Waksman Institute of Microbiology



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 classes of organisms amenable to genetic analysis such as bacteria and fungi (E. coli and yeast), animal systems (e.g., Drosophila), 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.



Waksman Institute of Microbiology Rutgers, The State University of New Jersey 190 Frelinghuysen Road, Piscataway, NJ, 08854, USA

> Phone: 848-445-3425, Fax: 732-445-5735 Email: info@waksman.rutgers.edu www.waksman.rutgers.edu

Director, Joachim Messing

Professors

G. Charles Dismukes Juan Dong Richard H. Ebright Andrea Gallavotti Kenneth Irvine Pal Maliga Kim McKim Bryce Nickels Richard W. Padgett Christopher Rongo Konstantin Severinov Andrew Singson Ruth Steward Andrew K. Vershon

Non-Resident Professors

Maureen Barr Robert Goodman

Professors Emeriti

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

Executive Director for Administration and Finance, Robert Rossi

Staff

MaryLynn Bianca, Administrative Assistant Maryann Bythell, Administrative Assistant Michelle Phillips, Senior Administrative Assistant Marge Piechota, Accountant Erin Sorge, Grant Proposal Assistant Jill Wachter, Administrative Assistant

Information Technology

Randy Newman, Core Facilities, Support Services and Information Technology Director Daja O'Bryant, Unit Computing Specialist Brian Schubert, System Administrator

Core Facilities

Kenneth Callanan, Fermentation Facility Nanci Kane, Confocal Imaging Core Facility Dibyendu Kumar, Genomics Core Facility Joshua Gager, Greenhouse Supervisor

Support Services

Rosita Law, Principal Lab Technician Avonell McKnight Laboratory Technician Ted Przybylski, Instrument Maker/Repairer

Rutgers Research and Educational Foundation

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

RREF Committee Members 2017-2018

Gregory Bender Ronald J. Garutti M. Wilma Harris Roberta Kanarick Tolulope A. Oyetunde Carole Sampson-Landers Edgar Torres

Executive Officers:

Dr. Debasish Dutta, Chancellor- New Brunswick for Dr. Robert Barchi President & Chair Mr. Peter Larson, Controller for J. Michael Gower, Executive VP of Finance and Administration and Treasurer Joachim Messing, Scientific Director Elizabeth Minott, Esq., Legal Counsel & Secretary Robert Rossi, Executive Director

CONTENTS

All hand

	About US Faculty & Staff	1 2
	REPORT OF THE DIRECTOR	4
	Advancing Our Research	10
	ANIMAL LABS	
	BARR LAB	11
	IRVINE LAB	13
	McKIM LAB	15
	PADGETT LAB	19
and a second second	RONGO LAB	22
Contraction of the second	SINGSON LAB	25
A AN	STEWARD LAB	27
	MICROBIAL LABS	
	FBRIGHT LAB	29
	NICKELSLAB	32
	SEVERINOV LAB	34
	VERSHON LAB	35
	PLANT LABS	
	DISMUKES LAB	36
	DONG LAB	39
	DOONER LAB	41
C.C.C.C.C.C.C.C.C.C.C.C.C.C.C.C.C.C.C.	GALLAVOTTI LAB	42
<u>Au</u>	MALIGA LAB	44
	MESSING LAB	46
	CODE FACILITIES	40
	CORE FACILITIES	7
	Training Future Leaders	53
	Charles and Johanna Busch Fellows	54
	Charles and Johanna Busch Fellows	55
	Benedict Michael Fellow	59
	WAKSMAN FACULTY COURSES	60
	WAKSMAN STUDENT SCHOLARS PROGRAM	61
	SHARING OUR DISCOVERIES	63
	WAKSMAN ANNUAL RETREAT	64
ALL	PRESENTATIONS & MEETING ABSTRACTS	67
And the second se	PATENTS & PUBLICATIONS	70

Cover Images: Oocyte with chromosome segregation defect, Janet Jang, McKim Lab; Innervation of neurons from the Drosophila brain, Ying Li,Padgett Lab; Patterns of gene expression in a Drosophila wing disc, Eunjoo Cho, Irvine Lab; Immature maize ear imaged by SEM, Gallavotti Lab; tdTomato localized in the nucleus of C. elegans intestine cell, Jing Lin, Padgett Lab; Protein polarization in stomatal divisions, Juan Dong, Dong Lab; Developmental defects in plant epidermis, Ying Zhang, Dong Lab; SEM images of Drosophila melanogaster eyes, Andrea Gallavotti and Nanci Kane; Storage protein bodies of corn endosperm, Zhiyong Zhang, Messing Lab

REPORT OF THE DIRECTOR

Joachím Messíng



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. Through the patent of streptomycin, and other antibiotics discovered in Dr. Waksman's laboratories, Rutgers received approximately \$16 million in royalties, which was used, in part, to build and endow the institute.

Organization

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 1st, 2013, we will have also the opportunity to make joint appointments with the Medical School. However, in the new Rutgers, the Waksman Institute of Microbiology will remain a unit of the New Brunswick campus rather than a unit of the Rutgers Medical School because of its broader life sciences mission. The faculty of the institute will also continue to participate in the various graduate programs, thereby remaining fully integrated into the state university system.

Administration

The Institutes administration consists of only seven people under Robert Rossi as Executive Director of Finance and Administration. The Director's Office shares the administrative assistant with the faculty, helping them with the preaward 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 personal action including visa needs for all foreign students and scientists. Finally, receiving of orders at the loading dock is covered and building maintenance requires work orders.



Core facilities, support service, IT

In support of the many and diverse research activities and considering the new University-wide budget model, I reorganized our core facilities, support services, and information technology (IT) with Randy Newman as the Director, who reports to Bob Rossi. Our Institute has a total of four facilities, two support, and two IT services.



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-theart facility that provides fermentation services to a multitude of clients including academic institutions, international pharmaceutical corporations, cosmetic companies, virtual enterprises and more. Our purpose is to provide, not for profit, specific niche services to produce biologics and similar products including purification, bulk production, scaleup and R&D. We operate independent of state support, using a fee for service arrangement with our clients. Our bioreactors 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 cGLP/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.

WGCF has three sequencers covering the full spectrum of NGS requirements. Platforms include Illumina's NextSeq500, MiSeq, and PacBio Sequel. The NextSeq500 sequencer can produce up to 150 GB of data per day in fragments up to 150 bp, and paired-ends up to 2x150 sizes. The throughput of the NextSeq500 is best suited for 'tag and count' experiments including genome re-sequencing, ChIP-seq (Chromatin ImmunoPrecipitation-sequencing to understand DNA-protein interactions), transcriptome sequencing for quantification of gene expression and alternative splicing, and microRNA abundance. Up to 20 transcriptomes or one human genome at 30x coverage can be sequenced on a single flow cell in 30 hours at the cost of ~\$5,000. On the other hand, the MiSeq has lower throughput, but longer read length, suitable for de-novo sequencing of small genomes, such as fungi and bacteria. MiSeq is also most appropriate for low throughput need including metagenome- and amplicon-sequencing applications. It has become evident now that several new grants would not have been possible without this investment. Furthermore, several members of the Institute received prominent symposia speaking invitations based on the next generation sequencing technology thereby raising the visibility of the Institute.

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 microscope 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 more 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 10Gb Ethernet and 8Gb Fibre Channel fabrics. Extensive server virtualization provided by VMware ESXi is used to make most efficient use of available physical hardware and minimize energy costs.

In addition to its on-site resources, the Institute 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, 10Gb 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.

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 2017/2018, the Institute consisted of fifteen resident, two non-resident, and six emeriti faculty members. The Institute accommodates five assistant research professors, seven visiting student/scholar researchers, eight research associates, twenty-seven postdoctoral researchers, twenty-two technical assistants, twenty-two graduate students. The Waksman Institute's total resident population is currently 120, which does not include the 49 undergraduate students that did independent research during the last year. There has been a notable drop in research associates, postdocs, and graduate students last year, probably due a cyclical turnover of trained people to the general workforce.

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, one was Assistant Professors, one Associate Professor, 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, five are Fellows of the American Academy of Microbiology, and eight Fellows of AAAS.

By the end of last academic year, Richard (Rick) Padgett retired as Professor of Molecular Biology and Biochemistry and became our seventh Professor Emeritus. He joined us as an Assistant Professor in 1991. Rick got his Ph.D. from the University of North Carolina with Clyde Hutchison III and Marshall Edgell. He now returns to North Carolina for his retirement. For his Ph.D., he was one of the first to clone and sequence mammalian globin genes. He then moved on to Harvard, where he switched to fly developmental genetics, especially, the TGF β pathway. In a critical experiment, he could complement a Drosophila mutation with the human bone morphogenic protein (BMP), exemplifying the conservation of function and raising the significance of Drosophila as a genetic model. At that time, we decided that the Institute would build a critical mass in genetic models. It was also Rick, who extended this research to C. elegans as a model. This initiative in animal genetic models at the Institute served as a platform for recruitment in the life sciences at Rutgers and the former UMDNJ. Today, Rutgers has one of the largest group of researchers in this area. Rick also was a fine example of collegiality and served for many years as the Co-Director of the Molecular Biosciences Graduate Program.

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 Clyde Hutchison III from the Venter Institute in San Diego here to give the lecture "Something old, something blue:

from genome sequencing to synthetic life" in honor of Rick's career. We also list the program of our annual retreat from September 12th, 2017. In addition, the institute sponsored The Microbiology Symposium, in New Brunswick, NJ, in February 2018 at Trayes Hall, Douglass College and the Plant Biology Graduate Program Lecture series in Foran Hall.

Recruitment & Funding

Recruitment for the replacement of Hugo Dooner in the Department of Plant Biology in the School of Environmental and Biological Sciences was finally approved by Dean Goodman and Chancellor Dutta, but got delayed again because of the construction of the Addition to the New Wing. Construction has been set into two phases, utilities and the actual building. The utilities work has now been completed and a contract has been awarded for the building construction to commence soon with a completion date of July 1st, 2019. We also obtained approval from Dean March of the School of Arts and Sciences for recruitment of somebody to replace Rick Padgett.

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 10-year period, total external funding of about \$10M has fluctuated significantly. Although there has been a slight increase last year compared to the year before, we expect that the coming year there will be 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.



Three years ago, the university changed its budget to the Responsibility Center Management or RCM system. The Institute has been designated RCM under Chancellor Richard Edwards and then Chancellor Debasish Dutta. As one can see from the graph, total external grants fluctuate from year to year and one cannot predict with certainty grant income from the preceding year. This is especially true if you have faculty changes such as retirements and delayed recruitment. Given the significant changes in external grants from year to year, it makes it extremely difficult to manage responsibly budget changes either as a deficit or a surplus, which would also contradict the current concept of RCM. Especially, if services (Cost Centers) are not subject to these fluctuations, it creates a budget imbalance. Indirect cost on grants is now being used to offset Allocated Costs, but it does not cover the cost of our infrastructure to support our research like the core facilities, research services, information technology, and many repairs. Because RCM units need a significant rebalancing of the Chancellor's annual allocation to cope with these fluctuations, to bridge faculty between grants, to retain and recruit faculty, we are now working with the University to determine the most effective way to budget and plan for these changes that we have no control of.

Awards/Honors

I am pleased to report several awards/honors of our students and faculty this year. Andrea Gallavotti got promoted to Associate Professor with tenure in the Department of Plant Biology and Pathology. He also received the Board of Trustees Research Fellowship for Scholarly Excellence, which recognizes one of the strongest promotion packets gone forward last year to the Promotion Review Committee (PRC) of the University. Maureen Barr, who received an outside offer from the Department of Biology at Indiana University, was successfully retained at the Institute with joint appointment at the SAS Department of Genetics. Rick Padgett was elected as Fellow of the American Association of the Advancement of Science. I became one of the Global Lecturers like Roy Vagelos and Mike Bishop at Zhejiang University in Hangzhou, China. Congratulations to these accomplishments of our students and members!

ADVANCING OUR RESEARCH



Plant & Photosynthetic Labs



Core Facilities



BARR LAB

Sensory Biology



Dr. Maureen Barr Genetics

Summary

Research: Cilia, extracellular vesicles, and animal behavior

My research program uses *C. elegans* as a model system to study fundamental questions in cilia cell biology and to model human polycystic kidney disease and other ciliopathies. Many of these ciliary genes and pathways act in the *C. elegans* nervous system to control behavior, therefore the Barr lab is also interested in neurogenetics, neuroplasticity, and stress-induced neuronal restructuring. Most recently, we have become fascinated with extracellular vesicles (EVs) – tiny communication devices that cells shed and release to influence the behavior of other cells, tissues, or even organisms. EVs are sub-micron sized particles that may be beneficial or toxic, depending on their cargo. Very little is known about EV cargo sorting, formation, or function, largely because their small size (~100nm) escapes detection by light microscopy. We have developed the only *in vivo* system to study EV biogenesis and bioactivity in a living animal, and are poised to make important discoveries that will have profound impact on human health and disease.

We have two ongoing NIH-funded research projects (DK59418 and DK116606). My lab is also supported by an NIH diversity supplement (to grad student K. Tiger), a NJ Commission on Spinal Cord Injury grant (to research assistant professor R. O'Hagan), 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).

Polycystins, Cilia, and Extracellular Vesicles in *C. elegans* R01 DK59418 Budget Period: 08/01/15-05/31/20

The long-term goal of this grant is to understand how cilia function. Cilia and extracellular vesicles (EVs) are signaling organelles that play important roles in human health and disease. While other animals require cilia for viability, *C. elegans* can survive entirely without cilia under laboratory conditions. We recently showed that *C. elegans* ciliated sensory neurons release bioactive, polycystin-containing EVs. In humans, mutations in the polycystin-encoding *PKD1* and *PKD2* genes cause autosomal dominant polycystic kidney disease (ADPKD). In *C. elegans* and mammals, the polycystins act in the same genetic pathway, act in a sensory capacity, localize to cilia, and are contained in secreted EVs, indicating ancient functions. EV shedding from ciliated cells is an evolutionarily conserved phenomenon, yet remarkably little is known about the relationship between the polycystins, cilia, and EVs and the fundamental biology of EVs. Hence, the nematode offers an excellent system in which to address central questions regarding the biology of cilia, EVs, and the polycystins.

Nephronophthisis-related ciliopathies and ciliary compartmentalization R01 DK116606 Budget Period: 12/15/17-12/31/21

Cilia are microtubule-based organelles that play essential roles in human development and health. Ciliopathies are caused by abnormalities in the structure or function of primary cilia, with polycystic kidney disease (PKD) being a common clinical phenotype. As cilia are found on most non-dividing cells in the human body, nephronophthisis-related ciliopathies (NPHP-RCs) display extrarenal manifestations including neurological disorders and retinal degeneration. The intraflagellar transport (IFT) machinery constructs all cilia and flagella, yet mechanisms contributing to ciliary diversity and specialization are poorly understood. We will test the hypothesis that the Tubulin Code – combinatorial use of tubulin isotypes and post-translational modifications – dictates ciliary structure, motor-based transport, and function. We discovered that tubulin glutamylation sculpts *C. elegans* ciliary axonemes, modulates IFT, and controls specialized ciliary functions including the release of extracellular vesicles. Mutation in the tubulin deglutamylase *ccpp-1* (cytosolic carboxypeptidase) results in ciliary hyperglutamylation and degeneration. *C. elegans ccpp-1* ciliary degeneration is suppressed by a mutation in any of three TTLL (tubulin tyrosine ligase-like) glutamylase genes,

indicating that regulated glutamylation is critically important for ciliary homeostasis. Pathological hyperglutamylation caused by CCP deglutamylase mutations are associated with human retinal degeneration and murine progressive neurodegeneration and sperm immotility. In humans, mutations in TTLL glutamylases or proteins required for their localization are implicated in pathologies including NPHP-RCs.

In mammals, exploring the role the tubulin code plays in generating ciliary diversity is a difficult task, due to technical limitations of obtaining ultrastructural information on cilia of native tissues and of visualizing ciliary structure, transport, and function. *C. elegans* is a transparent, multicellular animal with specialized cilia – features that enable both ultrastructural and *in vivo* subcellular imaging that is unprecedented in its simplicity and reproducibility. All genes we study have human counterparts, some implicated in kidney or ciliary diseases, while others have unexplored function with respect to NPHP-RCs and/or cilia. We are uniquely poised to examine the tubulin code in intact living animals. We isolated genetic suppressors of CCP1 deglutamylase deficiency, which causes neurodegeneration in mice and ciliary degeneration in *C. elegans*. Identifying the nature of these suppressors and determining the roles of these new genes will advance our understanding of molecular mechanisms regulating ciliary homeostasis, including MT glutamylation, MT dynamics and stability, and MT-based transport. Our genetically tractable model can make inroads where other systems have not, and advance frontiers of knowledge where little is known.

Dr. Maureen Barr, Professor

Phone: 732-445-1639 Email: barr@biology.rutgers.edu www.waksman.rutgers.edu/barr

Department of Genetics

145 Bevier Road, Piscataway, NJ 08854

Lab Members

Dr. Juan Wang, Research Assistant Professor Dr. Robert O'Hagan, Research Assistant Professor Dr. Jyothi Shilpa Akella, Postdoctoral Fellow Deanna De Vore, Graduate Fellow Kumar Tiger, Graduate Fellow Katlin Power, Graduate Fellow Gloria Androwski, Research Technician Yasmin Ramadan, Research Technician Amanda Guo, Undergraduate Assistant Anza Rizvi, Undergraduate Assistant Fatima Rizvi, Undergraduate Assistant



IRVINE LAB

Developmental Biology



Dr. Kenneth Irvine Molecular Biology & Biochemistry

Summary

During development, organs grow to a characteristic size and shape. This is essential for normal organ function, and the symptoms of many congenital syndromes stem from defects in organ growth or morphogenesis. Moreover, dysregulation of growth control is associated with tumorigenesis. A detailed understanding of organ growth and morphogenesis will also be required to create functional organs from stem cells, which is a key goal of regenerative medicine. Yet how characteristic and reproducible organ size and shape are achieved remains poorly understood. Key molecular insights into how growth is controlled have come from the identification and characterization in model systems of intercellular signaling pathways that are required for the normal control of organ growth. Many of these pathways are highly conserved among different phyla. We are engaged in projects whose long-term goals are to define relationships between patterning and growth in developing and regenerating organs and to determine how these patterning inputs are integrated with other factors that influence organ growth, such as nutrition and 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 and organoids.

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. A recent focus of our efforts on Hippo signaling have involved investigations of 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, and in 2011 the Piccolo lab reported studies implicating transcription factors of the Hippo pathway (YAP and TAZ) in mechanotransduction. However, the molecular mechanisms responsible were unknown. We identified the first biomechanical pathway that could link cytoskeletal tension to Hippo signaling by discovering that the localization and activity of the Drosophila Ajuba LIM protein (Jub), and the Warts kinase, are modulated by cytoskeletal tension, providing a direct link between myosin activity and organ growth. We have since 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. We have also characterized links between mechanical forces and Hippo signaling in mammalian cells, and discovered both conservation of this Jub biomechanical pathway and identified a role for it in cell density-dependent regulation of mammalian Hippo signaling, including contact-inhibition of cell proliferation. We also identified a contribution of cyclic stretch to regulation of Hippo signaling that is mediated through Ajuba family proteins. 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 also investigate 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, with a particular focus on the contributions of tissue mechanics.

Dr. Kenneth Irvine, Distinguished Professor

Phone: 848-445-2332 Email: irvine@waksman.rutgers.edu www.waksman.rutgers.edu/irvine

Lab Members

Dr. Cordelia Rauskolb, Assistant Research Professor Elmira Kirichenko, Research Technician Dr. Jyoti Misra, Postdoctoral Associate Dr. Consuelo Ibar, Postdoctoral Associate Dr. Bipin Tripathi, Postdoctoral Associate Zhenru Zhou, Graduate Fellow Srividya Venkatramanan, Graduate Assistant Katelyn Fleisch, Undergraduate Assistant Swathi Vasudevan, Undergraduate Assistant Tom Lehan, Undergraduate Assistant



MCKIM LAB Molecular Genetics of Meiotic Recombination and Chromosome Segregation



Dr. Kim McKim Genetics

Summary

At the reductional division of meiosis I, the homologous chromosomes segregate. Chiasmata, the result of meiotic crossing over between homologs, facilitate homolog orientation and segregation on the meiosis I spindle. Critical to meiosis is the assembly of microtubules (MTs) into a bipolar spindle, followed by the arrangement of homologous centromeres towards opposite poles, or bi-orientation. Bi-orientation during meiotic metaphase I establishes correct homologous chromosome segregation at anaphase 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. Our long-term goal is to determine the fundamental mechanisms that promote accurate chromosome segregation in oocytes.

Meiotic cohesins and the regulation of synaptonemal complex assembly in meiosis

In *Drosophila*, like many organisms, meiosis begins with the pairing of homologous chromosomes. The paired homologs are held together along their entire length by the synaptonemal complex (SC), which plays a critical role in



Figure 1: Model for two meiotic cohesin complexes. A) The structure of the two meiosis-specific complexes. The complex containing the kleisin C(2)M, Stromalin (SA/SCC3), and Nipped-B (SCC2) functions only in SC assembly. The complex containing SOLO, SUNN and ORD provides cohesion and some SC assembly. Both include SMC1 and SMC3. B) Cohesin rings may organize the loop structure of the axis. C) With cohesins at the base of the loops, they could interact with both the chromatin and lateral elements/ axis of the SC. D) Super resolution single section (OMX) showing C(2)M (green) in parallel tracks (from Lake and Hawley), indicating lateral element localization. Transverse element C(3)G (red) is between the axis containing C(2)M. Scale bar is 5 μ m.

regulating many aspects of meiotic prophase, including pairing, double strand break formation (DSB), repair and crossover frequency and distribution. The SC includes transverse elements that connect two homologous chromosomes and lateral elements that interact with the chromosome axis that includes cohesin-related proteins.

The premise for our studies on SC assembly is on our published results that SC assembly depends on a group of

cohesin proteins with meiosis-specific components and functions (Figure 1). Mitotic cohesin is made up of one complex with four subunits: SMC1, SMC3, Stromalin (SA) and the kleisin Rad21. Studies in Drosophila and mammals have shown that cohesins have functions in addition to holding sister chromatids together. The cohesin ring can entrap two strands of DNA from the same chromatid, forming a loop. This function of cohesins can organize chromatin into higher order physical domains, or "topologically associated domains" (TADs). This role can explain how cohesin function influences transcription in somatic cells and, during meiosis, organizes the axis and promotes SC assembly.

All SC assembly in *Drosophila* depends on cohesins SMC1 and SMC3. Our mutant analysis

has shown that SMC1 and SMC3 function in two pathways that independently promote SC assembly (Figure 1). One pathway depends on C(2)M, a kleisin family protein and two mitotic cohesins, Stromalin (SA) and Nipped-B. C(2) M belongs to a kleisin sub-family required for homolog but not sister chromatid interactions in meiosis. This group also includes mouse RAD21L and *C. elegans* COH-3 and COH-4. The second pathway depends on ORD, a cohesin protein that is not conserved, SUNN, which is distantly related to Stromalin, and SOLO, which has sequence motifs of a kleisin. SOLO may act like Rec8 and be the target of separase. We are currently investigating the structure and function of these two meiosis-specific cohesin complexes. The localization pattern and dynamics of these different cohesin complexes may dictate the pattern of SC assembly, which, through interactions with the chromosome axis, determines the frequency, distribution and locations of crossovers.

Our current work is following up on evidence that the function of the C(2)M cohesin complex is to promote SC assembly and meiotic crossing over. Meiotic chromosomes are organized into a linear array of loops. Recombination is proposed to occur in these loops, although interactions with axis-associated proteins is also required. A crucial gap in our understanding is how these loops are organized, their dynamics regulated and how they interact with the lateral elements of the SC. Recent findings that cohesins organize DNA into loops suggests they could play a critical role in meiotic chromosome organization and function. C(2)M has been shown to be in the chromosome axis by EM and super resolution microscopy (Figure 1). Organizing the meiotic chromatin into loops appears to have several important functions. By being at the base of the loops, the cohesins could promote physical linkages between the chromatin, the chromosome axis, and the SC. By interacting with the chromatin and being in the chromosome axis, C(2)M cohesin is positioned to mediate the effects of SC on meiotic recombination.

Using genetic and biochemical methods, we are investigating the structure of the meiotic cohesin complexes, determine what regulates the localization of these complexes, and reveal the relationship between the cohesin complexes and SC assembly. It is not known how the cohesins promote SC assembly, although we favor a model based on evidence in fission yeast that there is a direct interaction between cohesins and lateral element proteins of the SC. We have also found that, while cohesion is typically established during S-phase, and cannot be replaced if lost, C(2)M turnover occurs rapidly during prophase. This raises the possibility that a dynamic cohesin population underlies an adaptable chromosome axis that regulates meiotic crossing over. This could be important for interference, where one crossover event is able to repress additional crossovers on the same chromosome. These studies have an impact beyond meiosis by providing insights into non-cohesive roles for cohesins, such as organizing chromatin and interacting with other chromatin associated structural proteins.

Homolog bi-orientation and segregation in oocyte acentrosomal meiosis

In the oocytes of many animals, meiotic spindles are built without centrosomes. The absence of MTs growing from the poles necessitates alternative mechanisms for establishing spindle bipolarity and homologous centromere bi-orientation. Our long-term goal is to determine how chromatin-based signals initiate and organize the meiotic spindle in acentrosomal oocytes. In addition, we are studying the mechanisms of bi-orientation in meiosis. A premise of these studies is the finding that two types of kinetochore-microtubule attachments, lateral and end-on, are required for bi-orientation and segregation of homologs. We have proposed that bi-orientation occurs through lateral microtubule attachments between the kinetochores and the central spindle (Figure 2). To understand this process, we are studying



Figure 2: Models for meiotic spindle assembly, bi-orientation, and co-orientation of homologous chromosomes. A) Model of spindle structure and assembly. The spindle is composed of kinetochore and central spindle microtubules. The latter is composed of microtubules that make antiparallel overlaps. Bi-orienting pairs of centromeres first attach laterally to bundles of microtubules in the central spindle. They make end on attachments after attaching to opposite poles. The bi-orientation process could depend on the pairs of centromeres attaching to the same bundle of central spindle microtubules as shown. B) During prophase of meiosis I, cohesion is established between the chromosome arms (purple), the pericentromeric regions (red) and the centromeres (yellosw). Cohesion is lost at these three locations at different times as meiosis progresses.

important kinetochores proteins and proteins of the central spindle, which is composed of overlapping antiparallel microtubules adjacent to the chromosomes. By studying these essential aspects of meiosis, we expect to contribute critical insights into the mechanism of homolog bi-orientation and chromosome segregation.

The structure and function of the meiotic centromere and kinetochore (KT): 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. We have identified three activities that depend on SPC105R, kinetochore assembly, lateral microtubule attachments, and co-orientation. Co-orientation is the process where sister kinetochores are fused during meiosis I to promote attachment to MTs from the same pole (Figure 2). Co-orientation is absent from mitosis or meiosis II and is a defining characteristic of meiosis I, helping to determine whether the chromosomes will segregate reductionally or equationally.

We have found that PP2A is recruited by SPC105R and is required for maintaining sister centromere cohesion during meiosis I. Cohesin protection involves complex regulation because cohesion is differentially regulated at three locations during meiosis I: the centromeres, the pericentromeric regions, and the chromosome arms. We are currently testing the hypothesis that PP2A recruitment and cohesion protection depends on different proteins during the two divisions. In meiosis I, sister centromere cohesion depends on the kinetochore protein SPC10R/KNL1 and we are testing if cohesion on the arms depends on Dalmatian, the *Drosophila* homolog of cohesin regulator Soronin.

In addition to SPC105R, we have found that Protein Phosphatase 1 (PP1) is required to maintain sister centromere co-orientation during meiosis I in *Drosophila oocytes*. In PP1 depleted oocytes, sister centromeres precociously separate, and while this does depend on Aurora B kinase and, surprisingly, it does not depend on separase. In contrast, the precocious separation of centromeres in Spc105R RNAi oocytes does depend on separase. Our evidence is consistent with a model where centromere separation in PP1 RNAi meiosis I oocytes, and in wild-type meiosis II oocytes, is driven by forces that act in the presence of intact cohesin molecules. The cohesins at the sister centromeres are moved out of the way in a Separase independent manner. Consistent with this model, the precocious separation of sister centromeres in meiosis I of PP1 depleted oocytes depends on end-on attachment of microtubules to the kinetochores, as well as Polo kinase and BubR1, two proteins known to promote stable microtubule-kinetochore attachments. Thus, we have found evidence of a pathway for co-orientation that begins with cohesion but ends with PP1 regulating spindle forces and possibly remodeling of the centromere domain, but not cohesion release.

Characterize the relationship between CPC localization and bi-orientation: Aurora B regulates two sets of targets, the kinetochores and central spindle, either of which can assemble in the absence of the other (Figure 2). At each location, Aurora B activity is probably regulated by other kinases and phosphatases. To identify the phosphatases, we used a small molecule inhibitor of Aurora B, Binuclein 2 (BN2). When oocytes are treated with this drug, they lose their microtubules and kinetochores. We have found that Protein Phosphatase 1 (PP1) may antagonize Aurora B at the kinetochores in meiosis. In PP1 depleted oocytes also treated with BN2, the kinetochores were stabilized. These results suggest that PP1 antagonizes Aurora B for kinetochore assembly. PP1 does not antagonize Aurora B for all its function. For example, depletion of PP1 does not suppress the spindle loss caused by BN2 treatment, suggesting another phosphatase is antagonizing Aurora B in meiosis. Indeed, we found that the spindle microtubules were stabilized when Aurora B was inhibited by BN2 in *Pp2A* depleted oocytes, demonstrating that PP2A antagonizes the Aurora B spindle maintenance function.

During meiosis, Aurora B and the rest of the CPC localizes to the central spindle and the centromeres. To understand how the CPC is regulated and functions in these two critical locations, the centromeres and central spindle, we have been generating mutants of *Incenp* that alter the localization pattern of the CPC. These mutated transgenes contain silent changes in the sequence that makes them RNAi resistant. In females expressing RNAi to *Incenp*, no meiotic spindle forms. In females expressing an RNAi resistant form of the wild-type gene, the meiotic spindle forms normally.

When INCENP is targeted to the centromeres or kinetochores, only kinetochore microtubules form, and there is no central spindle. This result suggests that targeting the CPC to centromere/kinetochore is sufficient to form kinetochore microtubules but is not sufficient to promote central spindle formation. In addition, we deleted the microtubule binding domain of INCENP to disrupt the interaction with microtubules and maintain the CPC on the chromatin. or preventing the CPC interacting with microtubules. We also targeted the CPC to the central spindle by fusing the IN-box of *Incenp* with central spindle protein, either Subito (MKLP2 homolog) or Feo (Prc1 homolog). However, expressing these transgenes in *Incenp* RNAi background cannot form a spindle. This result demonstrates that the interaction between the CPC and the chromosomes is necessary to initiate spindle formation. We are currently characterizing transgenes that contain deletions of other possible protein-protein interaction sites in order to understand how the chromatin recruits the CPC and how the CPC builds the central spindle in meiosis. Our preliminary data suggests that the Borealin subunit and interactions with heterochromatin protein HP1 containing chromatin are critical.

Investigate how the central spindle interacts with kinetochores and promotes bi-orientation: Homolog bi-orientation depends on interactions between the kinetochore microtubules and the antiparallel microtubules present in the central spindle (Figure 2). This structure is maintained by Subito, a Kinesin 6 that can bundle antiparallel microtubules. In the absence of centrosomes in *Drosophila* oocytes, we have proposed that the kinesin 6 Subito is required for establishing spindle bipolarity and chromosome biorientation by assembling a robust central spindle during prometaphase I. Although the functions of the conserved motor domains of kinesins is well studied, less is known about the contribution of the poorly conserved N- and C- terminal domains to motor function. We recently published the results of an investigation into the contribution of these domains to kinesin 6 function in meiosis and early embryonic development. We found that the N-terminal domain has antagonistic elements that regulate localization of the motor to microtubules. Other parts of the N- and C-terminal domains are not required for microtubule localization but are required for motor function. Some of these elements of Subito are more important for either mitosis or meiosis, as revealed by separation-of-function mutants. One of the functions for both the N- and C-terminals domains is to restrict the CPC to the central spindle in a ring around the chromosomes. We also provide evidence that CDK1 phosphorylation of Subito regulates its activity associated with homolog bi-orientation. These results suggest the N- and C-terminal domains of Subito, while not required for localization to the central spindle microtubules, have important roles regulating Subito, by interacting with other spindle proteins and promoting activities such as bipolar spindle formation and homologous chromosome bi-orientation during meiosis.

Dr. Kim S. McKim, Professor

Phone: 848-445-1164 Email: mckim@waksman.rutgers.edu www.waksman.rutgers.edu/mckim

Lab Members

Janet Jang, Laboratory Researcher Lin-Ing Wang, Graduate Fellow Justin Mathew,Undergraduate Student Priya Patel, Undergraduate Student Amy Gladstein, Undergraduate Student Zachary Sisco, Undergraduate Student Tyler Defosse, Undergraduate Student Jay Joshi, Undergraduate Student

PADGETT LAB

Developmental and Molecular Genetics



Dr. Richard Padgett Molecular Biology & Biochemistry

Summary

My laboratory is interested in the control of cell growth and differentiation. Our studies focus primarily on transforming growth factor- β (TGF β) and its role in the control of cell growth.

TGF β controls many important developmental events in all animals, from sponges to vertebrates (Fig.1). Mis-regulation of the pathway is often a contributing factor in a variety of cancers and/or diseases. Our main focus is on determining how TGF β affects the growth of cells and how signaling strength of TGF β is regulated. Because of the powerful genetics and molecular tools available, we are using both *C. elegans* and *Drosophila* as experimental systems to study the TGF β -like signal transduction pathways.



Figure 1. Model for TGF β Signaling

Studies of the C. elegans I-Smad

TGF β is an animal invention and does not exist in yeast or plants. Evolutionary analysis of the TGF β pathway indicates that two complete 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. As expected, *C. elegans* has a putative I-Smad, tag-68, which has not been studied or characterized.

Using a deletion of tag-68, we examined the mutant phenotype of this gene. I-Smads attenuate TGF β signaling, so if it functions in the Sma/Mab pathway, one expects animals to be longer. Tag-68 mutants were outcrossed to remove unrelated mutations and measured for their body size. Animals were significantly longer than wild-type, but not as long as *lon-2* animals. Consistent with this finding is the observation that an intermediate mutant phenotype is observed for I-Smad mutants in other animals. Further experiments will focus on the developmental role tag-68 plays with the Sma/Mab pathway.

Studies of TGFβ Receptor Trafficking

We have used genetic screens in *C. elegans* to identify additional components of the TGF β -like pathways. Three different screens have been carried out in *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 screens 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 TGF β 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 TGF β 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 SMA-6 and DAF-4, the *C. elegans* TGF β receptors, but not with the TGF β ligand. Its physical association with the receptor could affect receptor stability or it could affect receptor trafficking (Fig.2). It is known that efficient signaling of pathways requires proper recycling of receptors, so SMA-10 could affect that process. In mutants of *sma-10*, we find that the receptors do not traffic properly and accumulate in vesicles. Further studies show that SMA-10 affects 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 TGF β receptors.

As a foundation for further SMA-10 studies, we examined the normal trafficking of TGF β receptors in the nematode. We find that the two TGF β receptors, type I and type II, traffic differently from each other. The type I



Figure 2. The C. elegans endocytic pathway.

receptor, SMA-6, is recycled through the retromer, which is a novel and unexpected finding. SMA-6 physically interacts directly with the core proteins of the retromer. Use of the retromer for trafficking provides an additional point for regulation of signaling strength, as both receptors are needed for signaling.

To gain clues about the role of SMA-10, we have determined its subcellular location. While it is found in most trafficking compartments at low levels, it is enriched in the late endosome/MVB vesicles. This suggests a possible role in degrading SMA-6. In support of this hypothesis, we found that mutations in SMA-10 lower the amount of ubiquitination on SMA-6, which is known to regulate degradation.

In an expansion of our endocytosis work, we have begun to look at naturally-occurring mutations in receptors of patients. These mutations are located near important motifs involved with receptor trafficking. These contribute to cancer or Marfan-like syndromes, depending on the particular patient. The hypothesis being examined is whether some of these diseases are due to improper trafficking of the receptors, which would change some of the common paradigms of these diseases. 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 signal, but are mis-trafficked. This data sheds light on the possible molecular defects in TGF β signaling that contribute to Marfan-like syndromes.

MicroRNA genes affect TGFβ-like Pathways in Drosophila

MicroRNA (miRNA) genes comprise at least 2% of animal genomes and represent an important aspect of gene regulation. In animals, they attenuate translation of target messages and often affect mRNA levels of most genes. We are exploring their role in regulating growth in Drosophila.

Previously, we identified *bantam* as a regulator of *Mad*, a signal transducer of the Dpp pathway in *Drosophila*. Mutations in the presumptive 3'UTR *Mad* binding sites prevent regulation by *bantam* in cell culture and in flies. Over-expression of *bantam* or a lack of *bantam* in clonal mutations in wing disks affects the level of downstream reporter genes.

We propose that regulation by *bantam* is a conserved feature of Dpp/BMP signaling. We have identified two vertebrate *bantam* homologs and show that they affect cell proliferation, possibly indicating a conserved function. We show that *bantam* and dpp exist in a feedback loop, regulating each other.

Development of a co-CRISPR technique in Drosophila

Genome editing using CRISPR has become a valuable tool in research, but identifying modifications can be time consuming and labor intensive. We developed a co-CRISPR strategy in *Drosophila* to simultaneously target a gene of interest and a marker gene, *ebony*, which is a recessive gene that produces dark body color. We found that *Drosophila*

broods containing higher numbers of CRISPR-induced *ebony* mutations ("jackpot" lines) are significantly enriched for indel events in a separate gene of interest (approximately 70%), while broods with few or no *ebony* offspring showed few mutations in the gene of interest. This co-CRISPR technique significantly improves the screening efficiency in identification of genome editing events in *Drosophila*. These studies are being expanded to develop similar tools in *Drosophila* and *C. elegans* using the newly discovered CRISPR/Cpf1 editing system and other variations of the Cas9 protein.



Figure 3. Genetic scheme for using Drosophila co-CRISPR.

Dr. Richard W. Padgett, Professor

Phone: 848-445-0251 Email: padgett@waksman.rutgers.edu www.waksman.rutgers.edu/padgett

Lab Members

Jing Lin, Graduate Fellow Nanci Kane, Laboratory Researcher Kelvin Liao, Undergraduate Honors Research Krishna Varre, Undergraduate Honors Research Arianne Bisnar, Undergraduate Honors Research



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



Dr. Christopher Rongo Genetics

Summary

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

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

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

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

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

Environment can impact nervous system function, and neurons can respond to accommodate a changing environment. Specifically, oxygen influences behavior in many organisms, and low oxygen levels (hypoxia) can have devastating consequences for neuron survival due to excitotoxicity from overactivated neurotransmitter receptors and impaired mitochondrial function. We have shown that hypoxia blocks the membrane recycling of glutamate-gated ion channels to synapses, thereby depressing glutamatergic signaling. Surprisingly, the canonical transcriptional factor that mediates most cellular hypoxia responses is not required for this effect. Instead, a specific isoform of the prolyl hydroxylase EGL-9, a key sensor for oxygen, recruits LIN-10, a known PDZ scaffolding protein, to endosomes, where together the two proteins promote glutamate receptor recycling. Our discovery demonstrates a novel way by which animals can sense and respond behaviorally to oxygen levels. It identifies a novel substrate of the EGL-9 prolyl hydroxylase. Finally, it indicates that neurons have signaling pathways that play a neuroprotective function to help minimize damage during ischemic events by using molecular and cellular mechanisms more diverse than originally appreciated.

It is also important to understand how mitochondria respond to oxygen deprivation given the critical role they play in

using oxygen to generate cellular energy. We have shown that neuronal mitochondria undergo DRP-1-dependent fission in response to anoxia and undergo refusion upon reoxygenation. The hypoxia response pathway, including EGL-9 and HIF-1, is not required for anoxia-induced fission, but does regulate mitochondrial reconstitution during reoxygenation. Anoxia results in mitochondrial oxidative stress, and the oxidative response factor SKN-1/Nrf is required for both rapid mitochondrial refusion and rapid functional recovery of the nervous system during reoxygenation. In response to anoxia, SKN-1 promotes the expression of the mitochondrial resident protein Stomatin-like 1 (STL-1), which helps facilitate mitochondrial dynamics following anoxia. This conserved anoxic stress response thus changes mitochondrial fission and fusion to help neurons survive the oxidative damage resulting from oxygen deprivation.

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.

Regulators Of Mitochondrial Dynamics In Neurons.

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

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

Dopamine Signaling Activates The UPS In Distal Epithelial Tissues.

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

We previously generated a GFP-based reporter system for UPS activity in C. *elegans*, allowing us to query UPS activity in specific tissues and at specific points along development. We found that epithelial cells undergo a dramatic increase in UPS activity as animals mature. We have also found that the humoral neurohormone/biogenic amine neurotransmitter dopamine promotes UPS activity in epithelia. In C. *elegans*, mechanosensory neurons release dopamine when nematodes encounter a potential bacterial food source. Dopamine in turn inhibits motoneuron activity through the dopamine receptors DOP-2 and DOP-3, resulting in a behavioral change that slows the animal down so that it can feed. We found that this released dopamine also activates the UPS in epithelial tissues, including the intestine and epidermis, through the dopamine receptors DOP-1 and DOP-4, and the cAMP-Response Element Binding Protein (CREB) transcription factor. This signaling pathway activates the expression of enzymes involved in xenobiotic

detoxification (e.g., cytochrome P450 enzymes) and innate immunity, which in turn promote protein polyubiquitin. While 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.

Dr. Christopher Rongo, Professor

Phone: 848-445-0955 Email: rongo@waksman.rutgers.edu www.waksman.rutgers.edu/rongo

Lab Members

Dr. Eunchan Park, Assistant Research Professor Dr. Kishore Joshi, Professor Dr. Mehul Vora, Research Associate Nathaly Salazar-Vasquez, Graduate Fellow James Mullin, Laboratory Research Assistant Arul Duggimpudi, Undergraduate Assistant Julia Schoeni, Undergraduate Assistant Neil Shah, Undergraduate Assistant Raphaella Ranjo, Undergraduate Assistant



SINGSON LAB Reproductive Biology, Cell-Cell Interactions



Dr. Andrew Singson Genetics

Summary

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

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

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

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

Sperm function

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

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

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

As we have been defining the molecular components of fertilization we have seen emerging parallels with other cellular systems. We have recently proposed the concept of a **fertilization synapse**. This framework takes into account the molecular and cellular complexity required for reproductive success (See Figure 1).



Figure 1. Sperm and egg genes required for fertilization. There are a surprising number of genes required at the surface of sperm and oocytes needed for fertilization. This has lead us to propose that these molecular components of fertilization are integrated into a fertilization synapse. Our ongoing genetic analysis suggests that there are still many more genes that impact fertilization. Two sperm transmembrane molecules that we recently cloned (*spe-13* and *spe-36*) are not shown.

Sperm activation

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

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 the *egg-3* gene. This will provide a genetic tool to not only better understand the regulation of the oocyte-to-embryo transition but will also help us identify additional components of the *egg-3* pathway.

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

Reproductive Life Span

We have 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.

Dr. Andrew Singson, Professor

Phone: 848-445-0836 Email: singson@waksman.rutgers.edu www.waksman.rutgers.edu/singson

Lab Members

Amber Krauchunas, Postdoctoral Associate Xue Mei, Postdoctoral Fellow Marina Druzhinina, Laboratory Technician Kendall Flanigan, Laboratory Technician



STEWARD LAB

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



Dr. Ruth Steward Molecular Biology & Biochemistry

Summary

Zfrp8, a new gene functioning in hematopoietic and ovarian stem cells in Drosophila, controls the assembly of specific ribonuclear 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 mouse embryo fibroblasts fail to grow and ultimately die, similar to the phenotype seen in Drosophila ovaries.

In yeast two-hybrid screens using Zfrp8 or PDCD2 as baits we identified Ribosomal Protein 2 (RPS2). We confirmed this interaction in fly and human tissue culture cells by co-immunoprecipitation. Further we found that in *Zfrp8* KD cells, the cytoplasmic levels of RpS2 and at least two other components of the 40S ribosomal subunit, RpS11, and RpS13, are reduced, suggesting that Zfrp8 may regulate their nuclear export, or the final cytoplasmic maturation steps that include mRNA binding and 60S-40S assembly. Despite the reduction of RpS2 levels in *Zfrp8* KD cells, expression of many proteins was maintained at relatively normal levels, suggesting that lack of Zfrp8 affects translation in a transcript specific manner. We also showed that Zfpr8/PDCD2 is required for efficient nuclear export of select transcripts. Based on the predicted chaperone activity of Zfrp8/PDCD2 and its interaction with RNA binding proteins, we propose that Zfrp8 plays a role in the assembly of transcript-specific RNPs and facilitates their nuclear export.

Another Zfrp8-interactor was NUFIP (Nuclear fragile X mental retardation-interacting protein) and we have been able to show that Zfrp8 forms a complex with NUFIP and FMRP (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 where it is essential in gonadal stem cell maintenance.

Zfrp8 forms a complex with Tet, a methylcytosine dioxygenase

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-methylcytisine (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. We induced mutations that eliminate either the large or small proteins specifically and also have induced a null mutation. The null allele, *Tet^{null}* genetically interacts with *Zfrp8*. *Tet^{null}* is 100% pupal lethal and removing one copy of *Zfrp8* suppresses this lethality; about 40% of animals survive to adulthood but die soon after eclosion.

In flies 5mC and consequently 5hmC is absent in DNA. In collaboration with Dr. Fuk's laboratory at the Free University of Brussels, we have mapped 5hmrC transcriptome wide in S2 Drosophila tissue culture cells. Significantly, in mRNA from Tet KD cells the 5hmC levels were reduced at least 4 fold in 80% of the modified transcripts compared to wild type. We conclude that Tet modifies specific transcripts and regulates the recruitment of Zfrp8 to these RNAs, thus controlling their processing and translation. We have now also ascertained 5hmC decorated mRNAs in Drosophila embryos and find that \sim 40% of the transcripts are identical to the 5hmrC-modified transcripts identified in S2 cells.

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. In parallel we have induced an CRISPR mutant in which one of the completely conserved Cs in the DNA binding domain of Tet is replaced by an A. Homozygotes for this *Tet*^{4XXC} allele show a partial phenotype, 40% of the adults survive, but are uncoordinated and die within 2 days. This result supports our hypothesis.

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 these proteins for a common function with Tet and also for potential interaction. The screen is designed so that we should identify genes that function together with Tet in RNA processing as well as genes that are regulated by Tet.

To address the tissue-specific requirement of Tet, we used different GAL4 drivers to knock down (KD) Tet ubiquitously, in muscles, or neurons and found that Tet is required in muscle precursor cells and in diverse neurons for normal function. These KD mutants show locomotion phenotypes in larvae and adults and our must recent indication is that Tet is essential for the normal outgrowth of specific axons.



Figure legend: Tet expression (red) shows significant overlap with the mesodermal marker Mef2 (blue), but its expression in mesodermal cells is lower than in neuronal cells. Stage 13 embryo; note the autofluorescence of the yolk mass on the dorsal side of the embryo (red).

Dr. Ruth Steward, Professor

Phone: 848-445-3917 Email: steward@waksman.rutgers.edu www.waksman.rutgers.edu/steward

Lab Members

Dr. Joe Kramer, Research Assistant Dr. Badri Nath Singh, Research Associate Hiep Tan, Graduate Student Le Nguyen, Laboratory Technician Sara Tenjerla, Undergraduate Student Ethan Chiang, Undergraduate Student Shahroze Khalil, Undergraduate Student



EBRIGHT LAB

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



Dr. Richard Ebright Chemistry & Chemical Biology

Summary

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

Structures of Transcription Complexes

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

Understanding bacterial transcription initiation will require understanding the structures of polypeptides in bacterial transcription initiation complexes and the

arrangements of these polypeptides relative to each other and relative to promoter DNA.

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

Mechanism of Transcription

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

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

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

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

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

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

initial transcription; by stepping in transcription elongation); (2) binding of the incoming nucleotide; (3) formation of the phosphodiester bond; and (4) release of pyrophosphate.

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

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

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

Regulation of Transcription

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

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

Inhibitors of Transcription; Antibacterial Drug Discovery

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

The rifamycin antibacterial agents--rifampin, rifapentine, rifabutin, and rifamixin bind to and inhibit bacterial RNAP. The rifamycins bind to a site on bacterial RNAP adjacent to the RNAP active center and prevent extension of RNA

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

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

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

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

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

Dr. Richard H. Ebright,

Board of Governors Professor of Chemistry and Chemical Biology Phone: 848-445-5179 Email: ebright@waksman.rutgers.edu www.waksman.rutgers.edu/ebright

Lab Members

Dr. Yon W. Ebright, Research Scientist Dr. Abhishek Mazumder, Postdoctoral Associate Dr. Jeremy Bird, Postdoctoral Associate Dr. David Degen, Postdoctoral Associate Dr. Wei Lin, Postdoctoral Associate Dr. Jared Winkelman, Postdoctoral Fellow Dr. Libing Yu, Postdoctoral Fellow Dr. Chengyuan Wang, Postdoctoral Associate Dr. Zhou (Joel) Yin, Postdoctoral Associate Adam Hasemeyer, Graduate Assistant Chih-Tsung Lin, Graduate Assistant Yu Liu, Graduate Fellow Shuya Yang, Graduate Fellow Hyejin Kim, Undergraduate Assistant Minsung Cho, Undergraduate Assistant Jiayu Shen, Research Technician Yu-Fen Huang, Research Technician David Kuster, Visiting Student

NICKELS LAB Regulation of Gene Expression in Bacteria



Dr. Bryce Nickels Genetics

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 sequencing 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 occurs *in vivo*, and shown that NCIN-mediated initiation has functional consequences by increasing RNA stability *in vivo*. We have further shown that NCIN-mediated initiation can occur with eukaryotic RNAP II, suggesting that NCIN-mediated "*ab initio* capping" may occur in all organisms.

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

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

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 <u>massively systematic transcript end</u> 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 4¹⁰ (~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 MAS-TER-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 <u>mapping by overexpression of an RNase in *Escherichia coli*), to define the cleavage consensus sequences of endoribonuclease toxins from the bacterial pathogen *Mycobacterium tuberculosis*.</u>

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 endoribonucleases. Therefore, to understand the physiological role of these *M. tu-berculosis* 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.

Dr. Bryce Nickels, Professor

Phone: 848-445-6852 Email: bnickels@waksman.rutgers.edu www.waksman.rutgers.edu/nickels

Lab Members

Dr. Irina Vvedenskaya, Assistant Research Professor

Dr. Leonid Minakhin, Assistant Research Professor Dr. Chirangini Pukhrambam, Research Technician Dr. Srujana Samhita Yadavalli, Research Associate Dr. Jeremy Bird, Postdoctoral Associate Dr. Jared Winkelman, Postdoctoral Fellow Kyle Skalenko, Graduate Student Hoa Vu, Graduate Student

SEVERINOV LAB

Mechanisms of Transcription in Microorganisms



Dr. Konstantin Severinov Molecular Biology & Biochemistry

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, concentrated on identification

of mechanisms that couple the interference and adaptation arms of CRISPR response.

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 will help 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.

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.

Structure-functional analysis of novel transcription enzymes

Through genome mining we identified several bacteriophage encoded RNA polymerases very distantly related to cellular enzymes. We use biochemical and structural methods to investigate these unusual enzymes and compare them to cellular RNA polymerases. The structure of one phage enzyme, multisubunit RNA polymerase encoded by a giant phage, has been determined to high resolution by combining X-ray crystallography and cryo electron microscopy methods.

Dr. Konstantin Severinov, Professor

Phone: 848-445-6095 Email: severik@waksman.rutgers.edu www.waksman.rutgers.edu/severin

Lab Members Dr. Konstantin Kuznedelov, Research Associate Dr. Vladimir Mekler, Research Associate Dr. Ekaterina Semenova, Research Associate Ishita Jain, Graduate Student Anna Shirieva, Visiting Graduate Student Alexander Salibi, Undergraduate Student

VERSHON LAB

Transcriptional Regulation in Yeast



Dr. Andrew Vershon Molecular Biology & Biochemistry

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 al/alpha2 complex, we identified binding sites downstream of two genes that require al/alpha2 for expression in diploid cells. We have shown that these sites regulate transcription of hap-loid-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.

Dr. Andrew Vershon, Professor

Phone: 848-445-2905 Email: vershon@waksman.rutgers.edu www.waksman.rutgers.edu/vershon

Lab Members April Rickle, Undergraduate Student
DISMUKES LAB Biological and Chemical Approaches to Renewable Energy Research



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

Summary

The Dismukes research group conducts fundamental and applied research in the areas of renewable energy production via biological and chemical approaches. Our strategy is to apply the principles of enzymatic catalysis and metabolic regulation to design bioinspired catalysts, reaction networks and microorganisms exhibiting improved performance that operate using electrical or solar energy power sources. The disciplinary approaches used are materials synthesis by design, electrochemistry and catalysis. The goal is to produce sustainable processes for renewable fuels and biomass production. Our laboratories are located in the Waksman Institute of Microbiology and the Wright-Rieman Chemical Laboratory at Rutgers University. In the 2017-June 2018 period the group was comprised of 34 researchers (listed below).

1) Photoautotrophic Carbon Fluxomics.

Our goal is to use flux balance analysis and isotopically nonstationary metabolic flux analysis (INST-MFA, figure) to quantita-

tively understand carbon flux distributions and pathway used by the cyanobacterium *Synechococcus* sp. PCC 7002 during photosynthesis. The outcome also helps discover new roles for existing metabolic pathways. Supported by NSF-MCB.

2) Optimizing Photosynthetic Light Conversion by Protein Subunit Engineering.



From the two differentially expressed cyanobacterial isoforms of the D1-PSII reaction center 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

3) Sustainable Biofuels.

The goal of this program is to control the metabolism of microbial photosynthetic organisms to efficiently produce biomass and fuels from sunlight and CO_2 . We apply genetic and environmental methods to modify the metabolic pathways to reroute the flux of fixed carbon and extract H_2 or carbon fuel precursors. We are collaborating with Christoph Benning's lab (Michigan State U.) to generate potential CO_2 . tolerant strains of *Nannochloropsis* by random mutagenesis combined with high throughput screening, as well as targeted genetic engineering of selected genes involved in fatty acid biosynthesis, TAG assembly and TAG catabolism pathways (graphic). Supported by the Global Climate & Energy Project.







4) Diversity of Photosynthetic Water Oxidizing Enzymes.

Our goal is to reveal the range of kinetic and energetic performance by photosynthetic water oxidation enzymes in vivo, selected from diverse microbial phototrophs, cyanobacteria and microalgae. The outcome is a fundamental understanding of the principles of light energy conversion to chemical energy and the mechanisms used to oxidize water in nature. Supported by DOE-BES.

5) Photosystem II and Water Oxidizing **Complex Photo-Assembly & Inorganic** Mutants.

This project aims to understand the biogenesis of the oxygenic reaction center (PSII) and the functions of the inorganic components comprising its catalytic site (WOC). We do so by substitution of the inorganic cofactors (Mn²⁺, Ca²⁺, Cl⁻, CO₂H⁻, H₂O) and examination of the consequences using multiple novel tools designed by our lab staff (graphic 3). Supported by the National Science Foundation, Chemistry of Life Processes.



6) Electrocatalysts for Water Splitting and CO2 Reduction.

Our goal is to apply the principles learned from enzymes to synthesize better catalysts for the generation of H₂ and O₂ from water and CO₂ conversion to fuels. These bioinspired catalysts must be 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 CO₂. Two synthetic projects shown here illustrate our ability to build both discrete molecular clusters of di-, tri- and tetra-nuclear Co₄O₄ cubanes using organometallic chemistry, and solid-state crystalline materials such as LiCoO₂ in two distinct polymorphs. Supported by the



DOE-EERE-SBIR program (see graphic 6) and Rutgers TechAdvance.

7) Tunable Photoanode-Photocathode-Catalyst-Interface Systems for Efficient Solar Water Splitting.



Our goal is to build a tandem solar fuel cell to split water into its elements using sunlight while achieving an overall efficiency of 10%. This will entail development of a dual absorber cell for red photons and near infrared photons coupled to OER and HER catalysts, respectively. Supported jointly by the National Science Foundation, Division of Chemical, Bioengineering, Environmental, and Transport Systems (CBET) and the U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Fuel Cell Technologies Office (graphic 7).

Dr. G. Charles Dismukes, Distinguished Professor

Phone: 848-445-6786 Email: dismukes@waksman.rutgers.edu www.waksman.rutgers.edu/dismukes http://rutchem.rutgers.edu/dismukes charles

Lab Members

Dr. Gennady Ananyev, Associate Research Professor Dr. Marie-Claire ten Veldhuis, Visiting Scholar, Assistant Professor Tech Univ. Delft, Netherlands Dr. Tie Shen, Visiting Scholar Dr. Bin Rui, Visiting Scholar, University of Science and Tech., Hefei, China Dr. Wooseok Ki, Visiting Scholar, Assistant Professor Stockton University Dr. Anders Laursen, Postdoc Associate Dr. Yunbing Ma, Postdoc Associate Dr, Joshua Flynn, Postdoc Associate Yuan Zhang, Graduate Fellow Colin Gates, Graduate Assistant Shinjae Hwang, Graduate Assistant Khue Tu Ho-Nguyen, Graduate Assistant Apostolos Zournas, Graduate Assistant Krishani Teeluck, Graduate Assistant Hengfei Gu, Graduate Assistant Hongxiang Lu, Visiting Graduate Assistant, Zhejiang Univ. Jonah Williams, Undergraduate Assistant Brendan Cullinane, Undergraduate Assistant George Dahdouh, Undergraduate Assistant Areeb Zaidi, Undergraduate Assistant

Edward Izett, Undergraduate Assistant

Anthony Heulitt, Undergraduate Assistant Sunjay Melkote, Undergraduate Assistant Anjali Modi, Undergraduate Assistant Devan Solanki, Undergraduate Assistant Jack Weber, Undergraduate Assistant Aatin Dhanda, Undergraduate Assistant Kyra Yap, Undergraduate Assistant Lauren Ostopowicz, Undergraduate Assistant Avinash Garlapati, Undergraduate Assistant Joshua Patelunas, Undergraduate Assistant Timothy Goetjen, Undergraduate Assistant Chris Dziedziak, Undergraduate Assistant Ajay Kashi, MSc Graduate Assistant



DONG LAB Cell Polarity and Asymmetric Division in Plants



Dr. Juan Dong Plant Biology

SUMMARY

Cell polarity, in both animals and plants, is of paramount importance for 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 Arabidop-

sis. 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-transmem-



brane 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 interested in building a model for plant cell polarity and its regulation in ACD. We are studying 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 includes the identification of additional polarized proteins and of mutants that display specific subsets of polarity defects.

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 (Figure 2A) 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.

Our previous results revealed the significance of BASL to be polarized at the cell cortex region for its function. To pursue the polarity determining factors from



Figure 2. Fluorescence recovery of BASL at the polarity crescent.

(A) Normalized FRAP curves for GFP-BASL, GFP-ROP2 and PIN3-GFP in the stomatal lineage cells. (B) Comparison of FRAP curves of BASL and phospho-variants. BASL_12356A indicates 5 Ser sites mutated to Ala (phospho-deficient). BASL_123456D stands for 6 Ser sites mutated to Asp (phospho-mimicking). Two specific sites were mutated to make BASL_14D. (C) Confocal images of 2-dpg seedlings showing subcellular localization of GFP-BASL (left) and GFP-BASL_14D (right). Note the nuclear localization is abolished in BASL_14D. (D) The expression of strongly polarized BASL_14D suppressed stomatal divisions. BASL protein itself, we finely analyzed and defined BASL subdomains and identify a conserved MAPK-docking motif that is required for BASL polarization. MAPKs are universal signaling regulators widely used in development and response programs in eukaryotes. We further demonstrated a MAPK-mediated phosphorylation signaling pathway is involved in the generation of BASL polarity. When six putative phosphosites were mutated, BASL no longer displayed polar localization pattern (retained in the nucleus) and lost its function in a complementation assay. We have also generated site mutations in BASL that resulted in the protein polarization at the plasma membrane only. The PM-only BASL variant was found much more stabilized (Figure 2B-C) and conferred strong regulation in stomatal divisions (Figure 2D). These mutants provided a foothold for the isolation of physical partners at specific intracellular compartment in the BASL trafficking network.

Mechanisms for asymmetric cell fate specification

Previous, we demonstrated that a canonical MAPK signaling pathway, including the MAPKKK YODA (YDA) and MAPK 3 and 6 (MPK3/6), is spatially concentrated by BASL polarization (Figure 3A). As SPCH is a direct target of MPK3/6, phosphorylated SPCH is subject to protein degradation and low activity. When GFP-BASL was crossed with SPCH-CFP, we found that the cells expressing polarized BASL showed reduced accumulation of nuclear SPCH, likely due to the enriched MAPK cascade in the polarized cells (Figure 3B). Conversely, in the absence of BASL, the differential expression of SPCH in two daughter cells was abolished (Figure 3B). Therefore, we provided a direct link between BASL and SPCH to interpret daughter fate differentiation in stomatal ACD.

Our lab will continue to use Arabidopsis as a model system, by studying BASL and the other 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.



Figure 3. BASL suppresses the master bHLH transcription factor SPCH. (A) Co-expression of CFP-BASL (cyan) with split YFP of DNyda and MPK6 (yellow) induced protein co-polarization. (B) Left, co-expression of SPCH-CFP and GFP-BASL in the stomatal lineage cells. Note the SPCH level is lowered in the cell expressing polarized BASL (insets). Right, the differential expression of SPCH in two daughter cells is diminished when BASL is absent.

Dr. Juan Dong, Assistant Professor

Phone: 848-445-7034 Email: dong@waksman.rutgers.edu www.waksman.rutgers.edu/dong

Lab Members

Dr. Xiaoyu Guo, Postdoctoral Associate Chao Bian, Graduate Fellow Dongmeng Li, Graduate Student Lu Wang, Graduate Student

DOONER LAB

Plant Genetics



Dr. Hugo Dooner Plant Biology

Summary

A sequence-indexed reverse genetics resource for maize

Our NSF-funded project to develop a reverse genetics resource for maize based on the transposable element *Ds* is on its tenth and final year. This community resource is allowing maize workers to fully exploit the maize genome sequence. The availability of lines with single gene knock-outs gives biologists a powerful tool in understanding the function of that gene. Thus, sequence-indexed collections of single insertions are critical resources for elucidating gene function in organisms with sequenced genomes. Our project is generating and sequence-indexing a collection of *Ds* transposon insertions using a cost-effective method that takes advantage of a three-dimensional pooling strategy and our in-house Waksman Genome Sequencing Facility.

Specifically, we are sequencing thousands of insertions of an engineered *Dsg* element that carries the jellyfish green fluorescent protein (GFP) to facilitate following

its movement in the genome (Figure 1). We accomplish this by high throughput sequencing of groups of 960 insertions arranged in three-dimensional DNA pools which are resolved by a specific software package (InsertionMapper) developed by our collaborators Charles Du and Wenwei Xiong at Montclair State University. To date, 14,184 *Dsg* insertions have been mapped to the reference genome. The location of newly mapped insertions is continuously being added to the websites of the Maize Genome Database (maizeGDB.org) and our project (acdsinsertions.org), where they are cross-referenced to stocks available from the Maize Stock Center. This summer we will be growing the last batch of sequence-indexed insertions to send to the Stock Center.



Figure 1. An engineered *Dsg* element carrying the jellyfish green fluorescent protein (GFP) to facilitate following its movement in the genome. The four pictures are views of the same ear sector, under natural light on the left and under blue light on the right to detect green fluorescence. The purple kernels in the bottom have had their crowns removed to expose the green fluorescent endosperm. All green fluorescent kernels carry the transposon Ds^* (GFP), the spotted kernels at the original location and the purple kernels at new locations in the genome.

We have also created a gene knockout resource for the community consisting of $86 Ds^*$ launching platforms carrying GFP that map to all 20 chromosome arms of the maize reference genome. This resource will allow simple visual selection of transpositions from many regions of the genome and will enable researchers to generate their own regional gene knock-out collections because Ac and Ds tend to transpose to nearby chromosomal sites.

Dr. Hugo Dooner, Distinguished Professor

Phone: 848-445-4684 Email: dooner@waksman.rutgers.edu www.waksman.rutgers.edu/dooner

Lab Members Harrison Wang, Laboratory Technician



GALLAVOTTI LAB

Mechanisms of Plant Development



Dr. Andrea Gallavotti Plant Biology

Summary

Molecular Mechanisms of Plant Development

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

In our laboratory we study the formation, activity and maintenance of meristems.

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

The role of the phytohormone auxin in maize development

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

As part of a collaborative research project sponsored by the National Science Foundation Plant Genome Research Program we are investigating the specificity of auxin function in developmental pathways and discovering new genes involved in auxin biology and meristem development. We used a newly developed genomic approach called DAP-seq to analyze the DNA binding behavior of the maize ARF family and to identify the direct targets of their regulation. Using this approach, we created the largest dataset of ARF targets in any plant species (Figure 1). Furthermore, we discovered that ARFs belonging to different phylogenetic groups have different binding behavior in terms of sequence recognition and genomic distribution.

Figure 1 legend: Example of ARF DAPseq data. Genome browser screenshot of the BIF1 locus showing ARF binding sites (orange and blue) in regions of accessible chromatin (as determined by ATAC-seq – grey bars).



Using traditional genetic screens, we have identified several genes that affect auxin function and meristem development. Notable among these are two Aux/IAA proteins (BIF1 and BIF4) that work in conjunction with ARF transcription factors, and a gene that encodes a mitochondrial localized protein and affects auxin transport and homeostasis. We have also identified key regulators of meristem size that eventually affect the number of seeds on maize ears.

Transcriptional repression in maize shoot development

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

The maize co-repressor REL2, a functional homolog of the TPL protein mentioned above, was originally isolated in a forward genetic screen for inflorescence defects. Mutations in the *REL2* gene give rise to pleiotropic defects throughout development, thus providing an excellent tool to study how plants use transcriptional repression mechanisms in numerous developmental processes. This research is sponsored by a grant from the Developmental Systems cluster of the National Science Foundation.

We identified a large number of transcription factors 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.

Dr. Andrea Gallavotti, Assistant Professor

Phone: 848-445-6421 Email: agallavotti@waksman.rutgers.edu www.waksman.rutgers.edu/gallavotti

Lab Members

Mary Galli, Laboratory Research Assistant Dr. Xue Liu, Postdoctoral Associate Dr. Zongliang Chen, Postdoctoral Associate Qiujie Liu, Graduate Fellow Sarah Trackman, Undergraduate Student Shaun Guru, Undergraduate Student Aditya Patil, Undergraduate Student Weibin Song, Visiting Scholar

MALIGA LAB

Plastid Molecular Genetics



Introduction

Plastids are semi-autonomous organelles with a relatively small (120-180 kb), highly polyploid genome present in 1,000 to 10,000 copies per cell. Over the past twenty years we have developed protocols for the transformation of the tobacco (*Nicotiana tabacum*) plastid genome based on integration of the transforming DNA by homologous recombination. We use plastid transformation to study plastid biology, transgene biosafety and biotechnological applications of plastid transgenes. Currently, we pursue research in the following areas.

Plastid transformation in Arabidopsis thaliana

Deletion of the *acc2* nuclear gene makes Arabidopsis hypersensitive to spectinomycin, the selective agent for the identification of transplastomic events. We have found that plastid transformation efficiency is 100x higher in the *acc2* knockout background than in wild type Arabidopsis. However, it was difficult to obtain

fertile transplastomic plants in the available Col-0 and Sav0 hypersensitive accessions (Yu et al. Plant Physiol. 175: 186, 2017). We deleted the *ACC2* gene using CRISPR/Cas9 in the regenerable RLD and Ws ecotypes and have shown that deletion of *ACC2* successfully makes the seedlings hypersensitive to spectinomycin (Figure 1). Selection for spectinomycin resistance in bombarded leaf cultures of the hypersensitive RLD and Ws backgrounds yielded putative transplastomic events at a high frequency. Classification of the clones as transplastomic lines or spontaneous spectinomycin resistant mutants and demonstration of seed transmission of spectinomycin resistance is in progress. Plastid transformation in Arabidopsis will facilitate studies of plastid-nuclear gene interactions and further engineering of the chloroplast genome to improve the efficiency of photosynthesis. The experiments are carried out in collaboration with Prof. Kerry A. Lutz, Farmingdale State College, Farmingdale, NY.



Figure 1. Deletion of the *ACC2* gene by CRISPR/Cas9 makes the plants hypersensitive to spectinomycin. (A) Wild-type RLD plants develop true leaves at the shoot apex on 100 mg/L spectinomycin. (B) The *acc2* knockout seedlings do not develop true leaves at the shoot apex due to inhibition of fatty acid biosynthesis. (C) The T-DNA carrying the CRISPR/Cas9 genes are segregated away in the white seedlings.

PPR10 RNA-Binding Protein for Regulated Gene Expression in Potato Amyloplasts

Constitutive, high-level expression of transgenes in the chloroplast compromises plant growth and interferes with development. Furthermore, plastid transgenes are expressed at extremely low levels in storage organs such as tubers and fruits. To boost expression of plastid transgenes in potato tubers, we constructed a two-part regulatory system in which a plastid GFP reporter gene is under the control of an engineered PPR protein expressed from a tuber specific promoter in the nucleus (Figure 2). Because the endogenous potato PPR10 protein does not recognize the introduced binding site, GFP accumulates only to low levels in leaves and tubers (Figure 3B). However, tuber-specific expression of the engineered PPR10 enhanced GFP accumulation to about 1.2% of total soluble protein in tubers, a ~20x increase, while having little impact on GFP production in leaves (Figure 3C). Thus, expression of chloroplast genes from an

engineered PPR10 binding site enables tuber-specific expression of recombinant proteins, without impact on vegetative growth. These experiments are carried out in collaboration with Prof. Alice Barkan, Oregon State University, Eugene, OR.



Figure 2. Tuber-specific protein expression regulated by the PPR10 RNA binding protein in potato. (A) In the leaf the engineered PPR10^{GG} RNA binding protein is not expressed due to the inactivity of the tuber-specific patatin promoter. (B) In the potato tuber, the PPR10GG protein is expressed from the patatin promoter which binds to the engineered PPR10^{GG} binding site (PBS^{GG)} of amyloplasts-encoded GFP facilitating high-level expression of GFP in tubers.



Figure 3. Expression of the engineered PPR10^{GG} protein selectively enhances GFP levels in tuber amyloplasts. GFP accumulation is shown as % of total soluble protein. (A) Wild type potato. (B) Amyloplast-encoded GFP expressed with the engineered binding site accumulates to low levels in leaves and tubers. (C) Expression of PPR10GG protein from the tuber-specific patatin promoter selectively enhances GFP accumulation from the engineered binding site in potato tubers.

Dr. Pal Maliga, Distinguished Professor

Phone: 848-445-5329 Email: maliga@waksman.rutgers.edu www.waksman.rutgers.edu/maliga

Lab Members

Dr. Zora Svab, Research Staff Dr. Aki Matsuoka, Postdoctoral Associate Lisa LaManna, Graduate Student Qiguo Yu, Graduate Fellow Amanda Chen, Undergraduate Student Mugdha Parulekar, Undergraduate Student Juliana DiGiacomo, Undergraduate Student



MESSING LAB Molecular Biology of Plant Development



Dr. Joachim Messing University Professor

Introduction

The Messing lab works in plant genetics and genomics. and has published in several categories including Shotgun DNA Sequencing, Bioenergy, Genome Structure, Protein Quality, RNAi, Genome Evolution, and Epigenetics. Publications during the last academic year reported in most of these categories.

Protein Quality

Interestingly, the emerging tariffs imposed on soybeans illustrate that a major shift in world food supply has occurred. Higher living standards raised the demand on animal protein, which in turn required an increase in soybean production. Why?

Livestock is fed with corn because it is the highest yielding grain on earth. Corn seeds are mainly composed of starch and about 10% protein. However,

the protein is deficient in certain essential amino acids, lysine and methionine. Essential amino acids are like vitamins, which animals and people must consume with their diet. For instance, lack of methionine causes lack of zinc retardation and has been shown to cause growth retardation in children in developing countries, where corn is a staple, a case, that is reminiscent of golden rice, which is enhanced with vitamin A. Therefore, corn is known for poor protein quality and requires supplementation of soybeans for a lysine balance, which explains the increased demand on soybeans. Although China is now the second largest producer of corn



after the US, it cannot produce enough soybeans. Moreover, soybean yield increases have been mainly achieved with GMO varieties that are herbicide tolerant and compete more efficiently for sun energy under dense planting. Although GMO soybeans can be imported to China, but not grown there.

Animal feed composed of 75% corn and 15% soybean still needs higher methionine levels, low in both seeds, which is produced chemically and is predicted to reach annual sales of USD 7.26 billion by 2022. Moreover, such supplementation would not be permitted for human consumption because the methionine is a racemic mixture. Therefore, we have investigated whether instead of increasing yield of crops we could improve protein quality. Such a strategy would allow us to reduce the demand of land and water needed to produce sufficient food, which also would have a positive impact on a stable climate.

Maize seed proteins have evolved to achieve the storage of reduced nitrogen and sulfur. Indeed, maize seed storage proteins, also called zeins, are a family of proteins that could be divided into two groups, alpha zeins, the major group representing 60% of the total protein, and beta, gamma, and delta zeins, the minor group, rich in cysteine and methionine, the two sulfur amino acids. To achieve a better balance of essential amino acids, would require the reduction of alpha zeins in favor of lysine-rich proteins and the enhancement of the minor group of zeins.

In previous studies, we had shown that maize inbreds vary in their seed methionine content, largely due to post-transcriptional regulation of gene expression of the 10 kD delta zein. Moreover, repression of higher levels of 10 kD zein mRNA appears to be dominant, but dominance can be avoided, when the non-coding part of the mRNA is replaced by one of the 27 kD gamma zein mRNA, raising the methionine levels of the 10 kD zein protein in seeds. However, this does not result in higher levels of total sulfur in the seed because higher levels of 10 kD zein led to a reduction of the other sulfur-rich proteins in the minor group of zeins. It appears that sulfur levels in the seed undergo a balancing of gene expression within the minor group of zeins. If seed methionine levels are regulated at the post-transcriptional level, the question arises whether this is due to the supply of the amino acid. Methionine is produced like all amino acids during photosynthesis and then transported to the seed during senescence. In the seed, free methionine levels stay low because it is incorporated into proteins during seed development. The 10 kD zein protein described above has more than 20% methionine residues, making it an important sink for free methionine that is produced during photosynthesis. We then asked what could limit the source of methionine?

Sulfur is an important mineral that is taken up by plants from the soil. Like nitrogen it is oxidized and must be reduced. Plants and bacteria are capable to do so. Photosynthesis is carried out in chloroplasts and there are two key enzymes required for reduction of sulfur and the formation of sulfur amino acids, APS reductase (APR) and Serine acetyltransferase (SAT). The first carries out the reduction from sulfate to sulfite and the second activates another amino acid, serine the precursor for cysteine. Cysteine can then be converted into methionine (see Figure; taken from Planta et al, 2017).



Given this metabolism, the question arose whether we could increase the reduction of sulfur and thereby raise the levels of methionine biosynthesis. This question had already been asked by the Leustek laboratory on Cook campus before, but resulted in maize plants retarded in growth. Growth interference was reasoned to be due to the accumulation of toxic intermediates because of the constitute expression of the corresponding bacterial enzymes in maize. If that were the case, we thought this could be circumvented by expressing the foreign genes only in leaf cells, where photosynthesis occurs.

Indeed, this was the solution and we found not only higher levels of the 10 kD zeins in the seed, but also could show that the endogenous APR mRNA was decreased, when bacterial APR mRNA was overexpressed in leaves. Therefore, the explanation appears to be that at least in maize sulfur reduction is feedback regulated, providing a threshold level of sulfur amino acids that can be synthesized. Moreover, increased sulfur reduction did not cause a rebalancing of the minor group of zeins as described above, but raised total sulfur in the seed. We therefore can conclude that the bottleneck in seed methionine is primarily the reduction in sulfur during photosynthesis and not the expression of seed storage proteins.

To further investigate the re-balancing of the minor group of zeins, we also crossed the enhanced APR PE5 line with a line expressing RNAi against the gamma zeins. The gamma zeins contain primarily cysteines rather than methionines. The lack of sulfur storage in gamma zeins appears to be compensated by the beta and delta zeins, but, to our surprise, lysine-rich proteins got also re-balanced raising the total lysine levels at the same time. Therefore, the combination of PE5 and gamma RNAi resulted in seeds that had a perfect kernel phenotype. In addition to elevated levels of lysine and methionine in the seed, kernels exhibited also a hard shell, which is absent in classical high-lysine corn but required for storage and transport of maize grain.

Potential connection between domestication and environment

Even with enhanced reduction of sulfur during photosynthesis, ultimately it is the availability of inorganic sulfur in the soil that is the limiting factor. Therefore, soil conditions play a critical role ensuring seed protein quality. Could changing soil conditions and the geographic distribution of plants have an impact on genome dynamics and thereby

genes that are critical for sulfur storage. In this respect, maize is a unique example of crop domestication because its predecessor, the teosintes, is not only still available, but can be crossed with modern maize, providing us with genetic approaches to identify those genes that underwent changes during domestication. We thought that we could compare the coding regions of the minor group of zeins between teosintes and modern lines of maize. Therefore, haplotypes of these genes in 60 maize and teosinte lines have been sequenced, exhibiting an uneven distribution of insertion/deletions and single nucleotide polymorphisms. Indeed, evolutionary analysis suggests that a subset of sulfur-rich zeins underwent environmental adaptation during the 9,000 years of maize domestication.

Gene balancing after polyploidization

Another evolutionary study is related to the polyploidy of maize. Maize arose from the hybridization of two diverged progenitors about 4.8 million years ago long before domestication 9,000 years ago. We previously have shown how homoeologous chromosomes underwent drastic rearrangement primary through a burst of retrotranspositions, chromosomal expansion and contraction. These changes also resulted in deletions of gene copies. We investigated whether such changes in gene copy number had a certain bias. We could show that a class of proteins that are sensitive to protein interactions like co-chaperones are subject to gene balancing pressures, which is conserved throughout evolution.

Biofuel studies

Besides the use of feed, grains have also become the target of alternative energy sources. One of the grasses, sorghum can be used as a model for investigating how reduced carbon is allocated during plant growth. Because sorghum varieties can differ significantly in the amount of stem sugar, it is possible to use genetics to identify genes that regulate carbon allocation in the stem during growth. Levels of sugar are usually measured with a hand-hold refractometer and scaled in Brix units. Because complex metabolisms during plant growth are regulated by many genes, measurements of stem sugar require large populations of progeny to be sampled at different time points of growth, represented by internodes, in random field plots, and in replicates. Therefore, a high-throughput method became essential to be developed for this purpose. Switching to a phenol-sulfuric acid assay, we could adapt sample analysis to a 96-well plate pipetting format and a robotic liquid handling system, which showed the variation in sugar accumulation among 35 different sorghum accessions.

Dr. Joachim Messing, Director Distinguished University Professor of Molecular Biology Selman A. Waksman Chair in Molecular Genetics

Phone: 848-445-4257 Email: messing@waksman.rutgers.edu www.waksman.rutgers.edu/messing

Lab Members

Professor Wenqin Wang, Visiting Scientist Dr. Jiaqiang Dong, Postdoctoral Fellow Dr. Yin Li, Postdoctoral Fellow Dr. Chenxu Liu, Postdoctoral Fellow Dr. Zhiyong Zhang, Postdoctoral Fellow Paul Fourounjian, Graduate Student Jose Planta, Graduate Student Jose Planta, Graduate Student Jennifer Ayer, Laboratory Technician Rushabh Mehta, Undergraduate Student Niketkumar Patel, Undergraduate Student Gyeong Min Lee, Undergraduate Student



CORE FACILITIES

Waksman Confocal Imaging Core Facility

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

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

The Waksman Core Facility has approximately 60 trained users, primarily Waksman researchers, from eighteen laboratories and is used an average of 65 hours per week. The Confocal Manager provides training, troubleshooting, and consultation on the use of our confocal microscopes. The future aim of the facility is to continue to provide exceptional imaging capabilities to Waksman researchers.



Nanci S. Kane, Manager

Laboratory Researcher IV Phone: 848-445-0250 Email: confocal@waksman.rutgers.edu www.waksman.rutgers.edu/confocal



Waksman Genomics Core Facility

Waksman Genomics Core Facility (WGCF) is a state-of-the-art laboratory facility, providing 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. One of the main driving force is to provide latest sequencing service across the entire spectrum of user ranging from highest throughput sequencing to small sequencing need. WGCF has three sequencers covering broad range of NGS requirements.

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



Sequel



MiSeq

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 leaning curve and focus on the result that are both statistically and biologically meaningful. WCGF 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



Complete Bacterial Genome using Sequel

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

Sequencing Applications	NextSeq500	MiSeq	PacBio Sequel
Wole Genome Resequencing	$\sqrt{\sqrt{\sqrt{2}}}$	\checkmark	$\checkmark\checkmark$
De Novo Sequencing	$\sqrt{\sqrt{\sqrt{2}}}$	\checkmark	ノノノノ
Exome Sequencing	ノノノノ		
Target Resequencing	$\checkmark\checkmark$	$\sqrt{\sqrt{\sqrt{1}}}$	
mRNA Sequencing	$\sqrt{\sqrt{\sqrt{2}}}$		$\checkmark\checkmark$
Small RNA Sequencing	$\sqrt{\sqrt{\sqrt{2}}}$		
ChIP Sequencing	ノノノノ		
Metagenome Analysis	\checkmark	\checkmark	
Methylation Analysis	$\sqrt{}$	$\sqrt{\sqrt{\sqrt{2}}}$	$\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{$

Dr. Dibyendu Kumar, Director Genomics

Core Facility/Research Associate Phone: 848-445-4737 Email: dk@waksman.rutgers.edu www.waksman.rutgers.edu/genomics

Lab Members

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



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.

Joshua Gager, Greenhouse Supervisor

Phone: 848-445-5293 Email: gager@waksman.rutgers.edu www.waksman.rutgers.edu/greenhouse

Cell and Cell Products Fermentation Facility

The state of the art Cell and Cell Products Fermentation Facility offers cost-effective services to both academic and industrial scientists.

For over 70 years, we have provided expertise in Fermentation Biotechnology to Pharma, Food /Flavor and Cosmetic enterprises across the US.

Our state of the art facility is flexible and versatile supplying biomolecules including; proteins, enzymes, antibiotics, growth factors, natural flavors and cosmetic substrates to industry engineering; microbiological and molecular groups.

Expert technicians follow users protocols or design customized processes to optimize research and production results.

Our bioreactor volumes range from 32 to 800 liters. This allows us to conduct both scale-up and production to maximize research potential both upstream and downstream processing.

We follow cGMP/cGLP guidelines, but only produce pre-clinical grade materials.



125 Liter Bioreactor



1000 Liter Tank



23 Liter Bioreactor

Kenneth Callanan, Director of Operations

Phone: 848-445-2925 Email: callanan@waksman.rutgers.edu www.waksman.rutgers.edu/fermentation

Lab Members

Amanda Rodriguez, Laboratory Researcher III and Lab Manager Dr. Sergey Druzhinin, Lab Researcher IV



TRAINING FUTURE LEADERS

Postdoctoral Fellowships



Predoctoral Fellowships



Graduate & Undergraduate Courses



High School Outreach/WSSP



CHARLES AND JOHANNA BUSCH FELLOWS

Postdoctoral Research



Xue Mei Singson Lab

Research summary

Fertilization is a fundamental process in sexual reproduction. Sperm and egg recognize and bind to each other, and fuse to form a zygote. The fertilized egg then activates, initiates the first mitosis and begins embryonic development. How the sperm and egg interact and how the egg activates have been longstanding questions in the field. In mammals, only several molecules are known to be required for sperm-egg fusion, with the sperm IZUMO and egg JUNO being the only known binding pair. The Singson lab studies gamete interactions and activations using the nematode *C. elegans*. Using forward genetic screens, we have identified quite a few "sperm function" genes that encode sperm surface molecules that are thought to mediate sperm-oocyte interaction. My project continues to identify gamete function genes through genetic screens, and I also use a reverse genetic method to hopefully identify oocyte genes that function during fertilization and egg activation.

spe-51(as39) is a sperm function mutant that shows the classic *spe-9* phenotype. Mutant hermaphrodites are sterile but can be rescued by mating with wild type males. The mutant sperm look indistinguishable from wild type, can activate

both *in vitro* and *in vivo*, and out compete hermaphrodite self sperm, similar to the wild type sperm. Using classical mapping strategies and whole genome sequencing, we identified T22B11.1 as the affected gene in *as39*. Interestingly, the SPE-51 protein is a single-pass transmembrane molecule that has an immunoglobulin-like fold. This structure resembles that of SPE-45, another sperm function molecule, and also the mammalian IZUMO. Ongoing work focuses on localization of SPE-51 and its interactions with other sperm surface proteins.

In *C. elegans*, EGG-1 and EGG2 are the only known egg surface proteins that are required for fertilization and they don't bind with known sperm surface proteins. In order to find more molecules, I looked at oocyte-expressed genes and sorted out all the transmembrane as well as GPI-anchored proteins. Here, I focus on paralogous pairs that may be functionally redundant and thus be missed in forward genetic screens. I have identified about 50 pairs, and I plan to use RNAi to test the functions of these candidates.

CHARLES AND JOHANNA BUSCH FELLOWS

Predoctoral Research



Chao Bian Dong Lab

Signaling events in stomatal development and patterning

Stomata guard cells control gas exchange between plants and the atmosphere. Stomatal development and patterning is tightly regulated by asymmetric cell division (ACD) in Arabidopsis. The initiation of stomatal ACD requires the activity of a bHLH transcriptional factor SPCH (MacAlister et al., 2007). Several kinases have been identified to directly regulate SPCH protein stability through phosphorylation, whereas corresponding phosphatases have not been identified until my recent research on the PP2A family. PP2A phosphatases form heterotrimeric complexes, including a scaffold subunit A (3 members in Arabdiopsis), a regulatory subunit B (17 members), and a catalytic subunit C (5 members) (Farkas et al., 2007). PP2A activity has diverse cellular functions, however, no evidence has shown that PP2A regulates stomatal development and patterning. I use multifaceted strategies that combine genetics, cell biology and biochemical assays to investigate how PP2A regulates stomatal differentiation and division in Arabidopsis.

In this study, multiple loss-of-function PP2A-A or PP2A-C subunit mutants generated by CRISPR-Cas9, RNAi, and T-DNA insertion methods consistently

reduced stomatal production, suggesting PP2A complex promotes stomatal development. The PP2A-specific inhibitor (Cantharidin) also suppresses stomatal production and, interestingly, suppresses the SPCH protein abundance without affecting its transcriptional expression. Further I found proteasome-specific inhibitor MG132 released the suppression on SPCH protein by PP2A inhibitor, supporting PP2A regulates SPCH protein stability. Genetic analysis by using CRISPR-pp2aa mutants with key components in stomatal pathway indicates PP2A functions downstream of MAPK cascades but upstream of SPCH. Moreover, I found non-phosphorylation SPCH variances are more resistant to Cantharidin compared with SPCH protein, suggesting the correct PP2A function is important for regulating SPCH phosphorylation status.

Thus my data strongly support the hypothesis that PP2A phosphatase complex de-phosphorylates and stabilizes SPCH to promote stomatal production. The discovery of PP2A function would be the first to report as a direct phosphatase of the SPCH master regulator in stomatal production. Therefore, my study is anticipated to shed new insights into the functions of classic phosphatases in plant development.



Qiguo Yu Maliga Lab

Plastid Transformation in Arabidopsis thaliana

Deletion of the *acc2* nuclear gene makes Arabidopsis hypersensitive to spectinomcyin, the selective agent for the identification of transplastomic events. We have found that plastid transformation efficiency is 100x higher in the acc2knockout background than in wild type Arabidopsis. However, it was difficult to obtain fertile transplastomic plants in the available Col-0 acc2-1 and Sav0 hypersensitive accessions (Yu et al. Plant Physiol. 175: 186, 2017). We deleted the *ACC2* gene using CRISPR/Cas9 in the regenerable RLD and Ws ecotypes and have shown that deletion of ACC2 makes the seedlings hypersensitive to spectinomycin. Selection for spectinomycin resistance in bombarded leaf cultures of the hypersensitive RLD and Ws backgrounds yielded putative transplastomic events at a high frequency. Classification of the clones as transplastomic lines or spontaneous spectinomycin resistant mutants and demonstration of seed transmission of spectinomycin resistance is in progress.

Engineered RNA-binding protein for high level tissue-specific gene expression in plastid

Constitutive, high-level expression of transgenes in the chloroplast compromises plant growth and interferes with development. Furthermore, plastid transgenes are expressed at extremely low levels in storage organs such as tubers and fruits. To boost expression of plastid transgenes in potato tubers, we constructed a two-part regulatory system in which a plastid GFP reporter is under the control of an engineered PPR protein expressed from a tuber specific promoter in the nucleus. Because the endogenous potato PPR10 protein does not recognize the introduced binding site, GFP accumulates only to low levels in leaves and tubers. However, tuber-specific expression of the engineered PPR10 enhanced GFP accumulation from 0.06% to about 3% of total soluble protein in tubers while having little impact on GFP production in leaves.



Yuan Zhang Dismukes Lab

Metabolic engineering of *Nannochloropsis Oceanica* CCMP1779 for biofuel production

Algae are the most productive photosynthetic organisms at solar energy conversion, by far. Many algal species are also known to accumulate high concentrations of oil (Triacylglycerides, TAGs) up to 50% or more of their cellular dry weight. Recently, the unicellular marine microalga *Nannochloropsis Oceanica* CCMP 1779 (hereafter *N. oceanica*) is drawing considerable interest as a biofuel resource due to its high lipid content and well-developed genetic tools (Liu, et al. 2013; Vieler, et al. 2012; Wang, et al. 2016). In this study, we explored two metabolic engineering approaches to enhance the lipid content for potential biofuel production. First, a yeast gene coding for cytosolic glycerol-3-phosphate dehydrogenase (G3PDH) was expressed in *N. oceanica* under the control of a bidirectional promoter. This modification led to up to a 3-fold increase in the cellular TAG content of the transgenic algal lines. Second, an endogenous gene coding for Citrate Synthase (CIS) was knocked down via RNA interference (RNAi). The suppression of CIS increased the cellular TAG level up to 2.3-fold in the transgenic lines. Furthermore, the double mutant co-expressing the yeast G3PDH

and the CIS inverted repeats for RNAi was constructed by utilizing a stacking vector with an endogenous bidirectional promoter. It was demonstrated that simultaneously regulate the two genes will further increase the desirable lipid content in comparison to single-gene regulation. The obtained results reveal the potential of metabolically modified microalgal biomass for biofuel production.



Jing Lin Padgett Lab

Some Disease-Associated Mutations of TGF-β Receptors Result in the Aberrant Trafficking

Transforming growth factor- β (TGF- β) plays an important role in cellular proliferation, differentiation and apoptosis. Over 30 years of research on this signaling family shows that TGF- β signal transduction activation depends on its kinase receptors, type I and type II, which will form a heteromeric receptor complex upon ligand binding. Many cancers and Marfan syndrome-like diseases are caused by different mutations in the kinase domain in either type I or type II receptor. Originally, it was thought these mutations inactivate the kinase activity in receptors causing the above diseases. However, recent studies have shown that mutant TGF- β receptors still retain kinase activity. Interestingly, receptor mutations that alter normal trafficking reside in the same region as many mutations found in MFS-like diseases and many TGF- β cancers. Based on this close proximity of trafficking mutations and disease mutations, I hypothesize that the abnormal trafficking of the TGF- β receptors may contribute to these diseases. To test this model we introduce these mutations in the orthologous receptors, encoded by the genes sma-6 and daf-4, which encode type I and type II receptors in

C. elegans respectively, and examine how their trafficking is altered. Our work showed that some of these mutations strongly affect the levels of the receptors as well as an altered delivery of receptors to the apical cell surface. Meanwhile, some of these missense mutations still retain kinase activity. These findings suggest a basis for the disease.



Qiujie Liu Gallavotti Lab

Research Summary

Zea mays is one of the most economically important crops in the world as well as an important model system for studying plant development. The shoot architecture of maize is primarily determined by apical and axillary meristems. Axillary meristems are groups of stem cells at the axils of true or modified leaves, which give rise to branches and flowers, thus directly affect maize yield. *barren* inflorescence mutants (*bifs*) are maize mutants defective in reproductive organogenesis and are characterized by a reduction in flower and branch number in both tassels and ears. The defects observed in several *bif* mutants are caused by mutations in genes regulating the plant hormone auxin, including biosynthesis, transport and signaling.

My project is to study a new *bif*-like mutant called *needle1 (ndl1)*, which is defective in inflorescence development. *ndl1* mutants develop tassels with fewer branches and spikelets, ears with unorganized kernels and partially barren tip and shorter primary roots. My SEM and *in situ* hybridization results suggest that axillary meristem initiation and development are defective in *ndl1* mutants. Moreover, *ndl1* is a temperature sensitive mutant and the strong

genetic interactions of *ndl1* with auxin signaling (*Bif1*) and biosynthesis mutants (*spi1*) suggest that *NDL1* may influence auxin biology.

NDL1 encodes a mitochondria-localized protein that mediates the degradation of a number of membrane proteins, functions in the assembly of respiratory complexes and affect the splicing of several mitochondrial genes. *ndl1* mutant inflorescences have high accumulation of Reactive Oxygen Species (ROS) as well as high expression of *ALTERNATIVE OXIDASE-2* (*AOX2*) and *SMALL HEAT-SHOCK* PROTEIN FAMILY (*HSP20*), suggesting defects in the respiratory chain.

This mutant should help us to better understand the relationship between mitochondria and maize inflorescence development, as well as the interaction of auxin pathway and mitochondria perturbation, which are critically important for maize development.



Lin-Ing Wang McKim Lab

Research Summary

In meiosis I, homologous chromosomes are guided to opposite poles, while sister chromatids are directed to the same poles for accurate segregation. A unique mechanism to ensure that sister chromatids co-orient and segregate to the same pole in metaphase I involves aligning sister kinetochores side-by-side. We have previously suggested that this sister centromere co-orientation depends on the kinetochore protein SPC105R/KNL1. We have now found that Protein Phosphatase 1 (PP1-87B) is required to maintain sister centromere co-orientation during meiosis I in Drosophila oocytes. In Pp1-87B RNAi oocytes, sister centromeres precociously separate. While this defect depends on Aurora B kinase, surprisingly, it does not depend on separase, which releases cohesins. In contrast, the precocious separation of centromeres in Spc105R RNAi oocytes is separase dependent. Our evidence is consistent with a model where centromere separation in *Pp1-87B* RNAi meiosis I oocytes, and in wild-type meiosis II oocytes, is driven by forces that act in the presence of intact cohesin molecules. However, the cohesins at the sister centromeres are moved out of the way in a separase and Wapl independent manner. Consistent with this model, the precocious separa-

tion of sister centromeres in meiosis I of *Pp1-87B* RNAi oocytes depends on end-on attachment of microtubules to the kinetochores, as well as Polo kinase and BubR1, two proteins known to promote stable microtubule-kinetochore attachments. Thus, we have found evidence of a pathway for co-orientation that begins with cohesion but ends with PP1-87B in regulating spindle forces and possibly in remodeling of the centromere domain, but not in cohesion release. In addition, we have also found C(3)G, the transverse element of the synaptonemal complex (SC), antagonizes PP1-87B in regulating co-orientation. Our results provide evidence for a functional role of the centromeric SC which lasts until metaphase I whereas the rest part of SC dissemble in late prophase.



Zhenru Zhou Irvine Lab

Research Summary

Understanding the mechanisms controlling tissue growth to form correct organ shape is a long-standing question in developmental biology. Altered tissue patterning due to loss of Ds-Fat signaling pathway can cause abnormal organ shape, such as shorter and rounder wings and legs in flies, and mitral valve prolapse and skeletal malfunction in humans.

The *Drosophila* wing normally has an elongated shape, which has been attributed to a preferential orientation of mitotic spindles along the proximal-distal axis. Orientation of mitotic spindles, and consequently of cell divisions, is believed to be a fundamental morphogenetic mechanism in multicellular organisms. The supposition that spindle orientation contributes to wing shape was suggested by observations that mutation or knock-down of components of the Ds-Fat pathway results in both rounder wings, and loss of the proximal-distal bias in spindle orientation. To directly evaluate the potential contribution of spindle orientation to wing morphogenesis, we assessed the consequences of loss of the *Drosophila* NuMA homolog Mud. We establish that loss of Mud randomizes spindle orientation, but has no effect on wing shape. Analysis of growth and cell dynamics in

developing discs and in ex vivo organ culture reveals that the absence of oriented cell divisions is compensated for by an increased contribution of cell rearrangements to wing shape. Our results indicate that oriented cell divisions play no significant role in wing morphogenesis, nor do they appear to play a significant role in the morphogenesis of other *Drosophila* appendages. Moreover, our results suggest that normal organ shape is not achieved through a summing of individual cell behaviors. Instead wing shape could be specified through establishment of tissue wide stresses that dictate an overall arrangement of cells without specifying the specific cell behaviors needed to achieve it.

BENEDICT MICHAEL FELLOW

Predoctoral Research



Nathaly M. Salazar-Vasquez Rongo Lab

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 programed 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.

Mitochondria are highly dynamic organelles existing in states of fusion and fission and moving 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, particularly in specialized cell types like neurons.

Mitochondrial function is regulated by changes in organelle size, number, and morphology, and these *mitochondrial dynamics* are the result of the balanced processes of organelle fission and fusion. 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.

WAKSMAN FACULTY COURSES

Undergraduate and Graduate

Undergraduate

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

Graduate

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

Faculty-Taught Graduate and Undergraduate Courses 2018-2019

Advanced Inorganic Chemistry Core Seminars in Plant Biology **Developmental Genetics** Essential Skills I Essential Skills II Experimental Methods in Molecular Biosciences Fundamentals of Molecular Biosciences Genetic Analysis II Harnessing Solar Energy Honors Introduction to Molecular Biology and Biochemistry Research Human Genetics Introduction to Molecular Biology and Biochemistry Research 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 Research in Chemistry Seminar in Molecular Biology and Biochemistry Structural Biology, Structural Biophysics, and Chemical Biology of Transcription The Genetics and Cell Biology of Fertilization Thesis Writing and Communication in Genetics

WAKSMAN STUDENT SCHOLARS PROGRAM

High School Outreach



Summary

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

For 25 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 2017 WSSP consisted of two interrelated parts: a Summer Institute (SI) and an Academic Year Program (AYP). In July 2017, 32 students and 6 teachers from 30 high schools attended a 13-day SI at the Waksman Institute. The SI consisted primarily of daily seminars and laboratory activities that focused on molecular biology and bioinformatics. The laboratory sessions introduced students and teachers to the content, background information, and laboratory procedures that were needed to carry out the research project at their schools during the academic year. Students and teachers used Internet resources to process and analyze their data. Some of the seminars that were presented dealt with bioethical issues and career opportunities in the fields of science, technology, engineering, and mathematics (STEM). Scientists met with participants to discuss recent research developments in the fields of plant ecology and Next Generation DNA sequencing.

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

Six 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 11, 2018 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 5 high schools in Maryland. A two-week summer Institute for 4 teachers and 14 students was conducted at the Law-rence Livermore National Laboratory, Livermore, CA, 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 1223 students from 48 different high schools in NJ, MD, PA, CA and HI participated in, and contributed to, the WSSP this past year.

The Research Question

The 2017 research project focused on identifying the genes and proteins of the duckweed, *Landoltia punctata*. Duckweeds are fresh water aquatic plants that can be potentially used in bioremediation and/or as biofuels. 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 2017-2018 SI and AYP, over 2600 plasmid clones were purified and 1722 were sequenced. To date, 1421 DNA sequences have been analyzed by the students. 907 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 2017, 22 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 2018 summer.

Dr. Andrew Vershon, Director WSSP, Professor

Susan Coletta, Educational Director

Phone: 848-445-2038 Email: coletta@waksman.rutgers.edu www.waksman.rutgers.edu/education/scholars

Dr. Janet Mead, Laboratory Director

John Brick, Laboratory Assistant April Rickle, Undergraduate Work Study Student



SHARING OUR DISCOVERIES

Waksman Annual Retreat



Presentations & Meeting Abstracts



Patents & Publications



WAKSMAN ANNUAL RETREAT

Rutgers University Inn & Conference Center

September 12, 2017

Presentations

- Konstantin Serverinov: Capturing DNA gyrase in flagrante delicto
- Kishore Joshi Rongo Lab: Biogenic Amine Signaling Promotes Protein Homeostasis
- Libing Yu Ebright Lab: The Mechanism of Transcription Start Site Selection
- Qiguo Yu Maliga Lab: Exploring the potential of Engineered PPR10 Protein to Regulate Plastid Transgene Expression
- Consuelo Ibar Irvine Lab: Tension-dependent regulation of mammalian Hippo signaling through LIMD1
- Amber Krauchunas Singson Lab: Sperm activation pathways in C. elegans
- Mary Galli Gallavotti Lab: Characterizing the genome-wide DNA binding behavior of maize ARF transcription factors using DAP-seq
- Jose Planta Messing Lab: Variations in seed storage protein accumulation enhance the nutritive value of maize kernels
- Dr. Badri Singh Steward Lab: Function of RNA Hydroxymethylation in Drosophila melanogaster from genome-wide analysis of TET dioxygenase
- Jeremy Bird Nickels Lab: CapZyme-Seq comprehensively defines promoter-sequence determinants for RNA 5' capping with NAD+
- Xiaoyu Guo Dong Lab: BSU phosphatases participate in the polarity complex in stomatal asymmetric division
- Juan Wang Barr Lab: Biogenesis and Function of Social Extracellular Vesicles (EVs)
- Min Tu Genomics Core Facility: Waksman Genomics Services

POSTER SESSION

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

Barr Lab

- Jyothi Shilpa Akella, Juan Wang, Fatima RIzvi, Ken Nguyen, David Hall, and Maureen Barr: RAB-28 regulates extracellular vesicle release in C.elegans.
- Deanna De Vore and Maureen Barr: Males need molecular promiscuity for mating: Extracellular matrix genes mec-1, mec-5, and mec-9 are not just for touch neurons
- Yasmin Ramadan, Robert O'Hagan, Amanda Gu, Elizabeth De Stasio, Harold Smith, Andy Golden and Maureen Barr: Pathways that regulate ciliary stability, structure, and transport in response to microtubule post-translational modifications

Dismukes Lab

- Xiao Qian, Bryan Mejia-Sosa, Tie Shen, Apostolos Zournas, Gennady M. Annanyev, Desmond Lun, G. Charles Dismukes, NSF-MCB: Light and Dark Side of Central Carbon Metabolism in Cyanobacterium Synechococcus sp. strain 7002
- Yuan Zhang, Gennady Ananyev, Yunbing Ma, Hoa Vu, Jun Cheng, G. Charles Dismukes, GCEP: Forward and reverse genetic engineering in Nannochloropsis oceanica for higher lipid production
- Gennady Ananyev, Colin Gates, Aaron Kaplan, and G. Charles Dismukes: Record Growth Rate in the Microalga Chlorella ohadii

Dong Lab

- Chao Bian and Juan Dong: PP2A phosphatases are novel positive regulators in Arabidopsis stomatal development
- Dongmeng Li and Juan Dong: New regulators for membrane trafficking in plant cell polarity

Dooner Lab

• Wenwei Xiong and Hugo Dooner: A sequenced-indexed reverse genetics resource for maize

Ebright Lab

- Wei Lin and Richard Ebright: Structures of Mycobacterium tuberculosis transcription initiation complexes: extracytoplasmic function (ECF) sigma factor Mycobacterium tuberculosis transcription initiation complex
- Yu (Jeff) Liu and Richard Ebright: Structural and mechanistic basis of reiterative transcription initiation
- Libing Yu and Richard Ebright: The Mechanism of Transcription Start Site Selection

Gallavotti Lab

- Qiujie Liu and Andrea Gallavotti: Characterization and cloning of a temperature sensitive maize mutant affecting inflorescence development
- Zongliang Chen and Andrea Gallavotti: The dominant effects of Barren inflorescence3 on initiation and maintenance of meristem development in maize
- Xue Liu and Andrea Gallavotti: The transcriptional co-repressor REL2 regulates meristem Initiation, determinacy and maintenance in maize inflorescences

Genomics Core Facility

• Yaping Feng and Dibyendu Kumar: Various analytical methods to expand the knowledge of gene expression regulation

Irvine Lab

• Zhenru Zhou and Kenneth Irvine: The mechanism of organ shape control

Maliga Lab

• Lisa Lamanna, Megan Kelly, Amanda Chen, Qiguo Yu and Pal Maliga: Generating fertile chloroplast transgenics in Arabidopsis

McKim Lab

- Janet Jang, Lin-Ing Wang, Arunika Das, Amy Gladstein and Kim S. McKim: Aurora B/phosphatase antagonism underlies maintenance of meiotic chromosome and spindle organization in Drosophila oocytes
- Justin Mathew, Nikunj K. Patel, Mercedes Gyuricza, Kim S. McKim: Exploring C(2)M and its ability to promote assembly of the synaptonemal complex

Messing Lab

- Paul Fourounjian and Joachim Messing: Small RNA and Degradome Sequencing of the aquatic plant Spirodela polyrhiza
- Yin Li and Joachim Messing: Transcriptome and metabolome of sugar accumulation during plant growth
- 23. Jiaqiang Dong and Joachim Messing: Investigation of the redundancy of transcription factors expressed during seed development
- Zhiyong Zhang and Joachim Messing: Functional study of teff alpha-globulins in maize endosperm

Nickels Lab

• Srujana S Yadavalli and Bryce Nickels: Antimicrobial peptides trigger a division block in E. coli through PhoQ signaling

Padgett Lab

• Jing Lin, Mehul Vora, Ryan Gleason, Nanci Kane, and Richard W. Padgett: Some Disease-Associated Mutations of TGF-β Receptors Result in the Aberrant Trafficking

Rongo Lab

• James Mullin and Christopher Rongo: Regulation of Neuronal Mitochondria by Mitophagy

Severinov Lab

- Anna Shiriaeva, Ekaterina Savitskaya, Ekaterina Semenova, Kirill A. Datsenko and Konstantin Severinov: RecBC influences CRISPR Adaptation in the Type I-E CRISPR-Cas System of Escherichia coli
- Ishita Jain, Leonid Minakhin, Vladimir Mekler, Vasily Sitnik, Natalia Rubanova, Kirill A. Datsenko, Konstantin Severinov and Ekaterina Semenova: Laying the seed of the Type V-B CRISPR-Cas system Cas12b

Singson Lab

• Xue Mei and Andrew Singson: Fertilization gene discovery in C. elegans

Waksman Institute Hosted Seminars

- Dr. Andrea Gallavotti, Department of Plant Biology, Rutgers University, "The Control of Maize Architecture: New Insights From Genetic and Geonomic Approaches "September 15, 2018
- Dr, Sven Bocklandt,Bionano Genomics, "Beyond NGS: Bionano Genome Mapping Reveals Structural
- Variation in Cancer and Genitic Disease." October 5, 2017
- Dr. Jun Xiao, Department of Biology, University of Pennsylvania, "Reprogramming of Cell Fate or Function in Response to Environmental or Developmental Cues." December 1, 2017
- Dr.Xing Wang Deng, Professor, Peking University, "Control of Arabidopsis Seedling Development: Emerging out of Darkness & Preparing for a Bright Future." February 16, 2018
- Dr. Jorge Dubcovsky, Plant Geneticist & Biologist, University of California, Davis, "Using Sequenced Mutant Populations to Improve Wheat." February 16, 2018
- Andrew Z. Fire, Nobel Prize Winner, Stanford University, "Opportunistic RNAs and Acquisitive Genomes." March 9, 2018
- Dr. Herta Steinkellner, Institute of Applied Genetics & Cell Biology, University of Natural Resources, "Engineering Protein Glycosylation in Plants." April 20, 2018
- Dr. John Doebley, Genetics, College of Agricultural and life Sciences, University of Wisconsin, Madison,
 "The Genetic Architecture of Maize Domestication: Low Hanging Fruit and Dark Matter"
- Dr. Clyde A. Hutchison Ill, J. Craig Venter Institute, La Jolla, CA "Something Old,Something Blue: From Genome Sequencing to Synthetic Life"

Waksman Student Scholars Programs

- Waksman Student Scholars Summer Institute, Waksman Institute, Rutgers University, July 5-July 21, 2017
- Waksman Institute Summer Experience (WISE June-17), Waksman Institute, Rutgers University, June 19-June 30, 2017.
- Waksman Institute Summer Experience (WISE August-17), Waksman Institute, Rutgers University, July 31- August 11, 2017.

Barr Lab

Albert Einstein College of Medicine, Bronx NY. De-

partment of Anatomy and Structural Biology Symposium for Dr. Peter Satir, October 2017

• Polycystic Kidney Disease Symposium, Kansas University Medical Center, June 29-July 2, 2018

Dismukes Lab

- 233th 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. 2017
- New York Metro Catalysis Society Symposium at Lehigh Univ, 3 posters, Easton PA, Mar 22, 2018
- Rutgers Energy Institute, Rutgers Univ, May 1, 2018
- Gordon Research Conference: Photosynthesis, Grand Hotel, Newry, ME, July 16-21, 2017
- Microbiology at Rutgers, Symposium, Rutgers SEBS, Feb 26, 2017

Dong Lab

- "Intracellular dynamics of polarized proteins in plant cells." Poster talk at Gordon Research Conference – Plant Molecular Biology, Holderness, NH.
- "NRPM, novel signaling regulators at the plasma membrane. 2018 Mid-Atlantic Society of Developmental Biology Region Meeting. Charlottesville, VA.
- "From a cell biological perspective: Function and regulation of MAPK signaling." Seminar at the Department of Horticultural Sciences, Texas A&M University, College Station, TX
- "Positive and negative regulators of cell polarity in Arabidopsis stomatal development." Seminar at the Department of Biology, U. of Pennsylvania. Plant Biology Seminar Series. Philadelphia, PA.
- "Attenuation of the polarity feedback loop in stomatal asymmetric cell division." Cold Spring Harbor Asia Conference on Plant Cell and Developmental Biology, Suzhou, China,
- "Dare to be different: regulators and mechanisms for asymmetric cell division in plants." Seminar at the Department of Plant and Microbial Biology, University of California, Berkeley
- "Positive and negative signaling at the plasma membrane for stomatal ACD." Seminar at the Department of Plant Biology, Carnegie Institution for Science, Stanford University

Dooner Lab

- Du, C., Xiong, W., Chatterjee, M., Li, Y., Wang, Q., Wang, H., He, L., Huang, J., Segal, G., and Dooner, H.K. "A single gene knock-out resource for maize: filling gaps in the genome with targeted Ds-GFP insertions". 2018 Maize Genetics Conference, St. Malo, Brittanny, France 3/22-3/25/2018.
- Li, Y., and Dooner, H.K., "Buried treasures: the maize transposable elements Dotted and Mrh".
 2018 Maize Genetics Conference, St. Malo, Brittanny, France 3/22-3/25/2018.

Ebright Lab

- "Antibacterial drug discovery targeting bacterial RNA polymerase: myxopyronin (Myx)." Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, New Jersey, 2017.
- "RNA polymerase: the molecular machine of transcription." Department of Chemistry, Columbia University, New York, New York, 2017.
- "Using nanopore tweezers to analyze translocation of RNA polymerase on DNA with sequence registration and sub nanometer, sub millisecond spatiotemporal resolution." Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 2017.
- "Therapeutics for drug-resistant bacteria: arylpropionyl phloroglucinols." Centers of Excellence in Translational Research Review Meeting, National Institute of Allergy and Infectious Diseases, Rockville Maryland, 2017.
- "Using nanopore tweezers to analyze translocation of RNA polymerase on DNA with sequence registration and sub nanometer, sub millisecond spatiotemporal resolution." Fusion Meeting on DNA and Interacting Proteins as Single Molecules, Cancun, Mexico, 2018.
- Bird, J., Vvedenskaya, I., Basu, U., Ramachandran, A., Krásny, L., Patel, S., Temiakov, D., Ebright, R.H., and Nickels, B. (2017) RNA capping by non-canonical initiating nucleotides (NCINs): promoter sequence determinants for NAD+ capping by bacterial RNAP, and NAD+ capping by mitochondrial RNAP. Molecular Genetics of Bacteria and Phages, Madison, Wisconsin, August 7-11, 2017.
- Nova, I., Mazumder, A., Laszlo, A., Derrington, I., Gundlach, J., and Ebright, R.H. (2018) Repurposing nanopore DNA sequencing to analyze RNA polymerase translocation at sub-nanometer, sub millisecond spatiotemporal resolution." Fusion Conference, DNA and Interacting Proteins as Single Molecules, Cancun, Mexico, February 21 24, 2018.
- Bird, J., Basu, U., Ramachandran, A.;, Grudzien-Nogalska, E., Kiledjian, M., Temiakov, D., Patel, S., Nickels, B., and Ebright, R.H. (2018) Ab initio RNA

5' NAD+ capping by mitochondrial RNA polymerases. EMBO Meeting on Molecular biology of Mitochondrial Gene Expression, Svartsjö, Sweden, May 20-24, 2018.

Degen, D., Taganov, K. Munguia, J., and Ebright, R.H. (2018) Improved Gram-negative antibacterial activity upon combination of LpxC inhibitor FG 944 and RNA polymerase inhibitors (rifampin or APY281). ASM Meeting, Atlanta, Georgia, June 7-11, 2018.

Gallavotti Lab

- Galli, M., Khakar, A., Chen, Z., Lu, Z., Sen, S., Joshi,
 T., Nemhauser, J.L., Schmitz, R.J., Gallavotti, A.
 Using DAP-seq to map genome-wide ARF transcriptional factor binding events in maize. Maize Genetics
 Conference Abstract 60:T13. Saint-Malo, France,
 March 22-25, 2018.
- Moss, B.L., Cates, R., Dali-Jensen, K., Klaeser, H.,
 Jackson, K., Chen, Z., Gallavotti, A. Functional
 characterization of maize auxin signaling modules in
 yeast. Maize Genetics Conference Abstract 60:P51.
 Saint-Malo, France, March 22-25, 2018.
- Monticolo, F., Zambelli, F., Galli, M., Minow, M.,
 Gallavotti, A., Colasanti, J., Pavesi, G., Rossi, V.
 INDETERMINATE1 direct targets and regulatory
 mechanisms. Maize Genetics Conference Abstract
 60:P58. Saint-Malo, France, March 22-25, 2018.
- Liu, Q., Galli, M., Gallavotti, A. Characterization and cloning of needle1 (ndl1), a temperature sensitive mutant affecting reproductive organogenesis. Maize Genetics Conference Abstract 60:P83. Saint-Malo, France, March 22-25, 2018.
 - Gallavotti, A. Auxin signaling in maize development: new insights from genetic and genomic approaches. University of Pennsylvania, Philadelphia, PA, November 30, 2017.
 - Sen, S., Galli, M., Gallavotti, A., Joshi, T. Development of an informatics analytics workflow for DAP-seq data exploration and validation for AUXIN RESPONSE FACTORS in maize. IEEE BIBM 2017 International Conference on Bioinformatics and Biomedicine, P230. Kansas City, MO, November 13-16, 2017.
 - Gallavotti, A., Liu, X., Camehl, I., Galli, M. New insights into auxin-dependent transcriptional regulation by the maize REL2 transcriptional corepressor.
 FASEB Mechanisms in Plant Development, Saxtons River, VT, July 30-August 4, 2017
- Gallavotti, A. Auxin signaling in maize development: new insights from genetic and genomic approaches. University of Missouri Columbia, Columbia, MO, November 13, 2017.

Irvine Lab

- Wieschaus Symposium, Princeton University, Princeton NJ, December 1 2017.
- EMBO Hippo Pathway Workshop, Rome Italy, October 25-29 2017.
- Cell Contact and Adhesion Gordon Conference. Proctor, NH, June 18-23, 2017.

Maliga Lab

- Pal Maliga, "Progress in Implementing plastid transformation in Arabidopsis thaliana". XXVI Plant and Animal Genome Conference, San Diego, CA, January 13-17, 2018.
- Pal Maliga, "Synthetic biology for regulated chloroplast gene expression". Systems Biology Workshop, AgriBio, Centre for AgriBioscience, La Trobe University, Melbourne, Victoria 3083, Australia, 14 – 23 May 2018.
- Pal Maliga, "Plastid transformation in Arabidopsis". Plant Biology 2018, Annual Meeting of the American Society of Plant Biologists, Montreal, Canada, July 14-18, 2018.
- Qiguo Yu1, Lisa Lamanna1, Megan Kelly1, Kerry Ann Lutz2 and Pal Maliga (2018) "Progress in Implementing Plastid Transformation in Arabidopsis thaliana." XXVI Plant and Animal Genome Conference, San Diego, CA, January 13-17, 2018.
- Lisa LaManna, Qiguo Yu, Megan Kelly, Kerry Lutz, and Pal Maliga. "Plastid Transformation in Arabidopsis thaliana". Plant Biology 2018, Annual Meeting of the American Society of Plant Biologists, Montreal, Canada, July 14-18, 2018.

McKim Lab

- National Institutes of Health (NIH/NIDDK),
- Bethesda, March 29, 2018.

Messing Lab

- Dong, J., Kumar, D., Tu, M. Feng, Y., Gei, F. Messing, J. (2018). Functional analysis of maize kernel development. Annual Maize Genetics Conference, St. Malo, France, March 2018.
- Xiang, X., Zhou, Y., Li, C., Leustek, T., Messing, J., Wang, W., and Wu, Y. (2018). Transcriptome analysis of the metabolically engineered high-met maize varieties. Annual Maize Genetics Conference, St. Malo, France, March 2018.
- Max Planck Institute of Molecular Plant Physiology, Golm, Germany, September 2017
- University of Regensburg, Regensburg, Germany, September 2017
- Henan Agricultural University, Zhengzhou, China, November 2017
- Shandong Agricultural University, Shandong, China,

November 2017

- Washington State University, Pullman, WA, April 2018
- Institute of Crop Science, Zhejiang Key Laboratory of Crop Germplasm, Zhejiang University, Hangzhou, China, June 2018
- Molecular Plant Symposium, Xi'an, China, June 2018

Rongo Lab

• Park, E.C., Tariq, I., and Rongo, C. Abstract title: Impaired Mitochondrial Transport in a C. elegans Tauopathy Model. Presented at the Cell Symposia "Multifaceted Mitochondria," June 4-6, 2018, Paradise Point, San Diego, CA.

Singson Lab

California Institute of Technology "Fertilization and gamete activation: The big bang of biology".

Patents

Dismukes

 2018 RU Patent published: Dismukes, G. C., Greenblatt, M., Laursen, A., Calvinho, K. USPTO Patent: PCT-US-2016-056026; Nickel Phosphide Catalysts For Direct Electrochemical CO2 Reduction To Hydrocarbons

Ebright

- Ebright, R., Ebright, Y., Feng, Y., and Degen, D. (2017) Antibacterial agents: salinamide derivatives. US Patent 9809626.
- Ebright, R. (2017) Antibacterial agents: combination of a rifamycin and a switch region inhibitor. US Patent 9839634.
- Ebright, R., Ebright, Y., Mandal, S., Wilde, R., and Li, Shengjian. (2018) Antibacterial agents: N(alpha)-aroyl-N-aryl-phenylalaninamides. US Patent 9919998.
- Ebright, R. (2018) Color-encoding and in-situ interrogation of matrix-coupled chemical compounds. US Patent 9945051.

Messing

- Messing, J. and Wu, Y. (2017). Composition and methods for rapid and efficient production of quality protein maize. US Patent # 9,603,317 B2.
- Messing, J. and Calvino, M. (2018). MIRNA 169 Compositions and methods for the regulation of carbohydrate metabolism and flowering in plants. US Patent # 9,949,488 B2.

Publications

Barr Lab

- O'Hagan, R., Silva., Nguyen, K.C.Q., Zhang, W., Bellotti, S., Ramadan., Y.H., Hall, D.H., and Barr, M.M. (2017) Glutamylation regulates transport, specializes function, and sculpts the structure of cilia. Current Biology 27: 3430-3441
- Wang, J. and Barr, M.M. (2018). EV mechanisms of cell-cell communication: what can we learn from model organisms? Essays in Biochemistry: 62(2):205-213.
- Barr, M.M., Garcia, L.R., and Portman, D.S (2018). Sexual dimorphism and sex differences in C. elegans neuronal development and behavior. Genetics. 208(3):909-935.
- De Vore, D.M., Knobel, K.M., Nguyen, K.C.Q., Hall,

D.H., and Barr, M.M. (2018) Extracellular matrix regulates morphogenesis and function of ciliated sensory organs in Caenorhabditis elegans. Genetics, under review.

Akella, J.S., Silva, M., Morsci, N.S., Nguyen, K.C.Q.,
Rice, W., Hall, D.H., and Barr, M.M. (2018) Cell
type-specific structural plasticity of the ciliary transition zone in C. elegans. Biology of the Cell, under review.

Dismukes Lab

- Rerouting of metabolism into desired cellular products by nutrient stress: Fluxes reveal the selected pathways in cyanobacterial photosynthesis. Xiao Qian, Yuan Zhang, Desmond S. Lun, & G. Charles Dismukes, ACS Synth. Biol. 2018, 7, 1465–1476.
- The Multiplicity of Roles for Dissolved Inorganic Carbon in Photosystem II Operation in the Hypercarbonate-requiring Cyanobacterium Arthrospira maxima. Ananyev, G., C. Gates, and G.C. Dismukes, Photosynthetica (Govindjee special edition), 2018. 56(X): p.1-12.
 - Photoassembly of the Mn4CaO5 catalytic core and its inorganic mutants. Understanding how and why photosynthetic oxygen is produced. Gates, C., G.
 Ananyev, and G.C. Dismukes, chapter in Metalloprotein Active Site Assembly, M.K. Johnson and R.A.
 Scott, Editors, John Wiley & Sons, Ltd,: Chichester, West Sussex PO19 8SQ, UK. 2017.
 - A. B. Laursen, R. B. Wexler, M. J. Whitaker, E. Izett, R. Rucker, H. Wang, J. Li, M. Greenblatt, A. M. Rappe and G.C. Dismukes, Climbing the volcano of electrocatalytic activity while avoiding catalyst corrosion: Ni3P a hydrogen evolution electrocatalyst stable in both acid and alkali. ACS Catalysis, 2018(8): p. 4408–4419.
- Photosystem II-cyclic electron flow powers exceptional photoprotection and record growth in the microalga Chlorella ohadii. Ananyev, G., C. Gates, A. Kaplan, and G.C. Dismukes, Biochimica et Biophysica Acta (BBA) Bioenergetics, 2017. 1858(11): p.873-883.

Dong Lab

- Zhang Y and Dong J# (2018) Cell polarity: compassing cell division and differentiation in plants.. Curr. Opin. Plant Biol. 45:127-135.
- Ge Z, Bergonci T, Zhao Y, Zou Y, Du S, Liu MC, Luo X, Ruan H, García-Valencia LE, Zhong S, Hou

S, Huang Q, Lai L, Moura DS, Gu H, Dong J, Wu HM, Dresselhaus T, Xiao J, Cheung AY, Qu L-J (2017) Arabidopsis pollen tube integrity and sperm release is regu-lated by RALF-mediated signaling. Science 358(6370):1596.

- Zhao C, Wang P, Si T, Hsu CC, Wang L, Zayed O, Yu Z, Zhu Y, Dong J, Tao WA, Zhu JK (2017) MAP Kinase Cascades Regulate the Cold Response by Modulating ICE1 Protein Stability. Dev. Cell, 2017 Oct 12. pii: S1534-5807(17)30783-9. doi: 10.1016/j. devcel.2017.09.024.
- Zhang J, Huang Q, Zhong S, Bleckmann A, Huang J, Guo X, Lin Q, Gu H, Dong J, Dresselhaus T, Qu L-J (2017) Sperm cells are passive cargo of the pollen tube in plant fertilization. Nat. Plants. 2017 Jun 6;3:17079. doi: 10.1038/nplants.2017.79.

Dooner Lab

 Huang, J.T., Q. Huang, W. Park, Y. Feng, D. Kumar, R. Meeley, and H.K. Dooner. 2017. Competitive ability of maize pollen grains requires paralogous serine threonine kinases STK1 and STK2. Genetics 207, 1361-1370

Ebright Lab

- Bird, J., Nickels, B., and Ebright, R. (2017) RNA capping by transcription initiation with non-canonical initiating nucleotides (NCINs): determination of relative efficiencies of transcription initiation with NCINs and NTPs. Bio-Protocol 7, e2336.
- Bucher, H., Ambrose, P., Chambers, H., Ebright, R., Jezek, A., Murray, B., Newland, J., Ostrowsky, B., and Rex, J. (2017) Developing antimicrobial drugs for resistant pathogens and unmet needs. J. Infect. Dis. 216, 228-236.
- Boucher, H., Ambrose, P., Chambers, H., Ebright, R., Jezek, A., Murray, B., Ostrowsky, B., Rex, J. (2017) Reply to Paul and Leibovici J. Infect. Dis. 217, 509-551.
- Yu, L., Winkelman, J., Pukhrambam, C., Strick, T., Nickels, B., and Ebright, R. (2017) The mechanism of transcription start site selection. eLife 6, e32038.
- Lin,W., Das, K., Degen, D., Mazumder, A., Duchi, D., Wang, D., Ebright, Y., Ebright, R.Y., Sineva, E., Gigliotti, M., Mandal, S., Jiang, Y., Liu, Y., Yin, R., Zhang, Z., Eng, E., Thomas, D., Donadio, S., Zhang, C., Kapanidis, A., and Ebright, R. (2018) Structural basis of transcription inhibition by fidaxomicin (lipiarmycin A3). Mol. Cell 70, 60-71.
- Bird, J., Vvedenskaya, I., Zhang, Y., Zhang, Y., Jiao, X., Krasny, L., Kiledjian, M., Taylor, D., Ebright, R., and Nickels, B. (2018) CapZyme-Seq" comprehensively defines promoter-sequence determinants for RNA 5' capping with NAD+. Mol. Cell 70, 553-564.

- Sosio, M., Gaspari, E., Iorio, M., Pessina, S., Medema, M., Bernasconi, A., Simone, M., Maffioli, S., Ebright, R., and Donadio, S. (2018) Analysis of the pseudouridimycin biosynthetic pathway provides insights into the formation of C-nucleoside antibiotics. Cell Chem. Biol. 25, 540-549.
- Duchi, D., Mazumder, A., Malinen, A., Ebright, R., and Kapanidis, A. (2018) Single-molecule fluorescence analysis of RNA polymerase clamp conformation: sub-second dynamic interconversion among three states and stabilization of a partly closed state by ppGpp. Nucl. Acids Res. (in press).
- Gabizon, R., Lee, A., Vahedian-Movahed, H., Ebright., R., and Bustamante, C. (2018) Pause sequences facilitate entry into off-pathway, long-lived pause states by reducing the on-pathway transcription rate of RNA polymerase. Nature Commun. (in press).

Gallavotti Lab

 Bartlett, A., O'Malley, R., Huang, S.C., Galli, M., Nery, J., Gallavotti, A., Ecker, J.R. "Mapping genome-wide transcription factor binding sites using DAP-seq". Nature Protocols 2017 (12), 1659-1672.

Genomics Core Facility

- Wang N, Zhang Y, Gedvilaite E, Loh JW, Lin T, Liu X, Liu CG, Kumar D, Donnelly R, Raymond K, Schuchman EH, Sleat DE, Lobel P, Xing J (2017). Using whole-exome sequencing to investigate the genetic bases of lysosomal storage diseases of unknown etiology. Hum Mutat. 38(11):1491-149
- Huang JT, Wang Q, Park W, Feng Y, Kumar D, Meeley R, Dooner HK. (2017) Competitive Ability of Maize Pollen Grains Require Paralogous Serine Threonine Protein Kinases STK1 and STK2. Genetics. 2017 Oct 6. pii: genetics.300358.
- Pareek CS, Błaszczyk P, Dziuba P, Czarnik U, Fraser L, Sobiech P, Pierzchała M, Feng Y, Kadarmideen HN, Kumar D. (2017) Single nucleotide polymorphism discovery in bovine liver using RNA-seq technology. PLoS One. Feb 24;12(2):e0172687
- Sims JS, Grinshpun B, Feng Y, Ung TH, Neira JA, Samanamud JL, Canoll P, Shen Y, Sims PA, Bruce JN (2016) Diversity and divergence of the glioma-infiltrating T-cell receptor repertoire. Proc Natl Acad Sci U S A. 13(25):3529-37.

Irvine Lab

 Rauskolb, C. and Irvine, K.D. 2018. Localization of Hippo signaling components in Drosophila by Flourescence and Immunofluorescence. Methods in Molecular Biology, in press.
- Ibar, C., Kirichenko, E., Keepers, B., Enners, E., Fleisch K. and Irvine, K.D. 2018. Tension-dependent regulation of mammalian Hippo signaling through LIMD1. J Cell Science, 131, jcs214700.
- Mao, Y., Sun, S. and Irvine, K.D. 2017. Role and Regulation of Yap in KrasG12D-induced lung cancer. Oncotarget 8, 110877-110889.
- Irvine, K.D. and Shraiman, B. I. 2017. Mechanical control of growth: ideas, facts and challenges, Development 144, 4238-4248.
- Bilder, D. and Irvine, K.D. 2017. Taking Stock of the Drosophila Research Ecosystem. Genetics 206, 1227-1236.

Maliga Lab

- Yu, Q., Lutz, K.A. and Maliga, P. (2017) Efficient Plastid Transformation Arabidopsis. Plant Physiol. 175(1):186-193.
- Hafizi A, Malboobi MA, Jalali-Javaran M, Maliga P, Alizadeh H. (2017) Covalent-display of an active chimeric-recombinant tissue plasminogen activator on polyhydroxybutyrate granules surface. Biotechnology letters 39(11):1683-1688.

McKim Lab

Das A., Cesario J., Hinman A.M., Jang J.K., McKim K.S., Kinesin 6 Regulation in Drosophila Female Meiosis by the Non-conserved N- and C-Terminal Domains. G3 (Bethesda). 2018 May 4;8(5):1555-1569

Messing Lab

- Planta, J., Xiang, X., Leustek, T., and Messing, J. (2017). Engineering sulfur storage in maize seed proteins without apparent yield loss. Proc. Natl. Acad. Rongo Lab Sci. USA PNAS 114, 11386-11391.
- Garcia, N. and Messing, J. (2017). TTT and PIKK complex genes reverted to single copy following polyploidization and retain function despite massive retrotransposition in maize. Frontiers in Plant Sci. 8:e1723.
- Larkins, B.A., Wu, Y., Song, R., and Messing, J. (2017). Maize seed storage proteins. In Maize Kernel Development, Larkins B.A., ed., CAB International, Boston MA, pp. 175-189.
- Planta, J. and Messing, J. (2017). Quality protein maize based on reducing sulfur in leaf cells. Genetics 207, 1687-1697.
- Xiang, X., Wu, Y., Planta, J., Messing, J., and Leustek, T. (2018). Overexpression of serine acetyltransferase in maize leaves alters zein expression increasing methionine content and nutritional value of kernels. Plant Biotechnology J. 16, 1057-1067.
- Li, X., Han, Y., Messing, J., Xu, J.-H. (2018). Genet-

ic diversity and evolution of reduced sulfur storage during maize domestication. Plant J. 94, 943-955.

Li, Y., Metha, R., and Messing, J. (2018) A new high throughput assay for determining soluble sugar in sorghum internode-extracted juice. Planta, in Press.

Nickels Lab

- Grudzien-Nogalska E, Bird JG, Nickels BE and Kiledjian M. 'NAD-capQ' Detection and Quantitation of NAD+-capped RNA. RNA (2018) in press.
- Desai M, Wurihan W, Di R, Fondell JD, Nickels BE, Bao X, and Fan H. A potential role for GrgA in regulation of σ 28-dependent transcription in the obligate intracellular bacterial pathogen Chlamydia trachomatis. Journal of Bacteriology (2018) in press.
- "CapZyme-Seq" comprehensively defines promoter-sequence determinants for RNA 5' capping with NAD+
- Vvedenskaya IO, Bird JG, Zhang Y, Zhang Y, Jiao X, • Barvík I, Krásný L, Kiledjian M, Taylor DM, Ebright RH and Nickels BE Molecular Cell (2018) 70, 553-564.

Padgett Lab

- Nanci S. Kane, Mehul Vora, Richard W. Padgett and Ying Li (2018). bantam microRNA is a Negative Regulator of the Drosophila decapentaplegic Pathway, Fly, in press.
- Cathy Savage-Dunn, Ryan J. Gleason, Jun Liu, and Richard W. Padgett (2018) Mutagenesis and Imaging studies of BMP Signaling Mechanisms in C. elegans. In Bone Morphogenetic Proteins Methods and Protocols, Springer.

Park, E.C. and Rongo, C. (In press) RPM-1 and DLK-1 Regulate Pioneer Axon Outgrowth by Controlling Wnt Signaling. Development.

Severinov Lab

- Metelev, M., Osterman, I. A., Ghilarov, D., Khabibullina, N. F., Yakimov, A., Florin, T., Shabalin, K., Utkina, I., Travin, D., Komarova, E. S., Serebryakova, M., Artamonova, T., Khodorkovskii, M., Konevega, A. L., Mankin, A. S., Sergiev, P. V., Severinov, K., and Polikanov, Y. S. (2017) Klebsazolicin inhibits 70S ribosome by significant obstruction of the peptide exit tunnel. Nat. Chem. Biol., 13, 1129-1136
- Ghilarov, D., Serebryakova, M., Stevenson, C. E. M., Hearnshaw, S. J., Volkov, D., Maxwell, A., Lawson, D. M., and Severinov, K. (2017) The origins of specificity in the microcin-processing protease TldD/E. Structure, 25, 1549-1561
 - Shmakov, S. A., Sitnik, V., Makarova, K. S., Wolf, Y. I., Severinov, K., and Koonin, E. V. (2017) The

CRISPR spacer space is dominated by sequences from the species-specific mobilome. Mbio, 8, e01397-17

- Tsibulskaya, D., Mokina, O., Kulikovsky, A., Piskunova Y., Severinov, K., Serebryakova, M., and Dubiley, S. (2017) Activation of Yersinia pseudotuberculosis Trojan-horse peptide-cytidine antibiotic by the Tld protease. J. Am. Chem. Soc., 139, 16178-16187
- Rodic, A., Blagojevic, B., Djordjevic, M., Severinov, K., and Djordjevic, M. (2017) Key features of CRISPR-Cas regulation lead to a highly efficient and temporally specific crRNA production. Front. Microbiol., 8, 2139
- Petushkov, I., Esyunina, D., Mekler, V., Severinov, K., Pupov, D., and Kulbachinskiy, A. (2017) Interplay between σ region 3.2 and secondary channel factors during promoter escape by bacterial RNA polymerase, Biochem. J., 474, 4053-4064
- Martynov, A., Severinov, K., and Ispolatov, Y. (2017) Optimal length of CRISPR array. PLOS Comp. Biol., 13, e1005891
- Ooi, W.-Y., Murayama, Y., Mekler, V., Minakhin, L., Severinov, K., Yokoyama, S., and Sekine, S. (2018) A Thermus phage protein inhibits host RNA polymerase by mimicking transcription bubble DNA. Nucleic Acids Res., 46, 431-441
- Krivoy, A., Rutkauskas, M., Kuznedelov, K., Musharova, O., Rouillon, C., Severinov, K., and Seidel, R. (2018) Primed CRISPR adaptation in Escherichia coli cells does not depend on conformational changes in the Cascade effector complex detected in vitro. Nucleic Acids Res., 46, 4087-4098
- Travin, D. Y., Metelev, M., Serebryakova, M., Osterman, I. A., Sergiev, P., Ghilarov, D., and Severinov, K. (2018) Biosynthesis of translation inhibitor Klebsazolicin proceeds through heterocyclisation and N-terminal amidine formation catalysed by a single YcaO enzyme. J. Am. Chem. Soc., 140, 5625-5633
- Shmakov, S. A., Makarova, K. S., Wolf, Y. I., Severinov, K., and Koonin. E. V. (2018) Towards comprehensive characterization of CRISPR-associated genes. Proc. Natl. Acad. Sci. USA, 115, E5307-E5316
- Wilcox, B., Osterman, I., Serebryakova, M., Lukyanov, D., Komarova, E., Gollan, B., Morozova, N., Sergiev, P., Helaine, S., Wolf, Y., Makarova, K., Dubiley, S., Borukhov, S., and Severinov, K. (2018) Escherichia coli ItaT is a Type II toxin that inhibits translation by acetylating isoleucyl-tRNAIle. Nucleic Acids Res., doi: 10.1093/nar/gky560.
- Jain, I., Minakhin, L., Mekler, V., Sitnik, V., Rubanova, N., Severinov, K., and Semenova, E. (2018) Defining the seed sequence of the Cas12b CRISPR-Cas effector complex. RNA Biol., in press
- Maikova, A., Severinov, K., and Soutourina, O.

(2018) New insights into functions and possible applications of Clostridium difficile CRISPR-Cas system. Front. Microbiol., in press

Steward Lab

Wang, F., Minakhina, S., Tran, H., Changela, N., Kramer, J., and Steward, R. Tet protein function during Drosophila development. PLOS One. 11;13(1):e0190367, 2018.

Singson Lab

- Y. Zhao*, C. Tan*, A. Krauchnas*, A. Scharf, N. Dietrich, K. Warnhoff, Z. Yuan, M. Druzhinina, S. Gu, L. Miao, A. Singson, R. Ellis, K. Kornfeld. (2018)
 The zinc transporter ZIPT-7.1/SPE-24 regulates
 sperm activation in nematodes PLOS Biology 16:
 e2005069. *Equal contribution. This manuscript was
 featured in a primer: D. Chu (2018) Zinc: A small
 molecule with a big impact on sperm function PLOS
 Biology 16 e2006204.
- M. Marcello, M. Druzhinina and A. Singson. (2018)The C. elegans sperm membrane protein interactome.Biology of Reproduction 98: 776-783.
- A. Krauchunas, E. Menendez, M. Druzhinina, J. Parry, J. Ni, S. Gu and A. Singson. (2018) Characterization of spe-43, a gene required for spermiogensis in Caenorhabditis elegans. Developmental Biology 436: 75-83.