis research

**Dong Lab**
- Xiaoyu, Gu, and Juan Dong: Cortical polarity segregates opposing functions of the BSU phosphatases in stomatal development.
- Dongmeng Li and Juan Dong: Coordinated functional regulation of small GTPases in establishing plant cell polarity
- Lu Wang and Juan Dong: The regulation of ARF-GEF by protein phosphorylation and phospholipid signaling in membrane trafficking

**Gallavotti Lab**
- Xue Liu, Mary Galli, Iris Camehl and Andrea Gallavotti: REL2-mediated transcriptional repression regulates vegetative and reproductive architecture in maize
- Weibin Song, Qiuju Liu and Andrea Gallavotti: Comprehensive reverse genetic analysis of the maize AUXIN RESPONSE FACTOR gene family

**Genomics Core Facility**
- Min Tu, Yaping Feng and Dibyendu Kumar: Accessory Equipment

**Irvine Lab**
- Jyoti Misra, Irvine lab: Early girls is a novel component of the Fat signaling pathway

**Maliga Lab**
- Qiguo Yu and Pal Maliga: PPR10 RNA-Binding Protein for Regulated Gene Expression in Potato Amyloplasts.
- Aki Matsuoka and Pal Maliga: Protein Export from Agrobacterium to Chloroplasts for the Excision of Target Sequences in the Chloroplast Genome.
- Lisa LaManna and Pal Maliga: A Multiplex CRISPR/Cas9 Gene Editing Platform to Obtain acc2-Knockouts in Brassica napus

**McKim Lab**
- Jessica Fellmeth and Kim McKim: Exploring the function of meiosis specific cohesins in promoting synaptonemal complex assembly
- Janet Jang and Kim McKim: Regulation of the meiotic spindle and sister centromere cohesion in oocytes by antagonism between PP2A and Aurora B kinase.
- Kim McKim: PP1-87B antagonizes Polo and BubR1 in controlling microtubule dynamics to achieve sister chromatid co-orientation in metaphase I in Drosophila oocyte

**Messing Lab**
- Jaquing Dong, Min Tu, Yaping Feng, Anna Zdepski, Fei Ge, Dibyendu Kumar, Janet P. Slovin, and Joachim Messing: Sequence identification of two mutations causing maize defective kernel using user-friendly pipelines applicable to large genomes
- Yin Li and Joachim Messing: Comparing transcriptomes of sugar-accumulating internodes between different sweet sorghum genotypes
- Zhiyong Zhang and Joachim Messing: Formation of large central storage vacuoles by teff α-globulin in maize starchy endosperm cells

**Nickels Lab**
- Jeremy Bird, Richard Ebright and Bryce Nickels: Mitochondrial RNA capping: highly efficient 5’-RNA capping with NAD+ and NADH by yeast and human mitochondrial RNA polymerase

**Padgett Lab**
- Jing Lin and Richard Padget: Some Marfan-like mutations result in the aberrant trafficking of TGF-β type II receptor
Rongo Lab
  • Kishore Joshi, Tarmie Matlack, Stephanie Pyonteck, Ralph Menzel, and Christopher Rongo: Biogenic Amine Signaling Promotes Ubiquitin-Proteasome System Activity
  • Eun Chan Park, Irafah Tariq and Christopher Rongo: The Hexosamine Pathway Ameliorates Tauopathy in C. elegans Neurons

Severinov Lab
  • Ishita Jain and Konstantin Severinov lab: Decoding the Mechanism of RNA-Targeting by Cas13a CRISPR-Cas System

Singson Lab
  • Amber Krauchunas and Adrew Singson: Characterization of a temperature-sensitive allele of egg-3

Steward Lab
  • Hiep Tran and Ruth Steward: Tet function in Drosophila
  • Ethan Chiang and Ruth Steward: Tet controls photoreceptor development in Drosophila

PRESENTATIONS & MEETING ABSTRACTS

Waksman Institute Hosted Seminars
  • Dr. Chao Wang, Ernest Maro School of Pharmacy, Dr. Dana C. Price, Department of Plant Biology, Dr. Erika Shor, New Jersey Medical School & Michael Weiland, Pacific Bioscience, The landscape of DNA methylome and transcriptome of prostate adenocarcinoma in prostate specific PTEN KO mice, Single molecule sequencing to improve algal genome assemblies, Using optical mapping and PacBio whole genome sequencing to understand the plastic genome of Candida glabrata & SMRT Sequencing: A Foundation for Discovery, November 1, 2018
  • Erin Barnhart, PhD, Department of Biology, New York University, “Cellular mechanisms for efficient coding in the Drosophila visual system”, January 14, 2019.
  • Amika Barber, PhD, Department of Neuroscience, University of Pennsylvania, School of Medicine, “When is it time to eat? How neuropeptides and fast neurotransmitters interact to transmit time-of-day signals” January 23, 2019.
  • Catherine Eichhorn, PhD, University of California - Los Angeles, “Structural insights into the 7SK core RNP, a major regulator of eukaryotic transcription” February 7, 2019.
  • Li He, PhD, Harvard Medical School, “Dynamic regulation of intestinal homeostasis: From gene expression to mechanical signaling”, February 20, 2019.
  • Ariel M. Pani, PhD, Biology Department and Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, “An integrative approach to dissect cell-cell signaling in vivo”, February 25, 2019.
  • Peri Kurshan, PhD, Biology Department, Stanford University, “Towards a molecular understanding of synapse formation”, February 27, 2019.
  • Xiuren Zhang, PhD, Department of Biochemistry & Biophysics, Institute for Plant Genomics & Biotechnology, Texas A&M University, “MicroRNA production in plants”, March 1, 2019
  • Han Wang, PhD, Division of Biology and Biomedical Engineering, California Institute of Technology, “Genetic and neural dissection of sleep in C. elegans”, March 6, 2019.
  • Srujana Samhita Yadavalli, PhD, Waksman Institute of Microbiology, Rutgers University, “Antimicrobial peptide stress response in E. coli”, March 12, 2019.
  • Jianxin Ma, PhD, Department of Agronomy, Center for Plant Biology, Purdue University, “Soybean translational genomics: From basic findings to applications”, March 22, 2019
  • Xuemei Chen, Professor, Department of Botany and Plant Sciences, University of California - Riverside, “Small RNA Networks in Plants”, May 1, 2019
  • Iva Greenwald, PhD, Biological Sciences, Columbia University, “New insights into gonadogenesis in C. elegans”, May 20, 2019.

Waksman Student Scholars Programs
  • Waksman Student Scholars Summer Institute, Waksman Institute, Rutgers University, July 5-7, 2018
  • Waksman Institute Summer Experience (WISE June 18), Waksman Institute, Rutgers University, June 20-July 3, 2018.
  • Waksman Institute Summer Experience (WISE August 18), Waksman Institute, Rutgers University, July 30-August 10, 2018.

Barr Lab

Dismukes Lab
  • 2019 DOE Hydrogen and Fuel Cell Technologies Research, Development &Demonstration; AMR.
  • 2019 National Renewable Energy Lab., Golden CO,
Bioenergy subgroup, April, host Jian Ping Yu
• 2019 Colorado School of Mines, Golden, CO, April, host M. Posselt
• 2019 Arizona State University, Bioscience Institute, Tempe, AZ, April 24
• 2019 Materials Research Society, Phoenix, AZ April 22-23
• 2019 Univ. Colorado Boulder, March, host C. Musgrave
• 2019 Rutgers Laboratory for Surface Modification, April 9-6 posters
• 2019 Catalysis Society of Metropolitan New York, March 22, 6 posters
• 2018 23rd Electrochemical Society Meeting, presentation in the division of Electrocatalysis, Seattle, WA, May.
• 2018 Eastern Regional Photosynthesis Meeting, Marine Biological Lab, Woods Hole, MA, May 5-6
• 2018 American Chemical Society National Meeting, Boston, MA, August 21-23.
• 2018 New York Metro Catalysis Society Symposium at Lehig Univ, 3 posters, Easton PA, Mar 22.
• 2018 Rutgers Energy Institute, Rutgers Univ, May 1.
• 2018 DOE Hydrogen and Fuel Cell Technologies Research, Development &Demonstration; AMR.

Dong Lab
• Seminar at Qingdao University, Qingdao, China. Title: From a cell biological perspective: Function and regulation of MAPK signaling

Dooner Lab

Ebright Lab
• “Structural Basis of Transcription Antitermination by Q.” Meeting on Post-Initiation Activities of RNA polymerases, Mountain Lake, Virginia, 2018.
• “RNA polymerase: the molecular machine of transcription.” UK RNAP Meeting, Oxford, United Kingdom, 2019
• Lin, W., Mandal, S., Jiang, Y., and Ebright, R.H. (2018) Crystal structure of an ECF-σ-factor-dependent pausing complex. ECF@25 Meeting, Marburg, Germany, September 21-26, 2018.
**PATENTS & PUBLICATIONS**

**Patents**

**Dissukes Lab**


**Ebright Lab**


**Publications**

**Barr Lab**


**Dissukes Lab**


Dong Lab


Dooner Lab


Ebright Lab


Gallavotti Lab


Genomics Core Facility


Irvin Lab


Maliga Lab


Mckim Lab


Messing Lab

Nickels Lab

Padgett Lab

Rongo Lab

Singson Lab