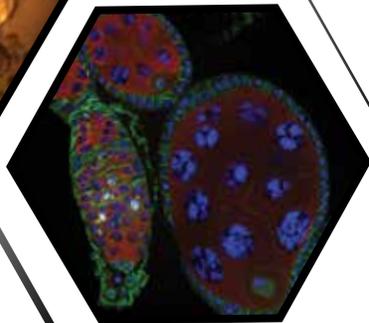
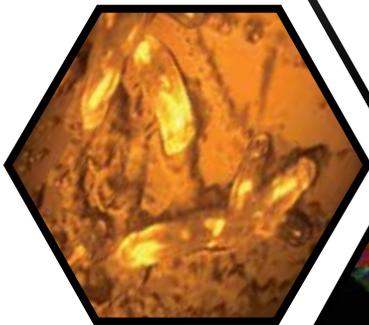
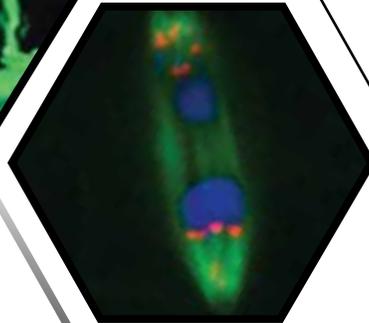
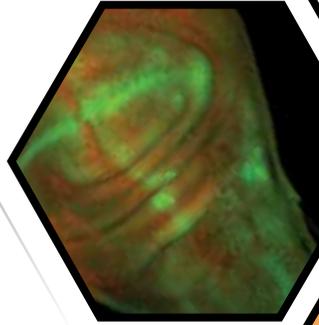


WAKSMAN INSTITUTE

of Microbiology



ANNUAL REPORT
2016-2017

RUTGERS
THE STATE UNIVERSITY
OF NEW JERSEY

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**

FACULTY & STAFF

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
Rosita Law, Unit Coordinator
Avonelle McKnight, Laboratory Technician
Eileen McLaughlin, Head Stock Clerk
Randy Newman, Information Technology Director
Daja O'Bryant, Unit Computing Specialist
Michelle Phillips, Senior Administrative Assistant
Marge Piechota, Accountant
Ted Przybylski, Instrument Maker/Repairer
Brian Schubert, System Administrator
Erin Sorge, Grant Coordinator
Jill Wachter, Administrative Assistant

Core Facilities

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

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 2016-2017

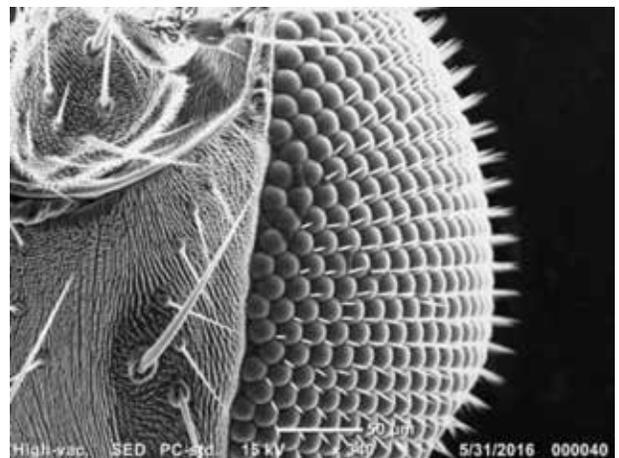
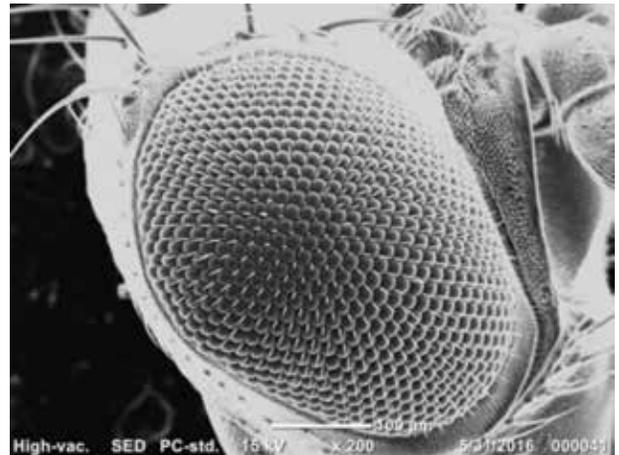
Alan M. Crosta, Jr.
Teresa A. Dolan
James F. Dougherty
Norman H. Edelman
Ronald J. Garutti
Debra Ann Lynch
Edgar Torres

Executive Officers:

Richard Edwards for Robert Barchi, President & Chair
Stephen DiPaolo Representing University Controller
Michael Gower, Exec VP for Finance & CFO
Joachim Messing, Scientific Director
Elizabeth Minott, Esq., Legal Counsel & Secretary
Robert Rossi, Executive Director

CONTENTS

ABOUT US	1
FACULTY & STAFF	2
REPORT OF THE DIRECTOR	4
ADVANCING OUR RESEARCH	8
ANIMAL LABS	
BARR LAB	9
IRVINE LAB	11
McKIM LAB	13
PADGETT LAB	17
RONGO LAB	20
SINGSON LAB	23
STEWART LAB	25
MICROBIAL LABS	
EBRIGHT LAB	27
NICKELS LAB	30
SEVERINOV LAB	32
VERSHON LAB	33
PLANT LABS	
DISMUKES LAB	34
DONG LAB	37
DOONER LAB	39
GALLAVOTTI LAB	40
MALIGA LAB	42
MESSING LAB	44
CORE FACILITIES	47
TRAINING FUTURE LEADERS	51
CHARLES AND JOHANNA BUSCH FELLOWS	52
BENEDICT MICHAEL FELLOW	58
WAKSMAN FACULTY COURSES	59
WAKSMAN STUDENT SCHOLARS PROGRAM	60
SHARING OUR DISCOVERIES	62
WAKSMAN ANNUAL RETREAT	63
PRESENTATIONS & MEETING ABSTRACTS	66
PATENTS & PUBLICATIONS	71



Images

Above: SEM images of *Drosophila melanogaster* eyes, Andrea Gallavotti and Nanci Kane

On the Cover: *Drosophila* wing disc, *Drosophila* adult mutant wings, Richard Padgett and Nanci Kane; Transplastomic *Arabidopsis*, Maliga Lab; McKim Lab: Anaphase 1 in *Drosophila*, *Drosophila* larvae Nanci Kane; Gyuricza Kim McKim and Nanci Kane

REPORT OF THE DIRECTOR

Joachim Messing



Dr. Joachim Messing, Director

Mission Statement

The Waksman Institute's mission is to conduct research in microbial molecular genetics, developmental molecular genetics, plant molecular genetics, and structural and computational biology. The Institute also provides a catalyst for general university initiatives, a life science infrastructure, undergraduate and graduate education, and a public service function for the state.

Background

The principal mission of the Waksman Institute is research. While the initial emphasis of the institute at its founding was microbiology, its focus soon turned toward molecular genetics, and was later broadened to include also multicellular organisms. Although 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 four 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 university to support 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.

Facilities at the Waksman Institute

The Waksman Institute's computing infrastructure has dedicated space on the fourth floor in the building's Old Wing. Randy Newman, Daja O'Bryant, and Brian Schubert are responsible for the deployment and hands-on maintenance of these resources as well as providing extensive user support.

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 750TB of enterprise class storage with an offsite backup location for disaster recovery. The servers, storage, and other devices communicate with each other using a combination of high-speed 10Gb Ethernet and 8Gb Fibre Channel fabrics. Extensive server virtualization, provided by VMware ESXi, is used where appropriate 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 Waksman computing staff is responsible for maintaining the high availability of these resources 24/7 with minimal downtime.

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.

The Waksman Genomics Core Facility (WGCF) is a sequencing facility 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. 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.

Together, WGCF has three sequencers covering the full spectrum of NGS requirements. The combination of instruments gives faculty flexibility to pick one that fits their needs and budgets. Available 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 ~\$5000. 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.

High throughput sequencing is an incredibly fast developing technology. With cutting edge equipment, the core connects scientists with the tools and expertise that can take their projects to the next level to enrich genomics research. The main goal is to reduce the startup time and degree of knowledge necessary for an individual user to design and execute experiments requiring next generation sequencing. 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.

The facility is managed and operated by Dibyendu Kumar with the assistance of Min Tu. To cope with the dramatic increase in data streaming and storage of both the cell biology core facility and the Genomics Core Facility we rely on the expertise of computational specialist Brian Schubert. The enormous increase in genomics and expression data also requires knowledge in data analysis application and the scripting of pipelines that can process the data expediently. This task is under the skilled hands of Yaping Feng.

Unique for New Jersey is a cell and cell products fermentation facility. Build in 1954 and renovated in 1984, the Cell and Cell Products Fermentation Facility, located within the Waksman Institute at Rutgers University is a state-of-the-art facility that provides fermentation services to a multitude of clients including academic institutions, international pharmaceutical corporations, cosmetic companies, virtual enterprises and more. Our purpose is to provide, not for profit, specific niche services for the production of biologics and similar products including purification, bulk production, scale-up and R&D. We operate independent of state support, using a fee for service arrangement with our clients. Our 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.

Personnel and faculty affiliations

In the academic year 2016/2017, the Institute consisted of fifteen resident, two non-resident, and six emeriti faculty members. The Institute accommodates nine assistant research professors, seven visiting researchers, seventeen research associates, twenty-seven postdoctoral researchers, twenty-four technical assistants, thirty-eight graduate students, and four visiting students. The Waksman Institute's total resident population is currently 133, which does not include the 48 undergraduate students that did independent research during the last year.

There are currently 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, 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 is Assistant Professor, one is Associate Professor, nine are Professors, four are Distinguished Professors, one is a Board of Governors Professor, and one a University Professor. I am also the first holder of the Selman Waksman Chair in Molecular Genetics. The Institute currently has six professor emeriti, who are all well, and periodically join us here for events. Three professors are members of the National Academy of Sciences (US), three of the American Academy of Arts and Sciences, one of the National Academy of Sciences Leopoldina (Germany), one of the Hungarian Academy of Sciences, five are Fellows of the American Academy of Microbiology, and seven Fellows of AAAS. One faculty member is Howard Hughes Institute Investigator.

It is with great sadness to report the passing of one faculty member, Carl A. Price, on September 25th, 2016. He died at the age of 89 in Somerset, New Jersey. Carl graduated from CalTech and got a Ph.D. from Harvard. He came to the Institute in 1976 and retired in 1999. For the coming year, we seek to replace Hugo Dooner, who retired in 2014.

Lectures

Because there are so many lecture series 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. We also list there the program of our annual retreat from September 8th, 2016. In addition, the institute sponsored The Microbiology Symposium, in New Brunswick, NJ, in February 2017 at Trayes Hall, Douglass College.

Recruitment & Funding

Although the Board of Governors had approved our plans for expansion of laboratory space, realization of these plans has been delayed in the design phase. Especially, the design of utilities had to be modified several times due to deferred maintenance of our existing buildings. We now anticipate utilities being updated and installed this Fall and construction to start in spring of 2018. Therefore, we begin the search for a new faculty member the coming year to fill the position that has been vacated by Hugo Dooner's retirement jointly with the Department of Plant Biology in the School of Environmental and Biological Sciences.

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 has fluctuated significantly, from \$6M to \$12M and now \$8M. These numbers only include basic research and not service grants (e.g. data bases). Current levels were strongly influence by the loss of two Howard Hughes Investigator appointments and the most recent



retirement of Hugo Dooner.

Two 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 now 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 retirement 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 between faculty and support structure. The Unrestricted Revenues have remained relatively the same but the University has increased the Allocated Costs resulting in drastic budget imbalances. 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. Juan Dong got promoted to Associate Professor with tenure in the Department of Plant Biology. Bryce Nickels with joint appointment in the SAS Department of Genetics got promoted to Full Professor. Chris Rongo, who received an outside offer to be the chair of the Department of Neuroscience at the University of Connecticut Medical School, was successfully retained at the Institute with joint appointment at the SAS Department of Genetics. Pal Maliga and Charles Dismukes were elected as Fellows of the American Association of the Advancement of Science. Charles Disumukes received a special recognition letter from the BASF Catalysis Division. Juan Dong received the Travel Award to attend the Auxin 2016 Conference in Sanya China. Kelvin Liao received the GSA Undergraduate Travel Award to the International C. elegans Conference. Congratulations to these accomplishments of our students and members!

ADVANCING OUR RESEARCH

- Animal Labs
-

- Microbial Labs
-

- Plant & Photosynthetic Labs
-

- Core Facilities
-

BARR LAB

Sensory Biology



Dr. Maureen Barr
Genetics

Summary

The Barr laboratory uses the nematode *C. elegans* to study fundamental questions in molecular genetics and cell biology (ciliogenesis, ciliary transport, and most recently, ciliary extracellular vesicles) and to model human genetic diseases. We consistently tackle major important problems that are biomedically relevant, and have a track record of groundbreaking discoveries. Many of the genes and pathways we study control *C. elegans* behaviors, therefore we are also interested in neurogenetics and neuroplasticity. Our studies have guided research into autosomal dominant polycystic kidney disease and other ciliopathies. Our research has unlocked insights into three exciting new areas in the lab: ciliary specialization, extracellular vesicles, and stress-induced neuronal restructuring.

SIGNIFICANCE: Cilia are found on most non-dividing cells in the body, perform a multiplicity of functions, and when faulty, cause a wide range of pathologies called ciliopathies. Some ciliopathies affect only subset of organs, such as Autosomal Dominant Polycystic Kidney Disease (ADPKD), while syndromes manifest in renal, neurological, skeletal, retinal, cardiac, and other abnormalities such the nephronophthisis (NPHP)-related ciliopathies. It has long been appreciated that cilia are organized into structurally and functionally distinct compartments called the basal body, the transition zone, and the ciliary shaft. In *C. elegans* and mammalian cilia, NPHP2/Inversin localizes to a proximal region of the ciliary shaft that is not identifiable by ultrastructural features. In our *C. elegans* model for NPHP-related ciliopathies, we showed the ciliary shaft is organized into discrete regions and modules, including the Inversin Compartment. While the Inversin Compartment is evolutionarily conserved from worm to man, mechanisms that define and establish the Inversin Compartment are unknown. Our genetically tractable model can make inroads where others have not, and advance frontiers of knowledge where little is known.

We demonstrated that the *C. elegans* Inversin Compartment regulates microtubule patterning and tubulin glutamylation. The Tubulin Code is proposed to employ tubulin post-translational modifications such as glutamylation and tubulin isoforms to regulate microtubule structure, motor-based transport, and function. Using *C. elegans* as a model system, we discovered that the Tubulin Code regulates ciliary structure, transport, and function. One ciliary specialization is the ability to produce extracellular vesicles. In the kidney, urinary extracellular vesicles carry disease-specific biomarkers for PKD including the polycystins, and ciliary- extracellular vesicle interactions have been proposed to play a central role in the biology of PKD. We found that specialized *C. elegans* cilia release bioactive polycystin-containing extracellular vesicles and the Tubulin Code controls extracellular vesicles release. The Barr laboratory continues use *C. elegans* as a springboard to identify mechanisms that establish ciliary compartmentalization and to determine the functions of conserved tubulin glutamylases and tubulin deglutamylases. Importantly, we are studying the connection between the Inversin compartment and glutamylation, which is implicated in human ciliopathies and is an unexplored area in cilia biology.

INNOVATION: How the Inversin Compartment is established and functions is not easily testable using other experimental systems. Likewise, how a ciliated cell writes and reads the Tubulin Code in its native context is entirely unknown. We developed the tools to study the Inversin Compartment and to tease apart the roles of writers, readers, and erasers of the Tubulin Code in an intact living animal. *C. elegans* is a transparent, multicellular animal with specialized cilia—features that enable subcellular imaging that is unprecedented in its simplicity and reproducibility. *C. elegans* specialized cilia shed and release bioactive polycystin-containing extracellular vesicles. The detection of extracellular vesicles is a challenge and obstacle because of their small size (100nm). To our knowledge, we possess the first and only system to visualize and monitor extracellular vesicle release in real time and are the only laboratory in the world studying extracellular vesicle biology *in vivo*. All of the genes we study have human counterparts, some implicated in kidney or ciliary diseases, while others have unexplored function. The connection between Inversin Compartment and microtubule glutamylation has never been examined, and we are uniquely poised to address this medically relevant issue. We isolated genetic suppressors of a tubulin deglutamylase deficiency, which causes neu-

rod degeneration in mice and ciliary degeneration in *C. elegans*. Identifying these suppressors and determining their functions will uncover ciliary homeostasis mechanisms. To complement our expertise in *C. elegans* molecular genetics, cell biology, and *in vivo* imaging, we established collaborations to extend our findings to study how glutamylation impacts motor-based transport in mammalian cilia and cell-free reconstitution systems.

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

Malan Silva, 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
and the analysis of gene function.

Summary

The control of growth is a fundamental, yet poorly understood, aspect of development. What dictates the size of a particular organ (e.g., how does a hand or a heart “know” how large it should be) or a particular organism (e.g., why is a mouse small and an elephant large)? Decades ago, regeneration experiments revealed an intimate relationship between organ patterning and organ growth, but the molecular basis for this relationship has remained elusive. More recently, 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

Our current research focuses on two intersecting signaling pathways, the Hippo pathway and the Dachous-Fat pathway. These pathways control the growth and shape of developing organs. We study both the molecular mechanisms of signal transduction and the roles of these pathways in different developmental and physiological contexts. The Hippo signaling pathway has emerged over the past decade as one of the most important growth regulatory pathways in animals.

In certain contexts, the Hippo pathway is regulated by the Fat pathway. The *fat* gene encodes a large transmembrane protein of the cadherin family. In addition to its influence on Hippo signaling, Fat also influences planar cell polarity (PCP), which is a polarization of cell structures and cell behaviors within the plane of a tissue. In this way, Fat modulates not only organ size but also organ shape (e.g., by influencing the orientation of cell divisions). Fat is regulated by two proteins expressed in gradients: Dachous (Ds), which like Fat is a large cadherin family protein and can bind to Fat, and Four-jointed (Fj), which we found is a novel Golgi-localized kinase that phosphorylates cadherin domains of Fat and Ds to modulate binding between them. One remarkable feature of Fat signaling is that rather than responding solely to the level of Ds and Fj, Fat is also regulated by the slope and vector of their expression gradients: the slope influences Hippo signaling and the vector influences PCP.

Clues to how this novel regulatory mechanism operates have come from the identification and characterization of downstream signaling components. Several years ago we identified Dachs as a key player in both Fat-Hippo and Fat-PCP signal transduction. Dachs, which is a myosin family protein, exhibits a polarized membrane localization that is regulated by Fat activity. Our studies indicate that the direction in which Dachs is polarized is governed by the vector of the Fj and Ds gradients and controls PCP signaling, whereas the extent to which Dachs is polarized is influenced by the slope of the Fj and Ds gradients and controls Hippo signaling. We have combined genetic, biochemical, and cell biological experiments to investigate how Dachs is regulated, and how Dachs accumulation influences Hippo signaling and PCP.

We have also investigated how other signaling pathways that modulate organ growth intersect with the Hippo signaling pathway. We identified molecular crosstalk between epidermal growth factor receptor (EGFR) signaling and Hippo signaling that promotes growth, which is of particular interest because activation of EGFR or some of its downstream effectors, like Ras, is observed in many human cancers, and we are exploring the significance of this cross-talk in cancer models.

Most recently, we have investigated how mechanical forces experienced by cells influence Hippo signaling, and thereby organ growth. In developing *Drosophila* tissues, we found that accumulation of a negative regulator of Warts, called Jub, at cell-cell junctions is dependent upon cytoskeletal tension. Jub then recruits Warts into junctions; formation of this Jub-Warts complex inhibits Warts activity, thereby promoting growth. This occurs in part because Warts is activated in specific membrane complexes, interaction with Jub prevents Warts from going to sites where it can be activated. Current studies in the lab investigate molecular processes involved in this tension-dependent regulation of Jub, how this pathway is deployed in different contexts, and its conservation in mammalian cells.

Homologs of many genes in Fat and Hippo signaling are conserved in mammals, but it was not initially clear whether mammals had a Fat signaling pathway equivalent to that in *Drosophila*, nor what the roles of this pathway were. To investigate this, we created a mutation in a murine *ds* homolog, *Dchs1*, and we and our collaborators have characterized it, together with mutations in a murine *fat* homolog, *Fat4*. Our analysis indicates that Dchs1 and Fat4 function as a ligand-receptor pair during mouse development, and we have identified novel requirements for Dchs1-Fat4 signaling in multiple organs, including the brain, ear, kidney, skeleton, intestine, heart, and lung.

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

Edward Enners, Research Technician

Dr. Yaopan Mao, Research Associate

Dr. Jyoti Misra, Postdoctoral Associate

Dr. Consuelo Ibar, Postdoctoral Fellow

Dr. Herve Alegot, Postdoctoral Fellow

Dr. Bipin Tripathi, Postdoctoral Associate

Yuanwang Pan, Graduate Fellow

Zhenru Zhou, Graduate Assistant

Srividya Venkatramanan, Graduate Assistant

Estelle Cervantes, Undergraduate Assistant

Christopher Markosian, Undergraduate Assistant

Katelyn Fleisch, Undergraduate Assistant

George Abdelmalek, Undergraduate Assistant

Rohan Shah, 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. Meiosis depends on the formation of microtubules (MTs) into a bipolar spindle and 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.

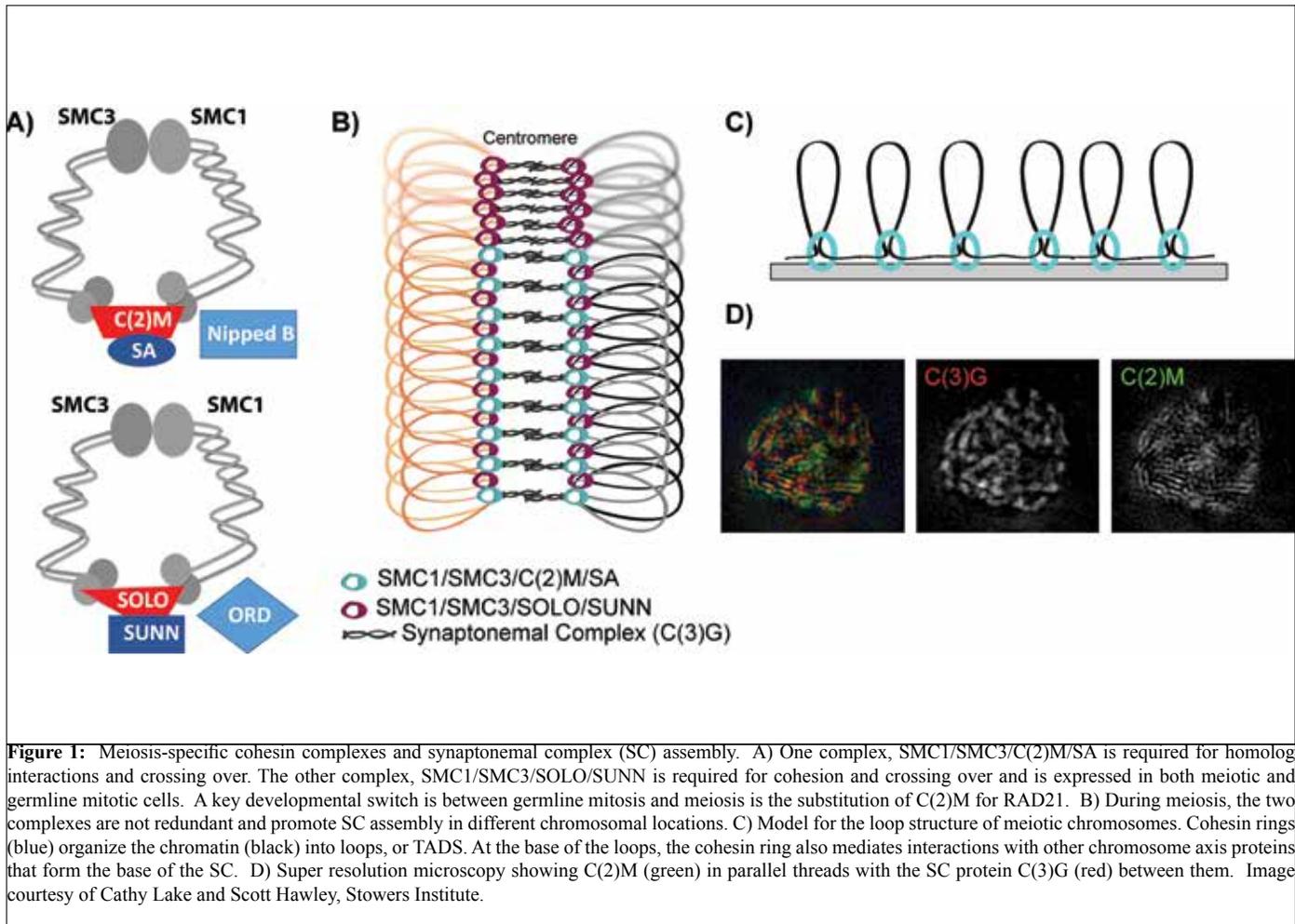
In the oocytes of many animals, meiotic spindles are built without centrosomes. The absence of MTs growing from the poles necessitates alternative mechanisms for establishing spindle bipolarity and homologous centromere bi-orientation. Our long-term goal is to determine the fundamental mechanisms that promote accurate chromosome segregation on the acentrosomal spindle of *Drosophila melanogaster* oocytes. We are particularly interested in the protein complexes and mechanisms of bi-orientation and the features of the oocyte spindle that make it susceptible to chromosome segregation errors. This involves understanding how chromatin-based signals initiate and organize the meiotic spindle in acentrosomal oocytes. Furthermore, this involves understanding the mechanism of bi-orientation in meiosis. We are interested in identifying the “weak points” that make the oocyte susceptible to nondisjunction. Characterizing these weak points is important to understand how meiosis works, and also important to understand why errors occur that lead to infertility.

Research in my laboratory uses genetic, biochemical and cytological approaches to study and understand meiosis in the model organism *Drosophila melanogaster*. In many cases we are studying genes required for both mitosis and meiosis, and thus are involved in cell proliferation and cancer. Because loss of mitotic proteins often causes lethality, their role in meiosis is relatively understudied in most systems. We have optimized methods such genetic mosaics and germline-specific RNAi to knock down and manipulate genes in the oocyte. With different genetic tools to control gene expression and shRNA constructs for RNAi, we can study oocyte-specific and stage-specific knockdowns of almost any gene during meiosis. Layered on this, we can use RNAi resistant transgenes to express mutated variants.

How do meiotic cohesin complexes promote synaptonemal complex assembly? (Figure 1)

In *Drosophila*, like many organisms, meiotic crossing over occurs between paired homologs held together along their entire length by the synaptonemal complex (SC). The SC interacts with and connects the axis of two homologous chromosomes and this structure plays a critical role in regulating many aspects of meiotic prophase, including pairing, double strand break formation (DSB), repair and crossover frequency and distribution. A crucial component of the chromosome axis is cohesin-related proteins. Mitotic cohesin is made up of one complex with four subunits: SMC1, SMC3, Stromalin (SA) and the kleisin Rad21. In meiosis, SC assembly depends on two groups of cohesin-related genes with meiosis-specific components and functions. We are investigating the hypothesis that the function of cohesins in SC assembly is related to a function revealed by other studies in *Drosophila*; that cohesins have functions in addition to holding sister chromatids together. Cohesins have a role organizing chromatin into higher order physical domains, or “topologically associated domains” (TADs). In this function, the cohesin ring entraps two strands of DNA from the same chromatid. It is this role that may explain how cohesin function influences transcription and, during meiosis, could organize the chromatin into loops, organize the axis and promote SC assembly.

All SC assembly in *Drosophila* depends on SMC1 and SMC3. Our mutant analysis has shown that SMC1 and SMC3 function in two pathways that independently promote SC assembly. One pathway depends on C(2)M, a kleisin family protein and two mitotic cohesins, Stromalin (SA) and Nipped-B. C(2)M was the first of a subgroup of kleisins 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 cohesion 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. In this proposal, we will test the hypothesis that these two pathways correspond to two meiosis-specific cohesin complexes: C(2)M/SA/Nipped-B and SOLO/SUNN/ORD.

Why there is a need for multiple cohesin complexes and how they promote SC assembly and genetic recombination is poorly understood. Interestingly, we have found the two sets of meiotic cohesins load at different sites and times on the chromosomes. Thus, the two proposed cohesin complexes differ not only in their meiotic functions, but also in where and when they are loaded onto the chromosomes. Unlike most other known meiotic proteins, the two groups of cohesins define distinct domains and promote SC in different places. Thus, the pattern and dynamics of these different cohesin complexes may dictate the pattern of SC assembly, which, through interactions with the chromosome axis, influences the frequency and distribution of crossovers.

The physical linkages between the chromatin, the chromosome axis, and the SC are not known. Meiotic chromosomes are organized as a linear array of loops. While the base of each loop is anchored in a specialized structural axis, a crucial gap in our understanding is how these loops are organized and their dynamics regulated. Recent findings have shown that cohesins can organize chromatin loops. Cohesins, by interacting with the chromatin and being in the chromosome axis, are in a position to mediate the effects of SC on recombination. Indeed, C(2)M has been shown to be in the chromosome axis by EM and super resolution microscopy. Each cohesin pathway is required for SC assembly at different sites, which may also reflect an underlying pattern of chromatin structure that regulates recombination and specifies crossover sites. Our studies are investigating a possible new paradigm in meiosis: that the meiotic cohesins have a non-cohesive role, more like the role mitotic cohesins have in regulating gene expression. While meiosis-specific cohesin complexes are loaded during S-phase, a second population, which may not depend on the first, is

dynamic and loaded during meiotic prophase and regulates when and where SC will assemble.

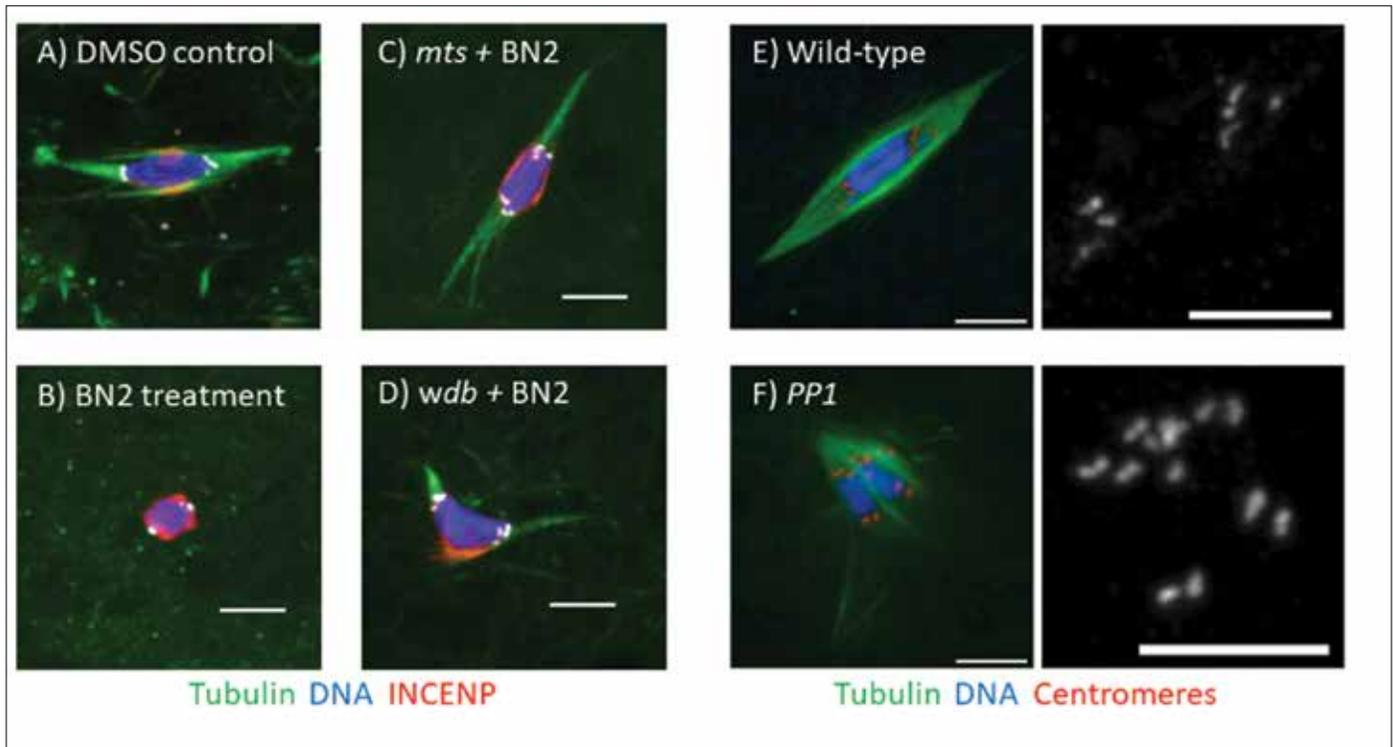


Figure 2: Phosphatases in oocyte meiosis. A) Wild-type oocyte with bipolar spindle microtubules (green) and the central spindle protein INCENP (red) around the chromosomes (blue). B) Treatment with the Aurora B inhibitor Binuclein 2 (BN2) causes loss of all spindle microtubules and INCENP associates with the chromosomes. C and D) Loss of PP2A (MTS and WDB are two subunits of PP2A) by RNAi partially suppresses the spindle loss caused by BN2. E) In wild-type, there are four pairs of chromosomes. The sister kinetochores are fused in meiosis I, resulting in the appearance of 8 centromere foci (CENP-C, red in merge and white in a higher magnification image). In PP1 RNAi oocytes, up to 16 CENP-C foci are observed, due to precocious separation of the centromeres. Scale bars are 5 μm.

How are the activities of kinases and phosphatases balanced to regulate meiotic spindle dynamics? (Figure 2)

The chromosomal passenger complex (CPC) is the basis for chromatin-mediated spindle assembly in *Drosophila* oocytes. The CPC is composed of four proteins: INCENP, Aurora B kinase, Survivin and Borealin. Tissue-specific RNAi was used to show that the CPC is required for the accumulation of microtubules (MTs) around the chromosomes. Indeed, the CPC promotes two pathways of spindle assembly, from the kinetochores (KTs) and non-KT chromatin. Correlating with these multiple sites of spindle assembly, the CPC localizes to two locations: forming a ring around the chromosomes in the central spindle, and at the centromeres. INCENP localizes to non-KT chromatin in *aurB* RNAi oocytes that lack MTs, suggesting that an interaction between the CPC and chromatin is important for spindle assembly. Our working model is that CPC activity is regulated by its localization to specific sites where it is needed.

A robust central spindle of antiparallel MTs that depends on the kinesin-6 *Subito* forms early in prometaphase I oocytes. In *subito* mutants, the central spindle does not form, resulting in monopolar or tripolar spindles. The meiotic central spindle may act as a “backbone” during metaphase, organizing the MTs into a bipolar structure in the absence of centrosomes. 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. Within the KMN network, two MT binding activities have been identified, one with the Ndc80 complex and the other with Spc105/KNL1. The results from our studies support a model where bi-orientation depends on lateral interactions between the KT and central spindle MTs. Lateral attachments establish bi-orientation and then end-on attachments maintain and segregate bi-orientated homologs. We suggest that lateral attachments with antiparallel MTs of the central spindle are required for homolog bi-orientation. The dependence of oocytes on lateral attachments for bi-orientation is conserved in meiosis of mouse and *C. elegans*. However, the mechanism of how lateral attachments lead to bi-orientation remains poorly understood.

Applying an inhibitor of Aurora B, Binucleine 2, caused effects similar to loss of Aurora B or INCENP by RNAi: loss

of the spindle in oocytes. This shows that sustained Aurora B activity is required during meiosis I to maintain the spindle and the KTs. The requirement of a kinase for spindle maintenance suggests spindle dynamics could be regulated by phosphatases. Using tissue-specific RNAi, a division of labor has been observed between two phosphatases, PP1 and PP2A, in regulating kinase activity in oocytes. The spindle microtubules were stabilized when Aurora B was inhibited in *Pp2A RNAi* oocytes, demonstrating that PP2A antagonizes the Aurora B spindle maintenance function. PP2A was also found to be required for maintaining sister chromatid cohesion during metaphase I, a role that appears to be independent of MEI-S332/SGO.

PP1-87B was found to antagonize the Aurora B function during metaphase I that maintains chromosome organization and regulates KT assembly. During these studies, we also found that PP1-87B is required for sister centromere co-orientation, the meiosis-specific function that promotes fusion of sister KTs in meiosis I to promote attachment to microtubules from the same pole. A similar phenotype had been previously observed in oocytes lacking the KT protein SPC10R. However, the mechanism of PP1-87B and SPC105R function in co-orientation appears to be different. The precocious separation of centromeres in *Spc105R RNAi* oocytes, and probably *Pp2A RNAi* oocytes, depends on separase, while in *PP1-87B RNAi* oocytes it does not. Therefore, SPC105R protects the centromeres from the activity of Separase. The precocious separation of sister centromeres in meiosis I of *PP1-87B RNAi* oocytes depends on the presence of microtubules (unlike *Spc105R RNAi* oocytes), Polo kinase and the SC protein C(3)G. These results show that multiple phosphatases maintain and regulate meiosis-specific spindle and chromosome structures in oocytes. In addition, we have found evidence of a pathway for co-orientation that begins with cohesion and SPC105R but ends with PP1-87B and spindle forces but not cohesion.

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

Mercedes Gyuricza, Graduate Student

Lin-Ing Wang, Graduate Student

Justin Mathew, Undergraduate Student

Amy Gladstein, Undergraduate Student

Jay Joshi, Undergraduate Student

Priya Patel, 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.

Studies of TGF β 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.

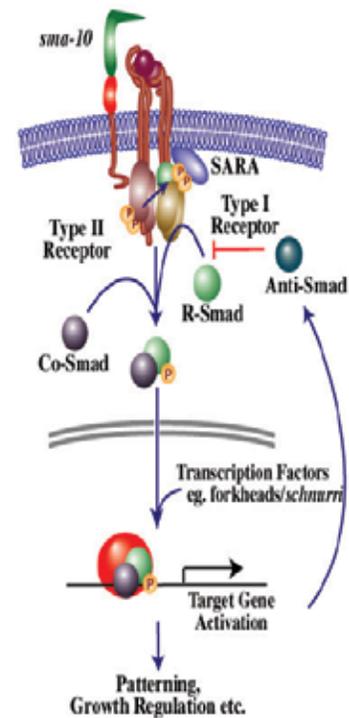


Figure 1: Model for TGF β Signaling

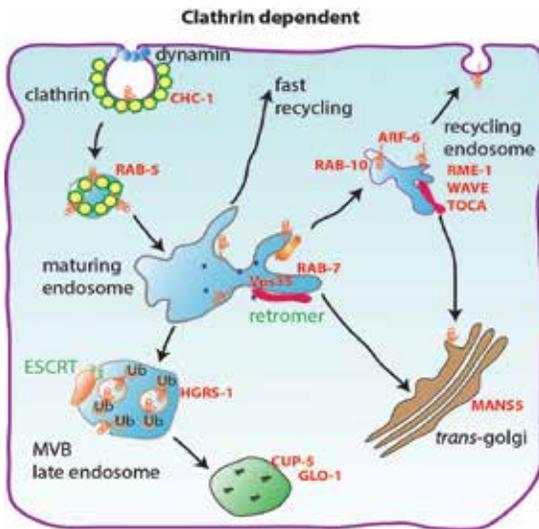


Figure 2: The *C. elegans* Endocytic Pathway

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 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 cause 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. Current experiments are designed to assay the signaling potential of these altered receptors.

LRIG function in *Drosophila* intestinal stem cells

BMP signaling plays an important role in intestinal stem cell (ISC) growth in both *Drosophila* and mammals. In mammals, a BMP signal originates from the intravillus mesenchyme that affects the villus epithelium. If BMP signaling is inhibited, the formation of numerous ectopic crypts is observed. The formation of these ectopic crypts is strikingly similar to the histopathology of patients with juvenile polyposis (JP). Further, many JP patients have mutations in BMP pathway genes, further connecting BMP with ISC regulation. In *Drosophila*, BMP is necessary to stop ISC growth after intestinal injury, similar to the overgrowth observed in mammals. Therefore, *Drosophila* will be a good model to examine conserved functions of LRIG.

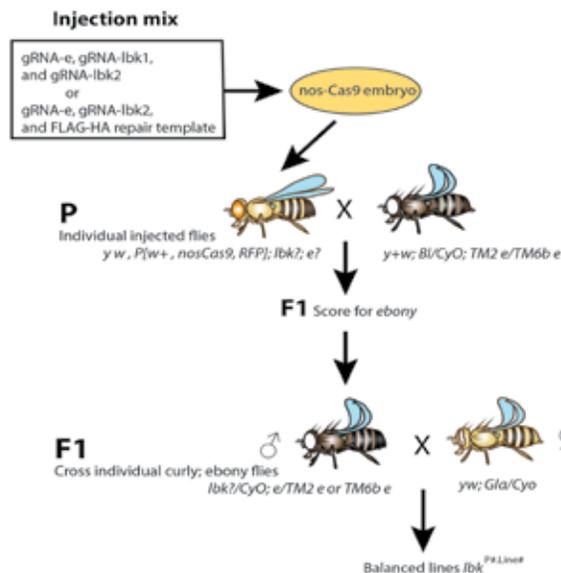


Figure 3. Genetic scheme for using *Drosophila* co-CRISPR.

In the *Drosophila* wing disc, we have shown that knockdown of fly LRIG reduces BMP signaling, showing that LRIG is required with BMP in the wing disc for normal growth. An important question is to determine if LRIG is required for all BMP signaling events, and whether it enhances or inhibits signaling, so we have chosen to examine its role in the fly intestine. For these experiments, we have used CRISPR to epitope tag the fly LRIG gene, LAMBIK, and to make loss of function mutations. These mutations are currently being examined for effects on intestinal function and BMP signaling.

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.

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

Mehul Vora, Postdoctoral Associate

Nanci Kane, Research Technician

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

Neuronal communication is the primary means by which our nervous system senses, interprets, remembers, and responds to the outside world and to our own internal physiology. Much of this communication occurs at chemical synapses, which are specialized signaling structures comprised of a presynaptic cell that releases neurotransmitters, and a postsynaptic cell that detects these neurotransmitters using receptor proteins. Synapses in turn are supported by cytoskeletal elements, which move synaptic proteins and other organelles between the cell body and the synapse, and by mitochondria, which provide energy and buffer calcium in support of synaptic signaling. Synapses are also regulated by protein turnover mechanisms, including the Ubiquitin Proteasome System (UPS), which maintains healthy proteostasis and aging by removing damaged and unfolded proteins. The underlying etiology of many neurological disorders and diseases, including ischemic stroke, Parkinson's Disease, and Alzheimer's Disease, are caused by defects in one or more of these key neurophysiological processes. A more complete understanding of these neurophysiological processes will facilitate better diagnosis and

treatment of multiple neurological disorders.

Our research is focused on understanding four areas of neurophysiology. First, we are interested in understanding how post-synaptic neurotransmitter receptors are localized to synapses. Second, we are interested in understanding how the movement and dynamics of mitochondria are mediated along axons and at synapses. Third, 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 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.

Glutamate Receptor Trafficking.

With regard to neurotransmitter receptor post-synaptic localization, my lab has had a long-standing interest in the mechanisms underlying the membrane trafficking of glutamate receptors (GluRs), which detect glutamate, the major excitatory neurotransmitter in our brain. We are particularly interested in how GluRs are localized to synapses because such glutamate receptor cell biology plays an important role in synaptic communication, synaptic plasticity, and learning and memory. In addition, glutamate receptors are implicated in several diseases of the nervous system, and are a primary neurodegenerative agent activated by mechanical damage (e.g., traumatic injury) and by oxygen deprivation (e.g., stroke). Thus, a better understanding of these receptors will facilitate the diagnosis, treatment, and prevention of diseases attributable to neurodegeneration, and help us better understand the mechanisms behind learning and memory.

Our focus has been to identify the factors that regulate GluR localization and function using a genetic approach in the nematode *C.elegans*. We use *C.elegans* because its simple nervous system, which is easily visualized through its transparent body, allows us to observe glutamate receptor trafficking 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 glutamate receptor 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.

Our lab studies the trafficking of GLR-1, a *C. elegans* glutamate receptor that functions in a simple touch circuit. We examine GLR-1 localization using transgenic nematodes that express a chimeric GLR-1:GFP protein, which is localized to synaptic connections. Using this transgene, we have screened for candidate genes that are required for proper GLR-1 localization. The process of glutamate receptor localization requires channel assembly and export from the ER, anterograde trafficking from cell body to synapse, anchoring at the synapse, endocytosis, recycling, and finally degradation. We have obtained mutants for genes that regulate all of these steps in GLR-1 localization. Using

additional subcellular markers, we showed that nearly all of the genes identified by this screen are relatively specific for GLR-1 localization, and do not impair protein trafficking or synapse formation in general. We have mapped and cloned many of these genes during the last few years, and we are currently studying how the retromer and the small GTPase RAB-6 promote recycling of endocytosed receptors, possibly through outpost Golgi along dendrites.

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. We have shown that hypoxia blocks the membrane recycling of GLR-1-containing GluRs to synapses and depresses 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 GLR-1 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, whereas fission is thought to be the first step on the way to mitophagy. Defects in mitochondrial dynamics have a clear role in Parkinson’s Disease, among others. Thus, an understanding of mitochondrial dynamics and movement is important for our understanding of neurological disorders and aging with mitochondrial etiology.

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 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). We 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/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 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, Research Associate

Nathaly Salazar-Vasquez, Graduate Fellow

Tarmie Matlack, Laboratory Manager & Research Assistant

James Mullin, Undergraduate Assistant

Arul Duggimpudi, Undergraduate Assistant

Julia Schoeni, Undergraduate Assistant

Neil Shah, 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 (see figure 1).

Sperm function

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

We continue to identify and characterize genes required for sperm function at fertilization taking advantage of the most up to date molecular tools. We have recently identified candidates for the *spe-9* class genes *spe-13*, *spe-36* and *spe-45* 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.

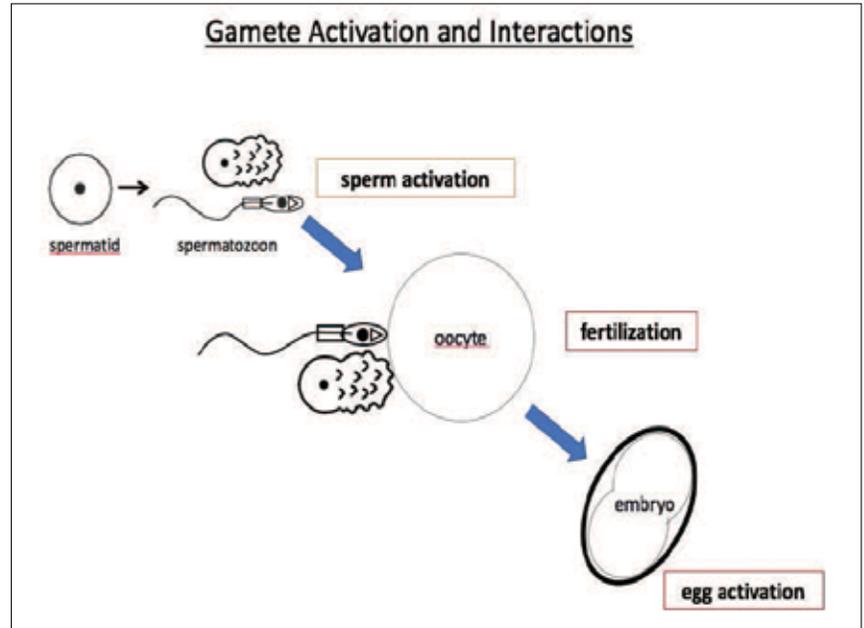


Figure 1: Main areas of research in the Singson Lab.

Sperm activation: converts a spermatid into a motile sperm. Fertilization: requires sperm and egg cell surface molecules.

Egg activation: requires the egg to detect sperm entry and activate the embryonic program. Egg activation is sometimes referred to as the “big bang” of development.

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

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

Sperm activation

Post meiotic sperm differentiation (spermiogenesis) is required for a haploid spermatid to build cellular structures required for motility and interactions with the egg. We recently cloned two new genes (*spe-24* and *spe-43*) that are required for *C. elegans* spermiogenesis. The *spe-24* 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 a temperature sensitive allele of the *egg-3* gene. This will provide a genetic tool to not only better understand the regulation of the oocyte-to-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 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 Fellow

Xue Mei, Postdoctoral Associate

Marina Druzhinina, Laboratory Technician

Kendal Flanigan, Laboratory Technician



STEWARD LAB

Stem Cells, Hematopoiesis, Oogenesis, and Epigenetic Control of Chromatin Integrity in *Drosophila*



Dr. Ruth Steward
Molecular Biology & Biochemistry

Summary

Zfrp8, a new gene functioning in hematopoietic and ovarian stem cells in *Drosophila*

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. Mutations in these conserved genes usually alter the development of the hematopoietic organ, the lymph gland, or the hemocyte differentiation program in the fly.

We have identified *Zfrp8* (PDCD2 in vertebrates) from its grossly enlarged lymph gland phenotype. Several loss-of-function alleles of *Zfrp8* cause hyperplasia of the lymph gland, abnormal differentiation of immature blood cells, and severe growth delay in other tissues. Using clonal analysis we determined that *Zfrp8/PDCD2* is essential for the maintenance of hematopoietic stem cells. The *Drosophila* and human proteins are 38% identical and we have shown that expression of human PDCD2 in flies rescues the *Zfrp8* mutant phenotype, underlining the structural and functional conservation of the proteins.

We have established that *Zfrp8* is essential in both hematopoietic and ovary stem cells, as mutant stem cells stop dividing and are ultimately lost. 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 we see in *Drosophila* ovaries.

Zfrp8 controls the assembly of specific ribonuclear complexes.

We performed yeast two-hybrid screens using *Zfrp8* or PDCD2 as baits and screened a *Drosophila* embryonic cDNA library or a human stem cell library for interactors. Ribosomal Protein 2 (RPS2) was identified in both screens. 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, export competency, or even 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 *Zfrp8/PDCD2* is required for efficient nuclear export of select transcripts, including some *TE-RNAs* and endogenous mRNAs.

Based on the predicted chaperone activity of *Zfrp8/PDCD2* and its interaction with RNA binding proteins, we propose that *Zfrp8* assists 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.

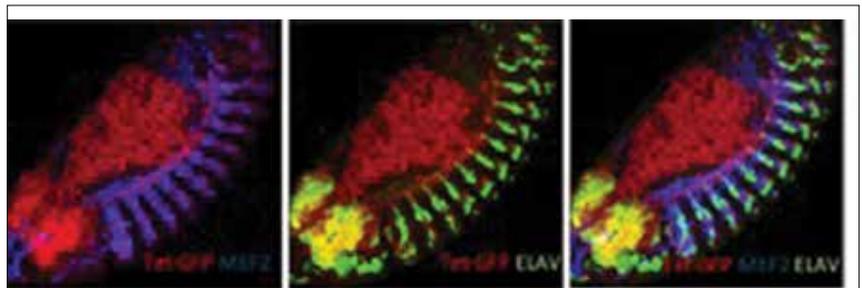


Figure: Tet expression (red) shows significant overlap with the mesodermal marker *Mef2* (blue), but its expression in mesodermal cells is lower than in neuronal cells. Stage 13 embryo; note the autofluorescence of the yolk mass on the dorsal side of the embryo (red).

We have identified components of the

Zfrp8 protein complex, including FMRP (Fragile-X Mental Retardation Protein), Nufip (Nuclear Fragile-X Mental Retardation Protein-interacting Protein) and Tral (Trailer Hitch). We showed that *Zfrp8* is required in the nucleus and for proper localization and activity of FMRP. Further we showed that *Zfrp8* genetically interacts with the translational regulators, Fmr1 and tral, in an antagonistic manner. These results suggest that Zfrp8 is required for nuclear export of the FMRP complex, and that continued association negatively regulates the activity of FMRP/Tral-dependent translational repression within the cytoplasm.

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-methylcytosine (5hmC). There are 3 TET proteins in vertebrates. They have well-documented functions in the maintenance of vertebrate stem cells. Tet proteins function as epigenetic regulators of TE activity and gene expression. 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 pupal lethal and removing one copy of *Zfrp8* suppresses this lethality; ~ 40% of animals survive to adulthood but die soon after eclosion. This result supports our protein interaction data and indicates that *Zfrp8* may have an antagonistic function to Tet.

In flies 5mC appears not to be present in DNA and our investigation of the presence of 5hmC in larval brain DNA, the tissue in which Tet is most highly expressed, failed to identify significant levels of 5hmC. A recent study shows that vertebrate Tet proteins can also convert 5mC to 5hmC in RNA. Inspired by this discovery, we have shown that 5hmC also exists in flies and depends on Tet activity. In collaboration with Dr. Fuk's laboratory at the Free University of Brussels, we have mapped 5hmC 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 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.

The Tet protein expression pattern (see Figure) and the partial loss of function phenotypes indicate that Tet functions in neurons and muscle development or function. To further 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.

Our hypothesis is that Tet, a DNA binding protein, may become localized to actively transcribed sites on the DNA and control the modification of the nascent RNAs. We have performed Chip-Seq experiments 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. Work in progress in the lab is aimed at identifying Tet-target mRNAs and mapping the modified cytosines within the RNA molecules.

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

Fei Wang, Graduate Fellow

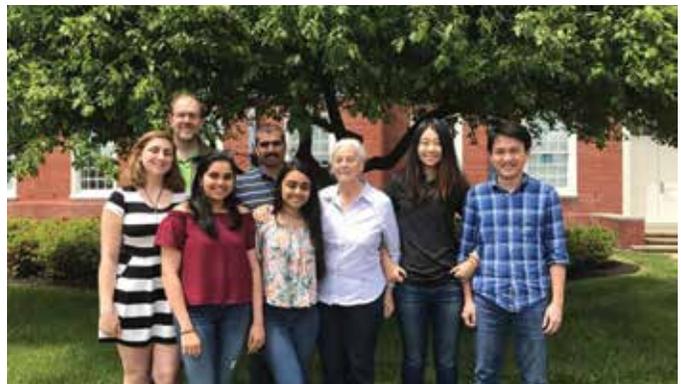
Hiep Tan, Graduate Student

Le Nguyen, Laboratory Technician

Andria Lifante, Undergraduate Student

Sara Tenjerla, Undergraduate Student

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

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

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

Inhibitors of Transcription; Antibacterial Drug Discovery

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

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

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

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

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

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

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. Yu Feng, Assistant Research Professor

Dr. Juan Shen, Assistant Research Professor

Dr. Abhishek Mazumder, Postdoctoral Associate

Dr. Jeremy Bird, Postdoctoral Associate

Dr. David Degen, Postdoctoral Associate

Dr. Soma Mandal, Postdoctoral Associate

Dr. Wei Lin, Postdoctoral Associate

Dr. Jared Winkelman, Postdoctoral Fellow

Dr. Libing Yu, Postdoctoral Associate

Adam Hasemeyer, Graduate Assistant

Miaoxin Lin, Graduate Assistant

Hanif Vahedian-Mohaved, Graduate Assistant

Yu Liu, Graduate Assistant

Barry Li, Undergraduate Assistant

Hyejin Kim, Undergraduate Assistant

Minsung Cho, Undergraduate Assistant

Jiayu Shen, Research Technician

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 massively systematic transcript end 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 4^{10} (~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. KMnO_4 , chloroacetaldehyde, DMS, hydroxyl radicals) are used to perform protein-DNA footprinting and high-throughput sequencing is used to detect RNAP-dependent changes in DNA accessibility on each member of the template library.

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

• ***Defining the role of endoribonuclease toxins in bacterial pathogenesis.***

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

Toxin/antitoxin (TA) systems are widespread in pathogens and have been implicated in virulence, survival during stress, and in promoting formation of a dormant state that is refractory to antibiotic treatment. In *M. tuberculosis*, there are greater than 80 TA systems. Furthermore, the majority of the toxins associated with these TA systems are homologues of sequence-specific endoribonucleases. Therefore, to understand the physiological role of these *M. tuberculosis* toxins, there is a need to define the cleavage specificity of each toxin. In current work performed in collaboration with Nancy Woychik (Rutgers) we are using MORE RNA-seq as a tool to determine the cleavage recognition sequences of the endoribonuclease toxins in *M. tuberculosis*, which, in turn, will provide a critical first step towards identification of the targets and physiological roles of these toxins.

Dr. Bryce Nickels, Professor

Phone: 848-445-6852

Email: bnickels@waksman.rutgers.edu

www.waksman.rutgers.edu/nickels

Dr. Jeremy Bird, Postdoctoral Associate

Dr. Jared Winkelman, Postdoctoral Fellow

Kyle Skalenko, Graduate Assistant

Lab Members

Dr. Irina Vvedenskaya, Assistant Research Professor

Dr. Leonid Minakhin, Assistant Research Professor

Dr. Chirangini Pukhrambam, Research Technician

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, 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*. Highly efficient experimental model systems to study CRISPR-Cas interference with development of various bacteriophages have been created in our laboratory and are being used to determine how the infection process is affected by the countering CRISPR-Cas at the molecular level. Powerful in vitro methods, including fluorescent beacon assays inspired by our work with RNA

polymerases, are being used to determine how various Cas effector proteins programmed with RNA guides locate their DNA targets. New CRISPR-Cas systems are being identified through bioinformatics searches and validated experimentally. Some of these systems have clear potential for applications in genome editing.

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.

Structure-functional analysis of novel transcription enzymes

Giant phages are a poorly characterized group of bacterial viruses with very large genomes. Phages of the family do not depend on bacterial host transcription apparatus for their development. We identified two families of unique multisubunit RNA polymerase in giant phage genomes. We use biochemical and structural methods to investigate these unusual enzymes and compare them to cellular RNA polymerases.

Dr. Konstantin Severinov, Professor

Phone: 848-445-6095

Email: severik@waksman.rutgers.edu

www.waksman.rutgers.edu/severinov

Lab Members

Dr. Konstantin Kuznedelov, Research Associate

Dr. Vladimir Mekler, Research Associate

Dr. Ekaterina Semenova, Research Associate

Dr. Kirill Datsenko, Research Assistant

Ishita Jain, Graduate Fellow

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 $\alpha 2$ and $\alpha 1$ 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 $\alpha 1/\alpha 2$ 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 $\alpha 1/\alpha 2$ complex, we identified binding sites downstream of two genes that require $\alpha 1/\alpha 2$ for expression in diploid cells. We have shown that these sites regulate transcription of haploid-specific, antisense ncRNAs that interfere with expression of their respective genes. Repression by these antisense transcripts only works in a *cis* configuration to the genes that they are regulating, suggesting that that mechanism of repression is not through the formation of double stranded RNA. Chromatin immunoprecipitation (ChIP) assays showed that the repression of the sense transcripts does not interfere with transcription factors binding to the promoter. We have identified regions within the open reading frame of both genes that are required for antisense-mediated repression. Inversion of this region in one of the genes causes a loss of repression, indicating that there is a context dependent orientation of this element that is required for repression. Our results suggest that these genes are being repressed through a novel mechanism.

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

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

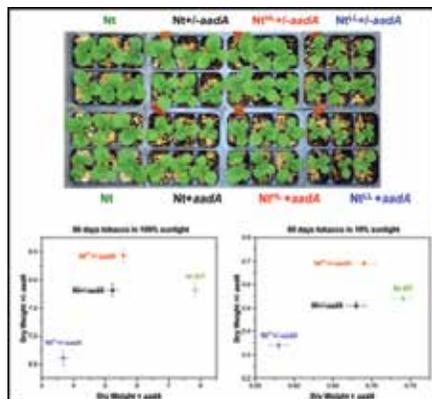
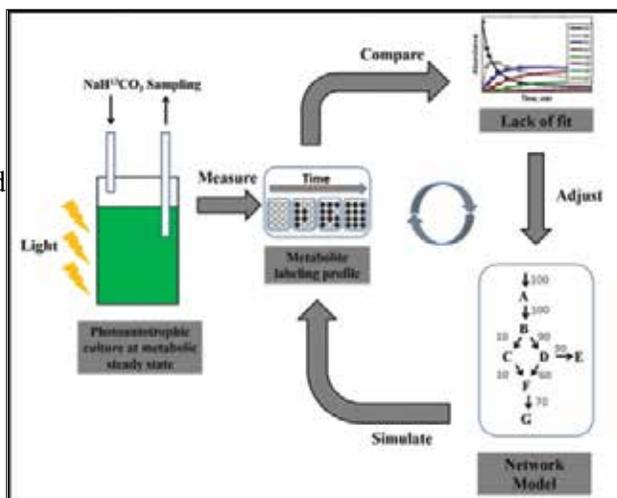


Dr. G. Charles Dismukes
Chemistry & Chemical Biology
Microbiology & Biochemistry

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 2016-June 2017 period the group was comprised of 46 researchers (listed below).

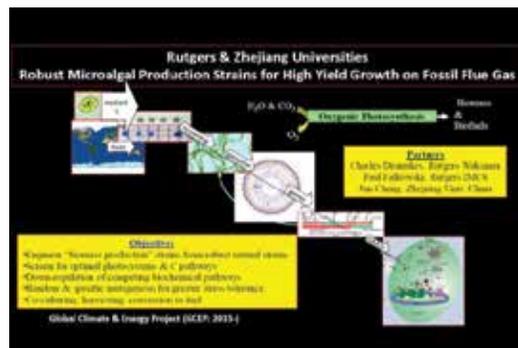
1) **Photoautotrophic Carbon Fluxomics.** Our goal is to use flux balance analysis and isotopically nonstationary metabolic flux analysis (INST-MFA, figure) to quantitatively understand carbon flux distributions and pathway used by the cyanobacterium *Synechococcus sp.* PCC 7002 during photosynthesis. The outcome also helps discover new roles for existing metabolic pathways. Supported by NSF-MCB.



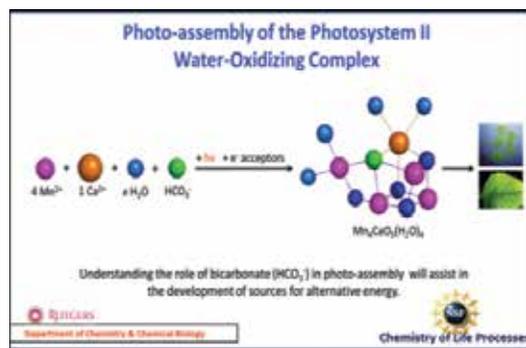
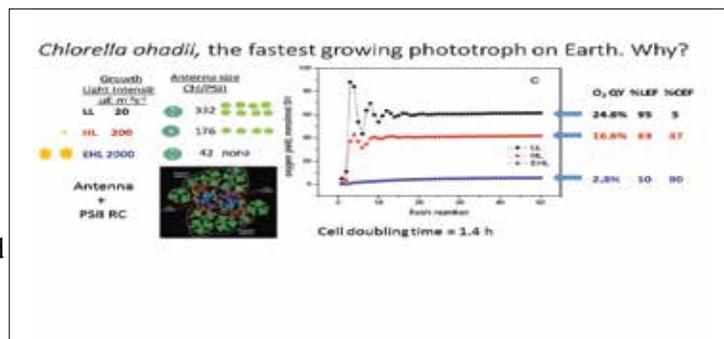
2) Optimizing Photosynthetic Light Conversion by Protein Subunit Engineering.

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 CO₂. We apply genetic and environmental methods to modify the metabolic pathways to reroute the flux of fixed carbon and extract H₂ or carbon fuel precursors. We are collaborating with Christoph Benning's lab (Michigan State U.) to generate potential CO₂-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.

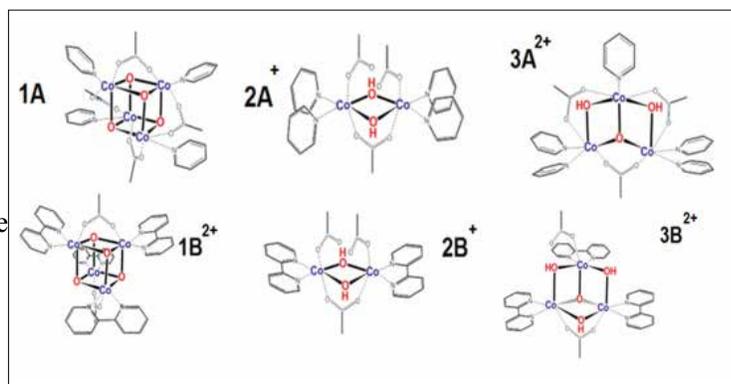


4) Diversity of Photosynthetic Water Oxidizing Enzymes. Our goal is to reveal the range of kinetic and energetic performance by photosynthetic water oxidation enzymes *in vivo*, selected from diverse microbial phototrophs, cyanobacteria and microalgae. The outcome is a fundamental understanding of the 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⁻, CO₃H⁻, 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

Tunable Photoanode-Photocathode-Catalyst-Interface Systems for Efficient Solar Water Splitting
GC Dismukes (PI), M Greenblatt and E Garfunkel (coPIs), Rutgers University, CBET-1433492

Light absorber: perovskite oxynitrides

- A: alkaline earth/rare earth
- B: Ti, V, Zr, Nb, Ta (d⁰)
- O, N

AB(O,N)₃, Optical Bandgap: 1.7 - 2.1 eV

Photoanode: LiCoO₂, AB(O,N)₃, TiO₂

Photocathode: LiCoO₂, TiO₂, Ni₂P₄

Electrolyte: alkaline electrolyte

Reaction: H₂O → H₂ + O₂

Fabrication: Using pulsed laser deposition, prepare photoanode with thin film structures shown above. Fabricate photocathode of p-silicon and Ni₂P₄ HER catalyst. Investigate two electrolyte systems: aqueous alkaline electrolyte solution (pH=14) and alkaline exchange membrane.

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

Dr. Tie Shen, Visiting Scholar, Assistant Professor

Dr. Bin Rui, Visiting Scholar, Associate Professor,

Dr. Ronald Pace, Visiting Scholar

Dr. Wooseok Ki, Visiting Scholar, Assistant Professor

Dr. Dmitry Shevela, Visiting Scholar

Dr. Anders Laursen, Research Associate

Dr. Voshadhi Amarasinghe, Research Associate

Dr. Yunbing Ma, Research Associate

Dr. Spencer Porter, Research Associate

Dr. G. K. Kenchappa, Research Associate

Dr. Joshua Flynn, Research Associate

Paul Smith, Graduate Assistant

Graeme Gardner, Graduate Assistant

Anagha Krishnan, Graduate Assistant

Xiao Qian, Graduate Assistant

Yuan Zhang, Graduate Assistant

Colin Gates, Graduate Assistant

Shinjae Hwang, Graduate Assistant

Karin Calvino, Graduate Assistant

Khue Tu Ho-Nguyen, Graduate Assistant

Apostolos Zournas, Graduate Assistant

Hongxiang Lu, Visiting Graduate Assistant

Hoa Vu, MS Graduate Assistant

Bryan Mejia-Sosa, Technician

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

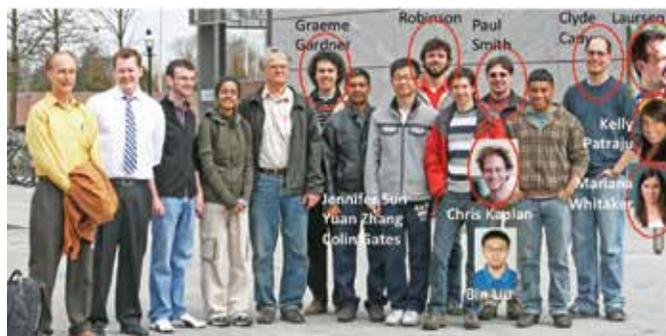
Timothy Goetjen, Undergraduate Assistant

Chris Dziedziak, Undergraduate Assistant

Shivam Kaushik, Undergraduate Assistant

Ajay Kashi, Undergraduate Assistant

Rachel Allen, Undergraduate 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. Establishment and maintenance of cell polarity is required for asymmetric cell division (ACD), an indispensable mechanism for multi-cellular organisms to generate cellular diversity by producing daughter cells with distinctive identities from a single mother cell. Our studies on the novel plant protein BASL (Breaking of Asymmetry in the Stomatal Lineage) provided strong evidence that plant cells also have the capability to polarize non-transmembrane proteins and utilize such polarized protein distribution to regulate asymmetric cell division.

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. This includes the identification of additional polarized proteins and of mutants that display specific subsets of polarity defects.

Regulatory mechanisms for BASL polar trafficking

We used the FRAP (Fluorescence Recovery After Photobleaching) technique to probe subcellular dynamics of the BASL protein and to compare with the other polarity proteins. Our data demonstrated that, unexpectedly, the recovery behavior of the non-membrane protein BASL at the cortical polarity site mirrored that of the member-embedded PIN proteins, but not that of the membrane-associated ROP proteins (Figure 2A), suggesting that BASL might rely on the membrane trafficking system for polarization. Interestingly, phosphorylation status of BASL seemed to influence its intracellular mobility (Figure 2B). One of the phospho-mimicking versions, BASL_14D, showed drastically reduced FRAP mobility (Figure 2B). Concomitantly, this version conferred the polar distribution only, as well as a stronger suppression of stomatal division and differentiation (Figure 2C-D).

BASL polarity at the cell cortex suppresses SPCH

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,

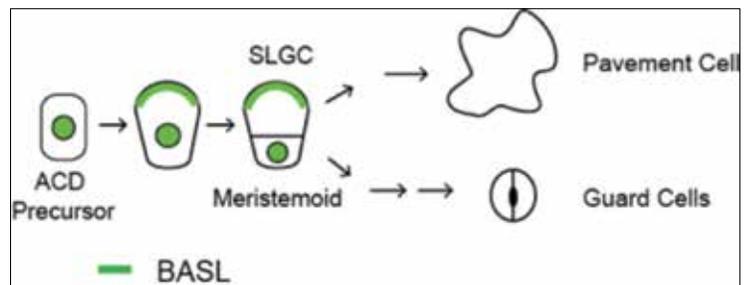


Figure 1: BASL localization and stomatal asymmetric cell fate

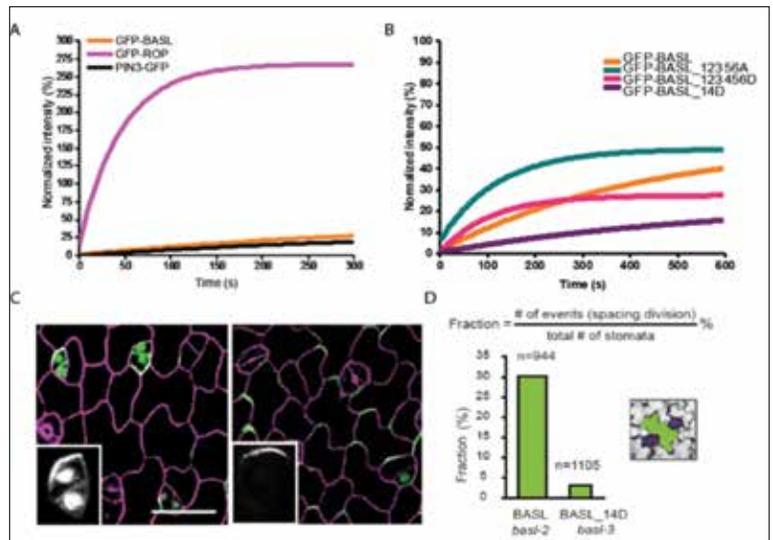
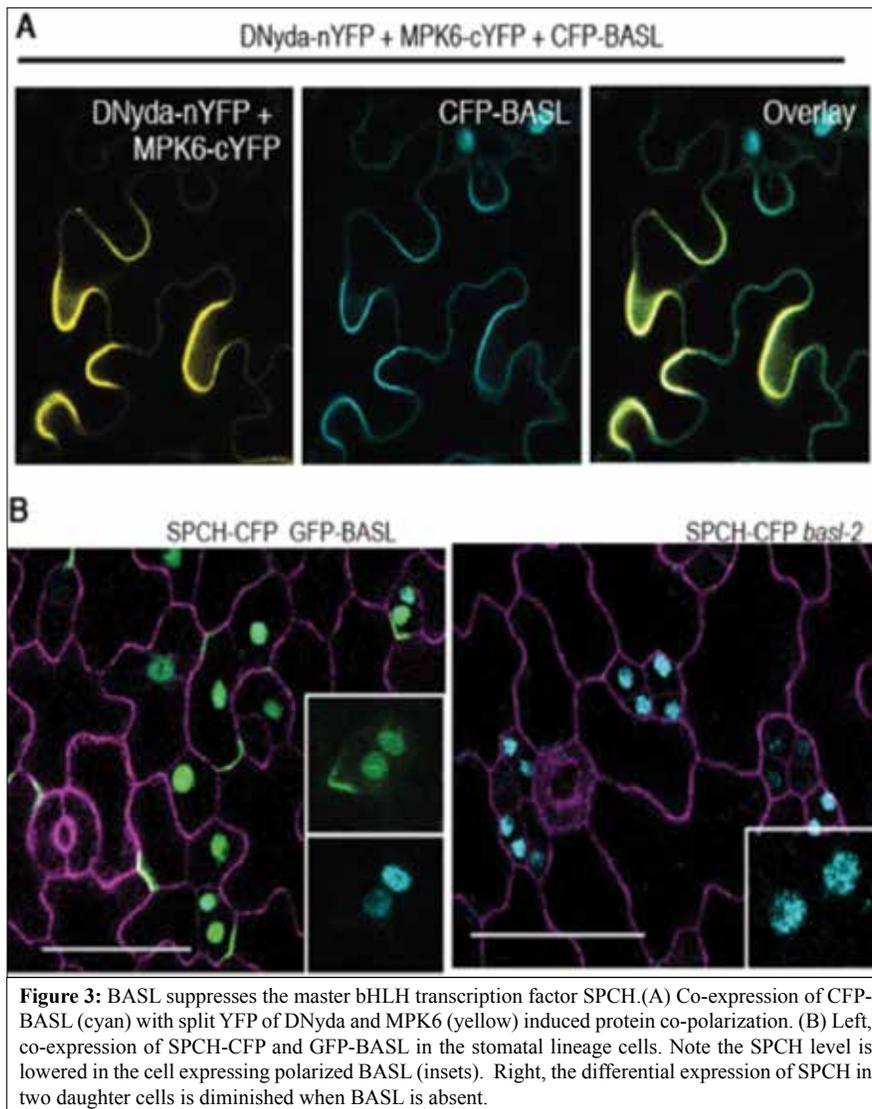


Figure 2. Fluorescence recovery of BASL at the polarity crescent. (A) Normalized FRAP curves for GFP-BASL, GFP-ROP2 and PIN3-GFP in the stomatal lineage cells. (B) Comparison of FRAP curves of BASL and phospho-variants. BASL_12356A indicates 5 Ser sites mutated to Ala (phospho-deficient). BASL_123456D 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.



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.

Dr. Juan Dong, Assistant Professor

Phone: 848-445-7034

Email: dong@waksman.rutgers.edu

www.waksman.rutgers.edu/dong

Lab Members

Dr. Ying Zhang, Postdoctoral Associate

Dr. Xueyi Xue, Postdoctoral Fellow

Dr. Xiaoyu Guo, Postdoctoral Associate

Wanchen Shao, Graduate Student

Dr. Kezhen Yang, Visiting Scholar

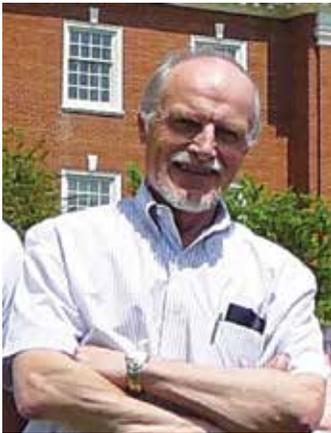
Chao Bian, Graduate Student

Dongmeng Li, Graduate Student

Lu Wang, Graduate Student

DOONER LAB

Plant Genetics



Dr. Hugo Dooner
Plant Biology

A sequence-indexed reverse genetics resource for maize

Our NSF-funded long term project is developing a reverse genetics resource for maize based on the transposable elements *Ac* and *Ds*. This is a highly desirable community resource that will allow maize workers to fully exploit the maize genome sequence. The availability of a mutant line in which a single gene has been disrupted 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. 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, 12,751 *Dsg* insertions have been mapped to the reference genome (Figure 1). 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.

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 element transposition from any region 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.

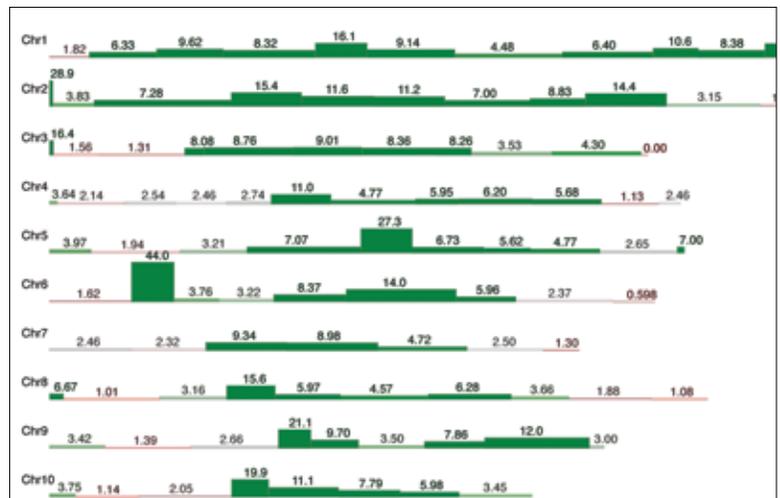


Figure 1. Distribution of mapped *Dsg* insertions by chromosome “bin” in all ten maize chromosomes. Each bin is ~20 centiMorgans long. Numbers above each bin are *Dsg* insertions per cM. The color scale corresponds to the z-score of insertions/cM of all bins, red indicating bins with significantly lower number of insertions than average. The width and height of each rectangle are proportional, respectively, to the actual cM of each bin and the number of insertions per cM in that bin.

Dr. Hugo Dooner, Distinguished Professor

Phone: 848-445-4684

Email: dooner@waksman.rutgers.edu

www.waksman.rutgers.edu/dooner

Lab Members

Dr. Mithu Chatterjee, Research Associate

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.

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.

We are part of a newly funded collaborative research project sponsored by the National Science Foundation Plant Genome Research Program to identify new genes involved in auxin biology and to investigate the specificity of auxin function in developmental pathways. Using forward genetic screens, we have identified BIF1 and BIF4, two Aux/IAA proteins that function synergistically to initiate the many specialized types of reproductive axillary meristems that form the highly complex inflorescences of maize. Aux/IAA work in conjunction with ARF transcription factors. Using a recently developed genomic approach called DAP-seq we are characterizing the DNA binding behavior of the maize ARF family to identify the direct targets of their regulation and to understand the molecular events that connect auxin to meristem initiation and development.

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.

REL2 is recruited by an array of transcription factors containing specific repressor motifs to repress the transcription of their target genes. We uncovered a large number of transcriptional regulators that interact with REL2 and they do so by 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.

Mechanisms of boron transport for maize development and productivity

Boron is an essential micronutrient for plant growth and development and plays an important role in the structure and maintenance of plant cell walls. In crops, proper boron nutrition is critical for obtaining high yields. The productivity of a variety of crops in nearly 80 countries is affected due to deficiency in boron, making it more widespread than deficiencies in any other plant micronutrient. While fertilization is one option to alleviate poor quality soils, the production and use of such chemicals is costly and can have negative effects on the environment. Furthermore, the range of optimal boron concentrations for plant growth is believed to be narrow and fertilization may therefore result in toxicity problems.

In maize, low levels of boron in the soil affect vegetative and reproductive development, eventually causing widespread sterility in its inflorescences. We characterized the boron efflux transporter RTE. Strong alleles of *rte* mutants produce tassels devoid of flowers and ears that wither during early development. To further understand how boron is transported and distributed during vegetative and reproductive development, we have identified five additional boron transporters in the maize genome. We discovered that one of these genes, RTE2, is a close duplicate of RTE. *rte rte2* double mutant plants are severely affected in shoot and root development (Figure 1) and these defects can be rescued by watering plants with an excess of boric acid. These results indicate that the combined action of both genes is required for shoot and root growth in boron deficient conditions. Elucidating the mechanisms of boron transport and its roles in plant growth will enable the design of new strategies to alleviate deficiency or toxicity problems and increase crop production in different areas of the world.



Figure 1. *rte rte2* mutant plants fail to form an apical inflorescence (tassel) when grown in soil with low levels of the micronutrient boron.

Dr. Andrea Gallavotti, Assistant Professor

Phone: 848-445-6421

Email: agallavotti@waksman.rutgers.edu

www.waksman.rutgers.edu/gallavotti

Lab Members

Mary Galli, Laboratory Researcher

Dr. Xue Liu, Postdoctoral Associate

Dr. Zongliang Chen, Postdoctoral Fellow

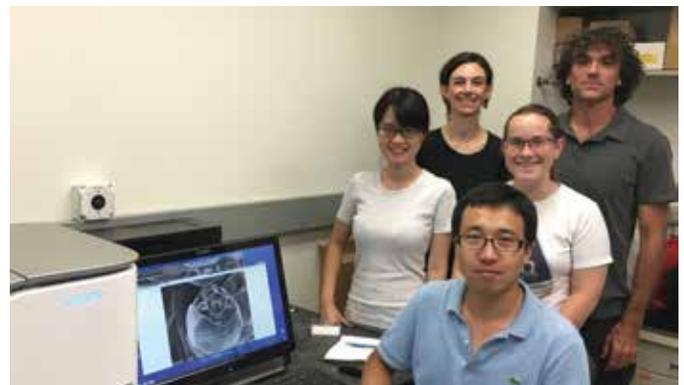
Qiujie Liu, Graduate Student

Caitlin Menello, Laboratory Assistant

Ryan Siegel, Laboratory Assistant

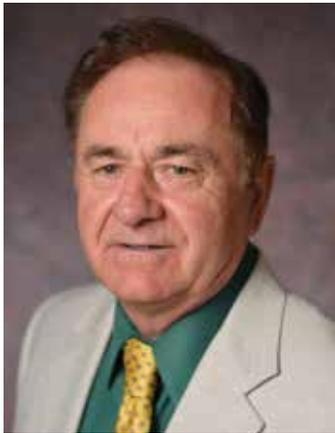
Ruhi Shah, Undergraduate Student

Aditya Patil, Undergraduate Student



MALIGA LAB

Plastid Molecular Genetics



Dr. Pal Maliga
Plant Biology

Summary

Plastids are semi-autonomous organelles with a relatively small (120-180 kb), highly polyploid genome present in 1,000 to 10,000 copies per cell. 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.

Progress in implementing plastid transformation in Arabidopsis thaliana

Plastid transformation is routine in tobacco, but 100-fold less frequent in *Arabidopsis*, preventing its use in plastid biology. A recent study revealed that null mutations in *ACC2*, encoding a plastid-targeted acetyl-CoA-carboxylase, cause hypersensitivity to spectinomycin. We hypothesized that plastid transformation efficiency should increase in the *acc2* background, because when *ACC2* is absent, fatty acid biosynthesis becomes dependent on translation of the plastid-encoded ACC β -Carboxylase subunit. We bombarded *ACC2*-defective *Arabidopsis* leaves with a vector carrying a selectable spectinomycin resistance (*aadA*) gene and *gfp*, encoding the green fluorescence protein GFP. Spectinomycin resistant clones were identified as green cell clusters on a spectinomycin medium. Plastid transformation was confirmed by GFP accumulation from the second open reading frame of a polycistronic mRNA, that would not be translated in the cytoplasm. We obtained one to two plastid transformation events per bombarded sample in spectinomycin hypersensitive Slavice (Sav-0) and Columbia *acc2* knockout backgrounds, an approximately 100-fold enhanced plastid transformation frequency. Sav-0 and Columbia are accessions in which plant regeneration is uncharacterized or difficult to obtain. A practical system for *Arabidopsis* plastid transformation will be obtained by creating an *ACC2* null background in a regenerable *Arabidopsis* accession. The recognition that the duplicated ACCase in *Arabidopsis* is an impediment to plastid transformation provides a rational template to implement plastid transformation in related recalcitrant crops. These experiments are carried out in collaboration with Prof. Kerry A. Lutz, Farmingdale State College, Farmingdale, NY.

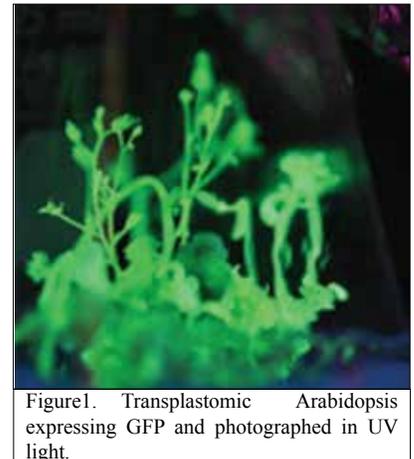


Figure 1. Transplastomic *Arabidopsis* expressing GFP and photographed in UV light.

Transgenic approach to probe gene regulation by PPR10 RNA binding protein in chloroplasts

We use a transgenic approach to test the utility of PPR10 binding site for transgene expression in a model dicistronic operon in which the 1st ORF encodes a selectable marker gene and the 2nd ORF the green fluorescent protein (GFP). The intergenic region in the operon is a 100-nt sequence, including 17 nucleotides required for PPR10 binding. We constructed operons with tobacco and maize sequences, which differ by one nucleotide in the binding region, and maize PPR10 binding site GG and AA variants (PBSZmGG and PBSZmAA), which do not bind the wild-type PPR10 protein *in vitro*. We report that the tobacco PPR10 protein discriminates between the tobacco and maize binding sites and preferentially stabilizes processed mRNA 5'-ends with the tobacco binding site. Processed mRNAs with mutant maize binding sites are degraded in chloroplasts, confirming the essential role of PPR10 binding in stabilization of mRNAs with processed ends. We also found that binding of mRNAs to a PPR10 binding site upstream of the AUG translation initiation codon facilitates translation; however, processing of dicistronic mRNAs is not required for efficient translation. Our data suggest that the highest ($\geq 20\times$) dynamic range between baseline and induced rates can be obtained by the combination of mRNA stabilization and enhanced translation mediated by PPR10 binding. We have

now incorporated the transgenes in potato plastids, and are testing tuber-specific accumulation of GFP regulated by PPR proteins under control of tuber-specific promoters. These experiments are carried out in collaboration with Prof. Alice Barkan, Oregon State University, Eugene, OR.

Dr. Pal Maliga, Distinguished Professor

Phone: 848-445-5329

Email: maliga@waksman.rutgers.edu

www.waksman.rutgers.edu/maliga

Lab Members

Dr. Zora Svab, Laboratory Researcher

Lisa Lamanna, Graduate Student

Qiguo Yu, Graduate Fellow

Yuan Zhang, Graduate Fellow

Akram Hafizi, Visiting Scientist

Amanda Chen, Undergraduate Student

Kliment Todosov, Undergraduate Student

Megan Kelly, 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, Shotgun DNA Sequencing, Genome Evolution

The grass family of species include some of the most important crops and their seeds provide the largest calorie intake for feed and food on earth. The major component of seeds are carbohydrates, but the nutritional quality is largely determined by the protein component. Seed proteins in this family of species are dominated by one group, called prolamins. Prolamins have diverged during evolution, resulting in proteins with different physical properties. The wheat proteins have the viscosity and elasticity required for bread making and noodles, a property that rice and maize prolamins have lost. A better understanding of these properties arises from comparative genomics of species

from different subfamilies of the grasses, also called the Poaceae (see Figure).

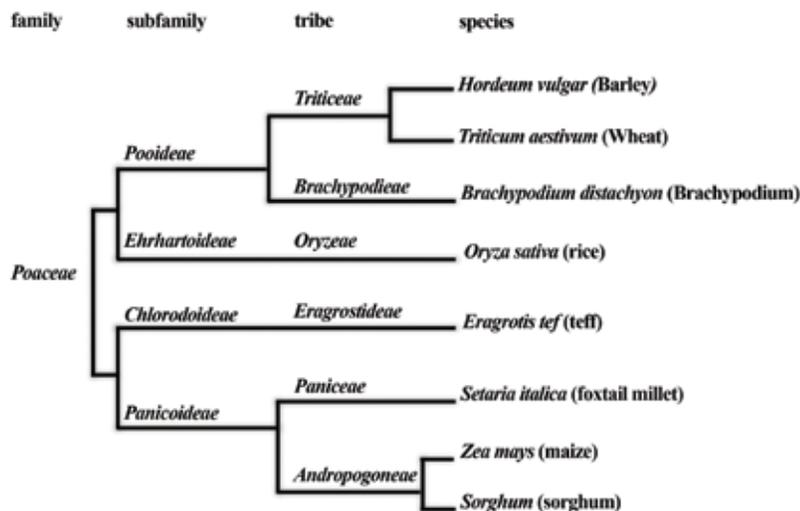


Figure 1. The Poaceae consists of several subfamilies that include the major grains used in food production. The relationship of the genomes of different species is illustrated in form of a phylogenetic tree (Figure from Zhang et al, Genome Biology and Evolution).

Previously, we have participated in the sequencing of grass genomes of rice, maize, sorghum, and Brachypodium. Now, we have also added the species of teff, a grain that is mostly consumed in Ethiopia. *Eragrostis tef* belongs to a different subfamily than wheat, rice, or maize. The Chlorodoideae are closer to the Panicoideae that includes the millets, sorghum and maize, than the Pooideae or the Ehrhartoideae (see Figure). Analysis of the prolamins in teff showed that they are indeed more closely related to maize prolamins than the wheat or rice prolamins. However, one major difference to maize emerged. Tef can be used to make flat bread, called injera, thereby demonstrating different properties than maize and more similarities to wheat. Therefore, we can now investigate how the properties of bread making was lost in maize.

Gene copy number and haplotype variation with PacBio

One of the youngest prolamin subfamily is the alpha prolamins that are absent in the Pooideae and Ehrhartoideae

and present in teff, the millets, sorghum, and maize. In maize, they are known as the alpha zeins. The alpha zeins are present in six chromosomal locations and except for one in tandem gene clusters. Tandem gene amplification, however, is quite common in eukaryotic genomes and difficult regions to sequence because of the conservation of gene copies. We have taken advantage of a new sequencing platform to investigate the variation in copy number of these gene clusters. A key feature of the PacBio sequencing system is the long reads, which help to reconstruct repetitive regions in chromosomes from shotgun DNA sequencing. Previously, it was necessary to prepare Bac clone libraries and use DNA fingerprinting to obtain overlapping BAC clones to selectively sequence chromosomal regions. With the PacBio platform we could perform whole genome shotgun sequencing without the need to create a physical map with overlapping BAC clones. This approach required us to make only one DNA preparation rather than the numerous ones associated with BAC clone libraries and DNA fingerprinting of them. Furthermore, we could prove the correct PacBio assembled chromosomal regions with another new technique, called BioNano. BioNano can create a whole genome physical map. It also requires only one genomic DNA preparation. The genomic DNA is stretched, fluorescently labeled at specific recognition sequence of seven nucleotides (GCTCTTC), which are then detected by the BioNano machine in situ so that distances can be compared among many molecules. This data allows us to concatenate molecules via their common restriction pattern and form a restriction map of chromosomal regions. The resulting physical map can then be used to confirm the correct assembly of PacBio sequences. We could compare three different maize inbred lines, which all differed in their tandem alpha zein gene clusters. Therefore, this new approach should also be generally applicable including to the human genome.

The PacBio platform is not only superior for studying tandem gene clusters, but also haplotype variation of gene families. The studies of prolamin transcripts have been hampered by short sequence reads because of their internal repeated blocks of amino acids. Again, it is the long sequence read capacity of the PacBio system that also applies to transcripts of genes. We could obtain from one PacBio sequencing experiment a total of 424 full-length prolamin transcripts from ten wheat cultivars. Comparison of the transcripts from different cultivars provides us with haplotypes that could be useful to reduce the load of toxic epitopes in gluten molecules for the benefit of celiac patients.

The connection between protein quality and yield

Expression of the alpha zein genes in maize described above prevents the storage of essential amino acids because they are poor in lysine, tryptophan, and methionine codons thereby lowering protein quality in maize. Therefore, a mutation discovered in the sixties that renders a transcription factor regulating alpha zeins inactive leads to an increase in lysine-rich proteins thereby improving the protein quality of maize. Because this mutation gives the kernel an opaque appearance, the corresponding transcription factor is called O2. Now, we found that this transcription factor also regulates starch synthesis. Furthermore, another transcription factor regulating zeins in maize, the prolamin box binding factor (PBF), is also linked to starch biosynthesis. This finding illustrates that improving protein quality can also result in kernel weight loss. To circumvent such a deleterious effect, we had previously applied a different approach of rebalancing the protein content in the maize kernel by using RNA interference, which is not only dominant but also very specific for its target.

New approach to develop allelic variation

The Dooner lab has developed a new maize mutant collection. We have selected a recessive mutation from this collection in the F2 generation as a defective kernel phenotype, also referred to as *dek38*. The critical feature of the new collection is that it is based on the properties of a transposable element system in maize discovered by Barbara McClintock. In the eighties, we could show that the non-autonomous element Ds tolerates internal deletion affecting the trans-acting function of the autonomous element Ac, but that the ends with the cis-acting functions are conserved between both. Building on this theme, the Dooner lab replaced the internal portion of the Ds element with a chimeric gene that expresses the green fluorescent protein GFP under a seed specific promoter so that one can distinguish this Ds element from others phenotypically as well as by its sequence. The *dek38* phenotype was linked to a gene, encoding a co-chaperone of the TTT complex, providing the first description of this function in plants. Rather than having to complement this function by transgenic means, the Ac/Ds system allowed us to excise the engineered element and generate alleles of the tagged gene as proof of linkage of the phenotype to the gene. Excisions gave rise to frame shifts and codon insertions of the tagged gene, thereby providing a useful approach to functional genomics.

Biofuel studies

Besides our studies with grasses as a major food supply, we are also investigating species that could be useful as a Biomass source. Biomass is directly dependent on growth efficiencies. In this respect, the fastest growing plants on earth are duckweeds, which are aquatic plants that float on water. Although algae have also photosynthesis and may be even faster in growth, they are more difficult to separate from water because of their size, whereas duckweeds are not. We have investigated the properties of them with the example of *Spirodela polyrhiza*, whose genome we previously sequenced. Because photosynthesis and growth are closely linked to the functions of organelles, we have investigated one aspect of gene expression in mitochondria and chloroplasts, RNA editing. For many genes, organelle transcripts must be modified so that they can be translated, a process called RNA editing. Interestingly, the genes that control this process are multigene families present in the nuclear rather than the organelle genomes, the pentatricopeptide-repeat proteins or PPR. We therefore have characterized this gene family in *Spirodela*, which appeared to be even larger in copy number than land plants, perhaps because of the enhanced exposure to UV light of aquatic plants.

Review and Essay

We also participated in the publication of a book “Achieving Sustainable Maize Cultivation” with the contribution of a chapter on maize protein traits. I also wrote an essay for the American Academy of Arts and Sciences on a subject of general interest.

Dr. Joachim Messing, Director 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

Dr. Jiaqiang Dong, Postdoctoral Fellow

Dr. Zhiyong Zhang, Postdoctoral Fellow

Dr. Yin Li, Postdoctoral Fellow

Fei Ge, Visiting Graduate Student

Paul Fourounjian, Graduate Student

Nelson Garcia, Graduate Student

Jose Planta, Graduate Student

Jennifer Ayer, Laboratory Research Technician

Chris Wakim, Undergraduate Student

Rushabh Mehta, Undergraduate Student



CORE FACILITIES

Waksman Confocal Imaging Core Facility

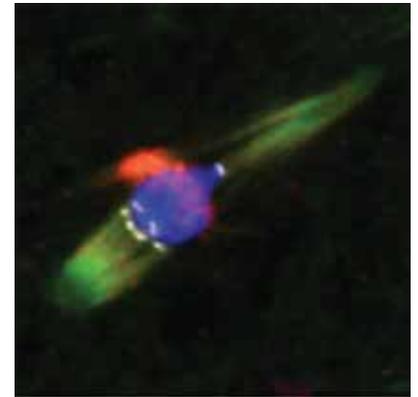
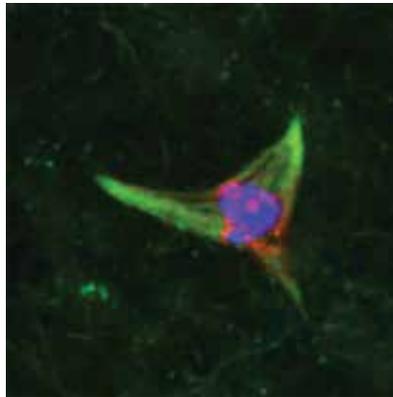
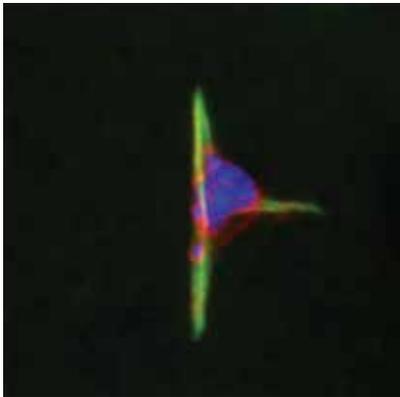


Padgett lab: Drosophila wing disc

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.

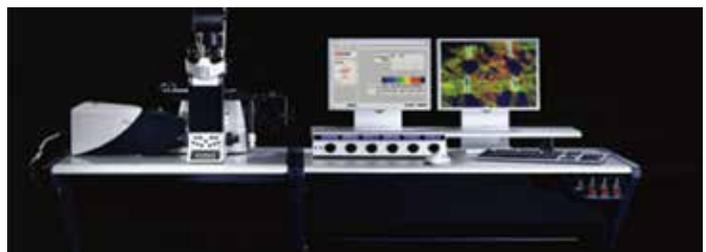
Both Leica TCS confocal models have inverted microscopes, spectral detection allowing for dynamic adjustment of detected emission wavelengths, sequential scanning to further reduce emission signal overlap, easy to use programs for creating Z-stacks and 3-D images, multiple programs for post-imaging, 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 50 trained users, primarily Waksman researchers, from twelve laboratories and is used an average of 70 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.

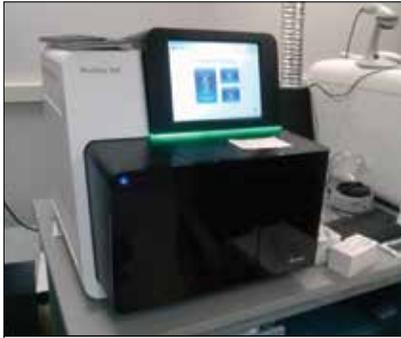


McKim lab: abnormal metaphase I
in Drosophila

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



NextSeq500



Sequel



MiSeq

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 forces 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.

WGCF recently acquired the Single Molecule Real-Time (SMRT) sequencer, the Sequel System made possible by generous financial support from the Chancellor's New Brunswick office. The Sequel uses Single Molecule Real Time (SMRT) technology to produce long reads, uniform coverage, and high consensus accuracy. The Sequel long 10-15kb reads will greatly enhance whole genome, full-length transcript, or long amplicons sequencing projects. Additionally, its SMRT sequencing technology can also be used to directly detect DNA base modification.

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

With these instruments, WGCF is capable of producing 150 Gb of data per day from over a billion reads. Data from DNA sequencing services are typically reported to customers within two weeks. Funding for the operation of the core facility comes from a combination of user fees and cost sharing from Waksman Institute keeping the instrument use cost low. This creates an exceptional opportunity for a researcher to perform experiments with state-of the art instruments.

User fees are used to cover part of instrument service contracts cost and consum-

able 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, Nano-Drop and Agilent bioanalyzer.

In Addition to the wet-lab services, Waksman Genomics Core Facility also provides powerful, accessible and intuitive analysis tools through its bioinformatics consulting and data analysis services. This allows researcher to bypass learning curve and focus on the result that are both statistically and biologically meaningful. 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 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
Whole Genome Resequencing	√√√√	√	√√
De Novo Sequencing	√√√	√	√ √ √ √
Human Exome Sequencing	√√√√		
Target Resequencing	√√	√√√	
mRNA Sequencing	√√√√		√√
Small RNA Sequencing	√√√		
ChIP Sequencing	√√√√		
Metagenome Analysis	√	√	
Methylation Analysis	√√	√√√	√√√√

Dr. Dibyendu Kumar, Director Genomics Core Facility/Research Associate

Phone: 848-445-4737

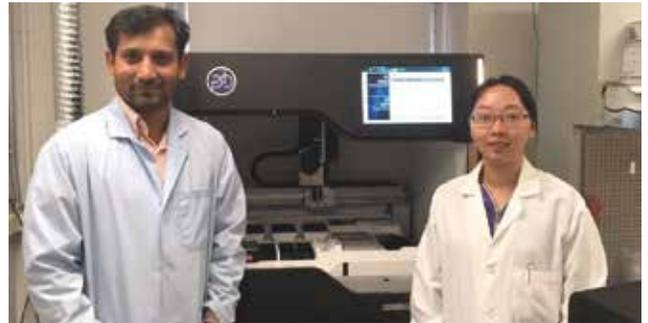
Email: dk@waksman.rutgers.edu

www.waksman.rutgers.edu/genomics

Facility Members

Dr. Yaping Feng, Bioinformatics Research Scientist, Research Associate

Dr. Min Tu, Lab Operations Scientist, Postdoctoral Fellow



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

For more than sixty years, the Fermentation Facility at Waksman Institute has served the private and public sector offering industrial fermentation expertise and services.

The facility has a multiclient base and produces a wide range of bulk biologics including; antimicrobials, cosmetic substrates, flavors/fragrances, biopesticides/bioherbicides and plasmid-derived; proteins, enzymes, growth factors and diagnostics to name just a few.

We provide a specific niche for companies with; minimal resources, limited space, limited expertise, novel proprietary technologies, scale-up restrictions, regulatory constraints or virtual structures.

We are equipped to handle most requests with the exceptions; pathogenic or opportunistic organisms and mammalian and insect cell lines. The facility is designated BL2-LS. Our bioreactor volumes range from 32 to 800L maximum operating volumes.

All projects are conducted under CDA/NDA agreements, which limit discussion of specific company or projects.

We operate as a not-for-profit, fee-for-service self-supportive lab and receive no direct funding from state or federal sources.

Purchase for 2017 include a third, forty liter Eppendorf Bioflo 510 bioreactor system.



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

Faculty Members

Amanda Rodriguez, Laboratory Researcher III and Lab Manager

Dr. Sergey Druzhinin, Lab Researcher IV

Ryan Rosen, Senior Lab Technician



TRAINING FUTURE LEADERS

- Postdoctoral Fellowships
- Predoctoral Fellowships
- Graduate & Undergraduate Courses
- High School Outreach/ WSSP

CHARLES AND JOHANNA BUSCH FELLOWS

Postdoctoral Research



Dr. Jared Winkelman
Ebright/Nickels Lab

Research Summary

During each phase of transcription (initiation, elongation, and termination), RNAP makes extensive interactions with nucleic acids and is responsive to sequence context. In addition, different RNAP functions during initiation, elongation, and termination can be rate limiting for specific 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 intriguing challenge. While previous studies have revealed many RNAP-nucleic acid interactions that affect transcription, a full understanding of the relationship between nucleic acid sequence and functional output remains a fundamental gap in our knowledge. Thus, my research seeks to leverage the capabilities of high-throughput sequencing technologies to address this knowledge gap. Over the past 4 decades many biochemical assays have been developed to monitor protein-nucleic acid complexes. These include several footprinting methods to monitor the surface of DNA protected by protein, crosslinking assays to monitor proximity

between amino acids and nucleotides, and DNA structure-sensitive chemical probes. The primary focus of my work over the past year has been to develop methods that combine these biochemical assays with high-complexity promoter libraries and high-throughput sequencing. These tools are currently being used to investigate the mechanism of RNA polymerase-promoter open complex formation, transcription start site selection, promoter escape, transcription pausing, and transcription termination. To date, we have successfully used unnatural-amino-acid-mediated protein-DNA crosslinking and transcription start site mapping to define the mechanism of transcription start site selection, the mechanism of reiterative transcription, and the sequence dependence of transcription pausing within the first 9-13 nt of RNA synthesis.



Dr. Xueyi Xue
Dong Lab

Research Summary

Stomatal development and patterning in *Arabidopsis* requires precisely controlled asymmetric cell division (ACD). The plant-specific polarity protein BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE (BASL) plays a central role in establishing physical asymmetry and differentiating daughter cell fate in stomatal ACD. The BASL protein, similar to the conserved PAR polarity proteins in animals, relies on its highly polarized localization to regulate orientated cell division. Our lab has generated preliminary RNA_{seq} data that showed differentially expressed genes in the absence and presence of BASL in *Arabidopsis* plants. We named these genes as Asymmetry-Related Genes (ARGs). The ARG enlisted a myriad of regulators, such as transcription factors, signaling molecules and microtubule associated proteins, all of which may contribute to stomatal ACD. I have been working on the protein families represented by the WRKY transcription factor (ARG42), the bHLH transcription factor (ARG65), the glycosylated protein (ARG48) and microtubule-binding IQD proteins (ARG56 and ARG64).

Recently my effort focuses on ARG48, which encodes a hydroxyproline-rich glycoprotein. ARG48 is specifically expressed in stomatal lineage cells and highly enriched in SPCHid2 (asymmetric divisions) but lowered in SPCHid2 *basl* (symmetric divisions). There are three homologous genes in the *Arabidopsis* genome. All of the ARG48 subfamily members localize to the plasma membrane and are seemingly enriched at lipid raft loci, suggesting their function in cell signaling and membrane organization. I have created quadruple mutants of the ARG48 family using CRISPR/Cas9. The *crispr_arg48* plants show strong stomatal cluster, which is similar to the receptor *er er11 er12* triple mutant. Our genetic analysis suggests that ARG48 functions downstream of the peptide ligands EPF1 and EPF2, while upstream of the MAPKK Kinase YODA. It remains to be elucidated the potential functional connection between the membrane receptor ER family and ARG48.

My studies suggested that the ARG48 glycoproteins may provide new regulatory mechanism for signaling events at the plasma membrane, in particular at the receptor level.



Dr. Amber Krauchunas
Singson Lab

Research Summary

In order to successfully fertilize an egg, most sperm must go through the processes of post-meiotic differentiation and activation whereby they gain polarity, motility, and fertilization competence. In *C. elegans*, differentiation and activation take place simultaneously during spermiogenesis, transforming round immobile spermatids into motile, fertilization-competent spermatozoa. Previous work has shown that *C. elegans* spermiogenesis can be initiated through either of two pathways: the spe-8 pathway or the try-5 pathway. In males, these two pathways are redundant and appear to act in parallel, but in hermaphrodites only the spe-8 pathway is utilized to activate self-sperm. While several genes in each pathway have already been discovered, our limited understanding of how the components work together to effect the cellular changes of spermiogenesis suggests that required genes still remain to be identified.

I have carried out a forward genetic screen for sterile mutants in *C. elegans*. At least four of these mutants have defects in sperm, since the sterile hermaphrodites produce progeny if mated to males with wild-type sperm. Complementation testing showed that one of these has a mutation in the spe-24 gene. It has been known that the spe-24 gene is required for spermiogenesis and mutations in spe-24 lead to sterility in both hermaphrodites and males. However, the molecular nature of spe-24 has remained unknown. I used a whole genome sequencing strategy to identify the genetic lesion of the ancestral spe-24(hc130) allele and found a loss of the translation start site in the gene zipt-7.1. The spe-24(as42) allele that I discovered in my screen has a unique point mutation in this same gene confirming that spe-24 is zipt-7.1. Sequence analysis indicates that ZIPT-7.1 is a zinc transporter and our collaborators have shown that ZIPT-7.1 can actively import zinc into mammalian cells and that zinc levels are low in zipt-7.1 spermatids compared to wild-type spermatids. These data show that zinc may be an important signaling or regulatory molecule during the process of sperm activation, that ZIPT-7.1 is necessary to load zinc into the developing spermatocyte, and that ZIPT-7.1 may also play a critical role in the release of zinc at the time of sperm activation. Future work will focus on characterizing the phenotypes and mutated genes in the three other sperm-defective mutants identified from the forward genetic screen.



Dr. Zongliang Chen
Gallavotti Lab

Research Summary

The initiation and maintenance of meristems, small groups of stem cells that form all lateral primordia, are essential for maize inflorescence development and can directly impact grain production. Indeed, the size of apical meristems is related to ear productivity since slightly larger meristems produce a higher number of kernel rows, without giving rise to developmental abnormalities. Meristem maintenance is established by a negative feedback loop between the CLAVATA (CLV) and WUSCHEL (WUS) genes. This pathway was first identified in *Arabidopsis*, and it appears conserved in higher plants. In meristems, the homeobox WUSCHEL protein up-regulates the expression of CLAVATA3. CLAVATA3 (CLV3) is a secreted signaling peptide that can be perceived by the plasma membrane-localized leucine-rich receptor (LRR) kinases CLAVATA1 (CLV1) and CLV2, which trigger a signaling cascade that in turn represses the expression of WUS. In maize, ZmWUS1, TD1 and FEA2 are co-orthologs of WUS, CLV1 and CLV2, respectively. Previous studies found that maize mutants with loss of function of TD1, FEA2 and other regulators of meristem size have bigger shoot meristems

and an increased number of lateral primordia.

I am characterizing a semi-dominant maize mutant, Barren inflorescence3 (Bif3), that shows severe developmental abnormalities in ears and tassels. Both inflorescences have defects in the initiation of new primordia and show enlarged apical meristems. This is an unusual finding since normally enlarged apical meristems give rise to more lateral primordia. Using positional cloning and transgenic complementation, I have found that the Bif3 phenotype may be caused by the overexpression of a meristem maintenance gene. By a combination of expression and genetic analysis I am investigating how the upregulation of this candidate gene is causing increased shoot meristem size while at the same time decreasing the number of lateral primordia formed. A thorough understanding of the molecular mechanisms regulating meristem size and function can lead to new strategies to improve yields in crop species.

CHARLES AND JOHANNA BUSCH FELLOWS

Predocctoral Research



Ishita Jain
Severinov Lab

Research Summary

Prokaryotes rely on CRISPR-Cas systems to provide adaptive immunity against foreign genetic elements such as phages and plasmids. CRISPR (Clustered regularly interspaced short palindromic repeats) loci consisting of direct repeat sequences that separate unique spacer sequences and cas (CRISPR-associated) genes comprise the essential components of this dynamic defense mechanism. CRISPR-Cas systems are evolutionary diverse and have been classified into two classes, several types and multiple subtypes.

The aim of this project was to explore the target recognition requirements of the *Bacillus thermoamylovorans* (Bth) C2c1 system (now renamed as Cas12b, one of the three new CRISPR-Cas systems discovered in our lab in collaboration with MIT and NIH scientists in 2015) by conducting interference studies for determining the extent of seed sequence required for target recognition. We used an *E. coli* strain devoid of its own CRISPR-Cas system and heterologously expressing the Bth C2c1 CRISPR-Cas

system, to transform a library of protospacer-plasmids carrying point mutations in every position of the 20 bp protospacer recognized by C2c1 effector charged with appropriate CRISPR RNA (crRNA). We performed deep sequencing utilizing the Waksman genomics facility, and saw decreased interference (revealed as increased frequency of corresponding reads in transformed cells) for mismatches in positions 1-5 and a G to T mismatch in the 15th position of the protospacer. The library data was confirmed by an *in vivo* transformation efficiency assay and *in vitro* target cleavage, permanganate probing and fluorescent beacon assays. In the end, the work allowed us to delineate a 5-nucleotide seed sequence at the 5' end of the guide RNA (downstream of protospacer PAM) and reveal the additional downstream (position 15) match requirement, which coincides with the position of target cleavage.

This information will be critical in designing and utilizing bioengineering tools based on the Bth C2c1 system and will allow selections of targets and guide RNAs with minimized off-target activities.



Qiguo Yu
Maliga Lab

Efficient Plastid Transformation in ACC2-Defective Arabidopsis

Plastid transformation is routine in tobacco, but 100-fold less frequent in *Arabidopsis*, preventing its use in plastid biology. A recent study revealed that null mutations in ACC2, encoding a plastid-targeted acetyl-CoA-carboxylase, cause hypersensitivity to spectinomycin. We hypothesized that plastid transformation efficiency should increase in the *acc2* background, because when ACC2 is absent, fatty acid biosynthesis becomes dependent on translation of the plastid-encoded ACC β -Carboxylase subunit. We bombarded ACC2-defective *Arabidopsis* leaves with a vector carrying a selectable spectinomycin resistance (*aadA*) gene and *gfp*, encoding the green fluorescence protein GFP. Spectinomycin resistant clones were identified as green cell clusters on a spectinomycin medium. Plastid transformation was confirmed by GFP accumulation from the second open reading frame of a polycistronic mRNA, that would not be translated in the cytoplasm. We obtained one to two plastid transformation events per bombarded sample in spectinomycin hypersensitive Slavice (Sav-0) and Columbia *acc2*

knockout backgrounds, an approximately 100-fold enhanced plastid transformation frequency. Sav-0 and Columbia are accessions in which plant regeneration is uncharacterized or difficult to obtain. A practical system for *Arabidopsis* plastid transformation will be obtained by creating an ACC2 null background in a regenerable *Arabidopsis* accession. The recognition that the duplicated ACCase in *Arabidopsis* is an impediment to plastid transformation provides a rational template to implement plastid transformation in related recalcitrant crops.



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 programmed cell death. Given their critical and diverse roles in cellular physiology, mitochondrial dysfunction contributes to the etiology of multiple diseases including many neurodegenerative diseases. This association occurs because neurons in particular have high-energy demands but do not store glycolytic reserves and instead rely on mitochondrial oxidative phosphorylation to meet these demands. Additionally, mitochondria can contribute to degeneration by producing reactive oxygen species that can lead to both cell damage and apoptosis.

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, 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. We are in the process of further validating and confirming the causative gene from these lists, as well as understanding the role it plays in mitochondrial transport in neurons as well as any interactions it might play with other known genes.



Jing Lin
Padgett Lab

Research Summary

Transforming growth factor- β (TGF- β) is a potent growth factor that plays important roles in various embryonic and developmental processes from invertebrates to mammals (Massague, 1998). TGF- β signaling depends on its kinase receptors, type I and type II receptors. Both receptors are heterodimeric transmembrane serine/threonine kinases, which contain an extracellular domain, a short transmembrane domain and an intracellular kinase domain (Shi and Massague, 2003). A subset of different cancers and diseases, including Marfan-like syndromes (MFS-like), are caused by mutations in the C-terminal part of the kinase domain of either receptor. For years, it was thought these mutations inactivate the kinase activity providing an explanation of the cause of TGF- β diseases. However, recent studies have shown that these mutant TGF- β receptors still retain some kinase activity. We noticed that some receptor trafficking mutations map to the same locations where TGF- β mutations are found in many cancers and patients with Marfan-like syndromes. I am testing the hypothesis that receptor trafficking is aberrant in these mutations, which could result in the observed mutant phenotypes reported. I

am introducing these mutations into the *C. elegans* receptor gene DAF-4, and using cellular tools to determine if there are any trafficking changes in these receptors. Currently I can show that BMP receptors mis-localize when Marfan-like syndrome mutations or cancer mutations are introduced into DAF-4, strongly supporting my hypothesis.

Reference:

Massague, J., 1998. TGF- β signal transduction. *Annu Rev Biochem* 67, 753-791.

Mátyás G., Arnold E, Carrel T., Baumgartner D., Boileau C, Berger W., Steinmann B., 2006. Identification and in silico analyses of novel TGFBR1 and TGFBR2 mutations in Marfan syndrome-related disorders. *Hum Mutat* 27,760-769.

Shi, Y., Massague, J., 2003. Mechanisms of TGF- β signaling from cell membrane to the nucleus. *Cell* 113, 685-700.



Fei Wang
Steward Lab

Research Summary

The TET (Ten-eleven translocation) 1, 2 and 3 proteins have been shown to function as DNA hydroxymethylases in vertebrates and their requirements have been documented extensively. Recently, the Tet proteins have been shown to also hydroxylate 5-methylcytosine in RNA. 5-hydroxymethylcytosine (5hmC) is enriched in messenger RNA but the function of this modification has yet to be elucidated. Due to the lack of Cytosine methylation in DNA, *Drosophila* serves as an ideal model to study the biological function of 5hmC. Here, we characterized the temporal and spatial expression and requirement of Tet throughout *Drosophila* development. We show that Tet is essential for viability as Tet complete loss-of-function animals die at the late pupal stage. Tet is highly expressed in neuronal tissues and at more moderate levels in somatic muscle precursors in embryos and larvae. Depletion of Tet in muscle precursors at early embryonic stages leads to defects in larval locomotion and late pupal lethality. Although Tet knock-down in neuronal tissue does not cause lethality, it is essential for neuronal function during development through its affects upon locomotion in larvae

and the circadian rhythm of adult flies. Further, we report the function of Tet in ovarian morphogenesis. Together, our findings provide basic insights into the biological function of 5hmC, a modification that is likely also regulated by Tet proteins in other species, and may illuminate observed neuronal and muscle phenotypes observed in vertebrates.



Mercedes Gyuricza
McKim Lab

Research Summary

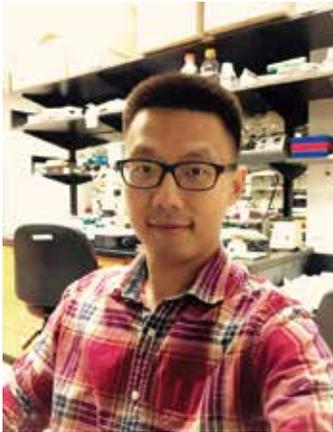
Meiosis is the process by which sexually reproducing diploid organisms make haploid gametes. During this process sister chromatids are held together by the cohesion complex and, homologous pairs, by the synaptonemal complex (SC). Both of these complexes are important for accurate segregation, as they ensure that the chromosomes can be attached to the proper spindle pole in metaphase. If the chromosomes are not attached to opposite spindle poles they will not segregate properly. Meiosis-specific cohesin complexes have been demonstrated in many organisms. These complexes are comprised of some meiosis specific subunits mixed with subunits that are also found in mitosis, making these complexes as a whole, unique ones. Although meiosis specific complexes have been identified in many organisms, the function of them is still not well understood. In *Drosophila*, there are two meiosis specific complexes, one that contains SMC1/SMC3/SUNN/SOLO and the other complex contains SMC1/SMC3/C(2)M/SA. It has been shown that the SUNN/SOLO complex is involved in keeping sister centromeres together, but the C(2)M/SA complex is not. The C(2)M/SA complex is responsible for a majority of the synaptonemal complex (SC) assembly. We have explored the structure of the C(2)M complex and how this complex promotes SC assembly.

We previously explored the function of the *Drosophila* meiosis-specific cohesin complexes (GYURICZA et al. 2016). The C(2)M/SA complex is not involved in keeping sister chromatid centromeres together, however, the complex is involved in assembly of the majority of the SC. Because the complex is not involved in the canonical role of cohesin complexes (sister chromatid cohesion), we are interested in testing if the complex has the same structure as the mitotic complex. Based on sequence alignments, C(2)M residue F89 should mediate the interaction with SMC3, while F524 and L528 should mediate the interaction with SMC1. In *S. cerevisiae*, the homologous amino acids are Y82, F528 and L532 respectively (HAERING et al. 2004; GLIGORIS et al. 2014). In order to test if the interactions between C(2)M and the SMC proteins are important for the function of the complex, we mutated each of these three residues to alanine and found that each of these point mutation were able to localize normally and rescue a c(2)M mutant. This indicates that C(2)M and the SMC proteins are not interacting in meiosis or the amino acids selected are not the ones mediating the interaction.

These experiments have provided new insight into the function of the meiosis-specific complex C(2)M/SA and the reason meiosis-specific complexes are present and conserved among so many organisms. We will test if a closed ring is required or if a “broken” ring structure is sufficient to do the job of the meiosis-specific cohesin.

BENEDICT MICHAEL FELLOW

Predoctoral Research



Yuanwang Pan
Irvine Lab

Research Summary

A fundamental question in developmental biology is how organs reach a correct size. But the mechanisms are not fully understood. *Drosophila* wing disc serves as a great model to address this question. During wing disc growth, the cell proliferation rates gradually slow down until finally stop. But what contributes to this growth cessation is not known. Recent study identified the Hippo pathway plays a key role in organ size control. However, how Hippo signaling is regulated during normal development is not clear. We propose that during wing disc development, mechanical stress gradually accumulates, which leads to the decrease in cytoskeletal tension and thus Yki activity through the biomechanical Hippo pathway. Therefore, the growth gradually stops.

We found that the activity of transcriptional activator Yki gradually decreases during wing disc development, which is consistent with the gradual decline in growth rates. In addition, we discovered that the biomechanical Hippo pathway could contribute to this change in Yki activity. Specifically, we showed that cells decrease cytoskeletal tension during disc develop, and we further found that cells become increasingly crowded in the center of wing disc. Interestingly, the spatial pattern of tension, Jub and Wts correlates with the pattern of cell size, Yki activity and proliferation. And the relative junctional Jub and Wts decline during disc growth. To test whether the decrease in cytoskeletal tension leads to the decline of Yki activity, we temporally altered tension in younger and older stage wing disc. We found cells respond differently to tension in young versus old wing discs. Finally, we discovered that increasing or decreasing cell density in half of wing disc is sufficient to alter the Hippo pathway activity. In summary, our study provides insight for how mechanical forces affect organ size determination through the Hippo pathway.

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

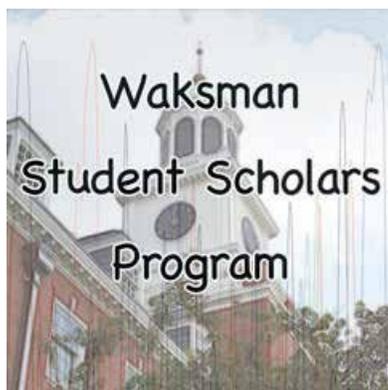
Graduate students from a wide range of programs conduct their Ph.D. or M.S. dissertation research projects with faculty at the Waksman Institute and are eligible for the Busch Graduate Fellowship Program to support their thesis research. Predoctoral candidates can be funded a maximum of four years, while 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 2016-2017

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

WAKSMAN STUDENT SCHOLARS PROGRAM

High School Outreach



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

For 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 2016 WSSP consisted of two interrelated parts: a Summer Institute (SI) and an Academic Year Program (AYP). In July 2016, 36 students and 12 teachers from 32 high schools attended a three-week 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. This last year the WSSP was awarded a grant from the Bayer Foundation to purchase some of the reagents, supplies, and DNA sequencing services to support the WSSP AYP activities.

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 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 6 teachers and 14 students was conducted at the Lawrence 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 1288 high school students participated in, and contributed to, the WSSP this past year.

The Research Question

The 2016 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 2016-2017 SI and AYP, over 2600 plasmid clones were purified and 1947 were sequenced. To date, 1063 DNA sequences have been analyzed by the students. 630 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 an independent two-week summer program called **Waksman Institute Summer Experience** (WISE) in which students perform the same research project as conducted in the WSSP. In August 2016, 35 students 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 2017 summer.

Upward Bound Program

In an independent outreach activity, the WSSP partnered with the Upward Bound Math Science Program at Rutgers University to hold a four-day DNA Workshop for 20 high school students. Students in the Workshop learned about DNA structure, molecular biology, and gene expression. They conducted experiments to purify and analyze a novel DNA fragment. At the conclusion of the Workshop they were able to present their results.

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

September 8, 2016

Rutgers University Inn & Conference Center

Presentations

- Konstantin Severinov “Following Waksman: Searching for New Antibiotics in the 21st Century”
- Colin Gates and G.C. Dismukes “Strontium Substitution in the Water-Oxidizing Complex of Photosystem II”
- Bob O’Hagan and Maureen Barr “Glutamylation of Microtubules Regulates Ciliary Trafficking, Structural Specialization of the Axoneme, and Extracellular Vesicle Release in *C. elegans* Sensory Neurons”
- Qiujie Liu and Andrea Gallavotti, “Characterization and Cloning of a Temperature Sensitive Maize Mutant Affecting Inflorescence Development”
- Lin-Ing Wang and Kim McKim “Chromatin-mediated Spindle Assembly in *Drosophila* Female Meiosis”
- Brian Gelfand and the Genomics Lab “Production of High Fidelity Custom Amplicon Libraries and Targeted Sequencing”
- Nathaly Salazar and C. Rongo, “A Genetic Screen to Identify Regulators of Mitochondrial Dynamics and Transport in *C. elegans* Neurons”
- Jose Planta, Thomas Leustek, and Joachim Messing “Leaf-specific Assimilation of Sulfur Limits Methionine Accumulation in the Maize Grain “
- Dongmeng Li and Juan Dong “Phosphoinositide Signaling in Plant Asymmetric Cell Division”
- Jyoti Misra and Ken Irvine “Vamana Couples Fat Signaling to the Hippo Pathway”
- Jared Winkelman and Bryce Nickels “Multiplexed Protein-DNA Crosslinking Reveals the Mechanism of Transcription Start Site Selection”
- Mehul Vora and Richard Padgett “Trafficking of TGF β Receptors”
- Richard H. Ebright “Repurposing Nanopore DNA Sequencing to Analyze Translocation of RNA Polymerase on DNA at Sub-nanometer, Sub-millisecond Spatiotemporal Resolution”

POSTER SESSION

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

Barr Lab

- α -tubulin isotype orchestrates ciliary microtubule architecture, IFT, and extracellular vesicle biology, Malan Silva, Natalia Morsci, Ken Nguyen, Christopher Rongo, Dave Hall and Maureen Barr
- *Caenorhabditis elegans* extracellular matrix proteins regulate polycystin localization/activity and cilia integrity, Deanna De Vore and Maureen Barr

Dismukes Lab

- Robust Microalgal Production Strains for High Yield Growth on Fossil Flue Gas: Toward Cost Effective Biofuels and CO₂ Mitigation, Yuan Zhang, Gennady Ananyev, Yunbing Ma, Hoa Vu, Jun Cheng and G. Charles Dismukes

Dong Lab

- The microtubule-associated protein YIP1 confers a negative regulation on the BASL-YDA polarity module in Arabidopsis stomatal development, Wanchen Shao and Juan Dong
- A MAPK substrate MASS family regulates stomatal patterning in Arabidopsis, Xueyi Xue and Juan Dong

Dooner Lab

- Massive rolling-circle amplification of centromeric Helitron in plant genome, Wenwei Xiong, Hugo Dooner and Charles Du
- A sequenced-indexed reverse genetics resource for maize, Mithu Chatterjee and Hugo Dooner

Ebright Lab

- Closing and opening of the RNA polymerase “trigger loop”: analysis using unnatural-amino-acid mutagenesis labelling and ensemble and single molecule fluorescence resonance energy transfer, Abhishek Mazumder, Miaoxin Lin, Anssi Malinen, Achillefs Kapanidis and Richard H. Ebright
- Closing and opening of the RNA polymerase trigger loop in solution, Miaoxin Lin, Abhishek Mazumder and Richard H. Ebright
- Sequence-specific interactions between RNA polymerase and the core recognition element, Hanif Vahedi an-Movahed, Yu Zhang, Irina Vvedenskaya, Jeremy Bird, Yuanchao Zhang, Seth Goldman, Craig Kaplan, Deanne Taylor, Bryce Nickels and Richard H. Ebright
- Novel small-molecule inhibitor of Mycobacterium tuberculosis RNA polymerase AAP1 Soma Mandal, Yu Feng, Yon W. Ebright, Shengjian Li, Richard Wilde, Sukhendu Mandal, Yi Jiang, Sujoy Chatterjee, Aashish Srivastava, David Degen, Meliza Talaue, Nancy Connell, Wei Lin, Yu Liu, Matthew Zimmerman, Veronique Dartois, Arkady Mustaev, Edinson Lucumi, Scott Diamond, Christopher Cooper, Khisimuzi Mdluli, Nader Fotouhi, Zhenkun Ma, Takushi Kaneko and Richard H. Ebright

Gallavotti Lab

- The Transcriptional Corepressor REL2 is Required for Maize Vegetative and Reproductive Development, Xue Liu and Andrea Gallavotti

Genomics Core Facility

- Integrated RNAseq data analysis, Min Tu and Dibyendu Kumar

Irvine Lab

- Mechanical Feedback, Yuanwang Pan and Ken Irvine.
- Cytoskeletal tension increases recruitment of Hippo pathway proteins: Ajuba LIM Proteins and LATS kinases, Consuelo Ibar, Benjamin P. Keepers, Elmira Kirichenko, Edward W. Enners and Kenneth D. Irvine

McKim Lab

- Cooperation between kinesin motors promotes spindle symmetry and chromosome organization in oocytes, Sarah Radford, Allysa Go and Kim McKim
- Dynamic and stable cohesins regulate synaptonemal complex assembly and chromosome segregation,” Mercedes Gyuricza and Kim McKim
- CDK1 Regulation of the Central Spindle, Anna Maria Hinman, Janet Jang and Kim McKim
- The meiosis-specific kleisin C(2)M may be the key to meiosis, Nikunj Patel, Mercedes Gyuricza and Kim McKim

Messing Lab

- Analysis of alpha zein gene loci in the W22 inbred using single-molecule real-time sequencing technology, Jiaqiang Dong, Yaping Feng, Dibyendu Kumar, Wei Zhang, Tingting Zhu, Mingcheng Luo, and Joachim Messing
- A maize kernel mutant with impaired phosphatidylinositol 3-kinase-related protein kinase (PIKK) function, Nelson Garcia, Yubin Li, Hugo Dooner, and Joachim Messing
- Maize endosperm-specific transcription factors O2 and PBF network the regulation of protein and starch synthesis, Zhiyong Zhang and Joachim Messing
- Transcriptome study of soluble sugar accumulation in sorghum stem, Yin Li and Joachim Messing

Nickels Lab

- The mechanism of RNA 5' capping with NAD⁺, NADH, and desphospho-CoA, Jeremy Bird, Richard Ebright and Bryce Nickels

Padgett Lab

- Co-CRISPR Techniques in Drosophila Nanci Kane and Richard Padgett

Rongo Lab

- Role Of Biogenic amines in Protein Homeostasis, Kishore Joshi and Chris Rongo
- Mitochondrial transport in C. elegans neurons requires function fusion/fission dynamics, Natalia Morsci and Chris Rongo
- The Hexosamine Pathway Metabolite N-Acetyl Glucosamine Alleviates Tauopathy in C. elegans, Ifrah Tariq and Chris Rongo
- TGF-Beta signaling modulates protein homeostasis in target tissues, Janelle Thomas and Chris Rongo

Severinov Lab

- Novel C2c1 CRISPR-Cas System: Requirements For Target Recognition And Spacer Acquisition, Ishita Jain and Konstantin Severinov

Singson Lab

- Characterization and identification of new genes required for sperm activation in C. elegans lab name, Amber Krauchunas and Andrew Singson

PRESENTATIONS & MEETING ABSTRACTS

Waksman Institute Hosted Seminars

- Dr. David Ish-Horowicz, MRC Lb for Molecular Cell Biology, University College, London. “Post-transcriptional regulation during *Drosophila* neurogenesis.” September, 19 2016
- Dr. Juan Dong “All about polarity: regulators and mechanisms for stomatal asymmetric cell division in *Arabidopsis*.” October, 5 2016
- Dr. Xiaobo Li, Princeton University. “Understanding photosynthetic energy capture, conversion and storage with advanced functional genomics tools.” February 24, 2017
- Singson and Dr. Sam Gu, Ronald Ellis, Rowen University. “The evolution of self-fertility in nematodes.” March 28, 2017
- Li-Jia Qu, Peking University. “Identification of major factors controlling male-female interactions in *Arabidopsis*.” April 11, 2017
- Dr. Paul Ciclitira, University College of London. “Celiac Disease.” May 11, 2017
- Dr. Gavin J. Wright, Cell Surface Signaling Laboratory, Wellcome Trust Sanger Institute, Cambridge, UK. “Identification of cell surface receptor-ligand pairs that are essential for cellular recognition processes: From malaria to mammalian fertilization.” June 5, 2017
- Eric F. Joyce, Ph.D., Department of Genetics, University of Pennsylvania, Philadelphia, PA. “Unraveling the molecular basis of chromosome interactions.” June 19, 2017

Waksman Student Scholars Programs

- Waksman Student Scholars Summer Institute, Waksman Institute, Rutgers University, July 5-July 22, 2016
- Upward Bound Math-Science DNA Workshop, Waksman Institute, Rutgers University, June 28-July 1, 2016.
- Waksman Institute Summer Experience (WISE), Waksman Institute, Rutgers University, August 1- August 12, 2016.

Barr Lab

- Cilia2016: “Cilia, from Fundamental Biology to Human Disease,” Amsterdam, NL, October 2016
- Science Friday Seminar Series, Rider University, NJ, October 2016
- University of Indiana, Biology Department Seminar Series, November 2016
- American Society of Nephrology Annual Meeting, Session on “Establishing the cilium and its unique signaling functions,” Chicago, IL, November 2016
- American Society for Cell Biology Annual Meeting, Subgroup on “Cilia, Signaling, and Human Disease,” San Francisco, CA, December 2016
- American Society for Cell Biology Annual Meeting, Subgroup on “Patterning the Cytoskeleton,” San Francisco,

CA, December 2016

- Department of Pharmacology and Center for Smell and Taste at the University of Florida, Symposium on “Biology and Disease of Ciliated Senses”, January 2017
- Memorial Sloan-Kettering Cancer Center, NYC, NY, Cell Biology Seminar Series, March 2017
- International Symposium on Neural Precursor Cell Fate Determination, Differentiation and Neuronal Circuit Formation. Hangzhou, China, April 2017
- Keynote Speaker at Cold Spring Harbor Asia Conference on Cilia & Centrosomes. Suzhou, China, April 2017
- Yale University, New Haven CT. Molecular, Cellular and Developmental Biology Department. May 2017

Dismukes Lab

- Gordon Research Conference: Photosynthesis, Grand Hotel, Newry, ME, 2017
- DOE Hydrogen and Fuel Cell Technologies Research, Development and Demonstrations; AMR 2017
- Eastern Regional Photosynthesis Conference, poster presentation, Marine Biological Lab, Woods Hole, MA, 2017
- TIFR Colloquium, Tata Institute for Fundamental Research, Mumbai, India, Jan 4, convenor: Jyotishman Dasgupta. 2017
- 2017 keynote speaker, SABIC-Society of Asian Bioinorganic Chemistry, Calcutta, India, convenors: A Dey and S. Mazumdar, 2017
- Rutgers NJPIRG Student Chapter, the future of renewable energy, 2017
- New York Metro Catalysis Society Symposium at Exxon-Mobil, 2 posters, Clinton, NJ. March 22. 2017
- Microbiology at Rutgers, Symposium, Rutgers SEBS, 2017
- Rutgers Laboratory of Surface Modification Symposium, Rutgers, March 7. 2017 Rutgers Energy Institute Annual Symposium, 2017
- Keynote address: The Bio-Economy: Technology and Policy Paths Forward, Sept 30, convenor: Gal Hochman, Rutgers University, 2016
- Speaker Rutgers Newark, Dept of Chemistry 2016
- Keynote speaker: American Chemical Society National Meeting, Philadelphia, PA, Inorganic Symposium, convenor: Michael Zdilla, 2016
- Keynote and session chair, International Hydrogen Production Conf., Hanzhou, China. 2016
- 229th ECS Meeting. 2 oral papers and 1 poster in the divisions of Physical and Analytical Electrochemistry, Electrocatalysis, and Photoelectrochemistry, 2016
- New York Metro Catalysis Society Symposium at Rutgers, 4 posters, New Brunswick. 2016
- Microbiology at Rutgers, Symposium, Rutgers SEBS, 2016

- Rutgers Laboratory of Surface Modification Symposium, Rutgers, 2016
- Eastern Regional Photosynthesis Meeting, Marine Biological Lab, Woods Hole, MA, 2016
- National Renewable Energy Laboratory, Golden, CO, hosted by Andriy Zakutayev, 2016
- Rutgers Energy Institute Symposium, 2016
- International Hydrogen Production Conf., Hanzhou, China; paper & chaired session. 2016
- Launch of the Rutgers-Zhejiang joint project for Global Climate and Energy Project and tour of the Yantai bioreactor facility. Zhejiang University, Hanzhou, China, 2016
- 229th Electrochemical Society Meeting. 2 oral papers and 1 poster in three divisions, 2016

Dong Lab

- “Attenuation of the polarity feedback loop in stomatal asymmetric cell division” Cold Spring Harbor Asia Conference on Plant Cell and Developmental Biology, Suzhou, China, 2017
- “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, 2017
- “Positive and negative signaling at the plasma membrane for stomatal ACD.” Seminar at the Department of Plant Biology, Carnegie Institution for Science, Stanford University, 2017
- “Novel functions of a microtubule-associated protein YIP1 in the regulation of protein polarization.” The Auxin Meeting 2016, Sanya China, 2016
- “Cell polarity and MAPK signaling in plant asymmetric cell division.” Plant Biology 2016 Meeting, American Society of Plant Physiology, Austin, TX, 2016
- “BASL differentiates asymmetric cell fate through MAPKs and SPCH in stomatal development.” International Conference on Arabidopsis Research, Gyeongju, Korea, 2016
- “Cell polarity and MAPK signaling in plant asymmetric cell division.” Seminar at the Department of Molecular Biology, Princeton University. Princeton, NJ, 2016
- “Cell polarity and MAPK signaling in plant asymmetric cell division.” Seminar at Delaware Biotechnology Institute, University of Delaware. Newark, DE, 2016
- “Cell polarity and MAPK signaling in plant asymmetric cell division” Seminar at the Department of Plant Biology, University of California, Davis. Davis, CA, 2016
- “Cell polarity and asymmetric cell division in Arabidopsis.” Seminar at Center for Plant Cell Biology, University of California, Riverside. Riverside, CA, 2016

Dooner Lab

- Chatterjee, M., Li, Y., Wang, Q., Xiong, W., Wang, H., Huang, J., He, L., Segal, G., Du, C., Dooner, H.K. “A sequence-indexed reverse genetics resource for maize”. 2017 Maize Genetics Conference, St. Louis, MO 3/9-12/2017.
- Garcia, N., Li, Y., Dooner, H.K., Messing, J. “The maize

dek34-Dsg encodes Tti2, a member of the TTT complex essential for PIKK stability 2017 Maize Genetics Conference, St. Louis, MO 3/9-12/2017.

Ebright Lab

- “RNA polymerase: the molecular machine of transcription.” Harden Meeting, Machines on Genes, Manchester, United Kingdom, 2016.
- “Antibacterial drug discovery targeting bacterial RNA polymerase: myxopyronin (Myx),” Pharmaceutical Sciences Symposium, Rutgers University, Piscataway, New Jersey, 2016.
- “Structural studies of transcription initiation and activation.” Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, China, 2016.
- “Anti-tuberculosis drug discovery targeting transcription.” Institut Pasteur of Shanghai, Chinese Academy of Sciences, Shanghai, China, 2016.
- “Repurposing nanopore DNA sequencing to analyze translocation of RNA polymerase on DNA at sub-nanometer, sub-millisecond spatiotemporal resolution.” Mechanistic basis of transcription start-site selection,” Meeting on Post-Initiation Activities of RNA Polymerases, Mt. Lake, Virginia, 2016.
- “Repurposing nanopore DNA sequencing to analyze translocation of RNA polymerase on DNA at sub-nanometer, sub-millisecond spatiotemporal resolution.” Zing Nucleic Acids Conference, Tampa, Florida, 2016.
- “Using nanopore tweezers to analyze translocation of RNA polymerase on DNA with sequence registration and sub-nanometer, sub-millisecond spatiotemporal resolution.” Single-Molecule Biophysics 2017, Aspen, Colorado, 2017.
- “Structural basis of Mycobacterium tuberculosis transcription and transcription inhibition.” New York Structural Biology Winter Meeting, New York, New York, 2017.
- “Structural studies of transcription initiation and activation, and anti-tuberculosis drug discovery targeting transcription.” Department of Biochemistry, Duke University, Durham, North Carolina, 2017.
- “RNA polymerase: the molecular machine of transcription.” 2017, Microbial Genetics and Genomics VII, Asilomar, California, 2017.
- “Antibacterial drug discovery targeting bacterial RNA polymerase: myxopyronin (Myx),” Antimicrobial Resistance Webinar, Mid-Atlantic Society of Toxicology, Spring House, PA, 2017.
- “Antibacterial drug discovery targeting bacterial RNA polymerase: myxopyronin (Myx).” Institute for Quantitative Biomedicine Symposium, Antimicrobial Resistance, Rutgers University, Piscataway, New Jersey, 2015.
- “Using nanopore tweezers to analyze RNAP translocation in transcription elongation, pausing, and termination with sub-nanometer, sub-millisecond spatiotemporal resolution.” FASEB Meeting on Transcription, Saxtons River, Vermont, 2017.

- Zhang, Y., Feng, Y., Mazumder, A., Lin, M., and Ebright, R.H. (2015) Structural biology and structural biophysics of transcription. Harden Meeting on Machines on Genes. Manchester, United Kingdom, July 31-August 5, 2016.
- Vahedian-Mohaved, H., Zhang, Y., Vvedenskaya, I., Bird, J., Knobloch, J., Goldman, S., Zhang, Y.C., Taylor, D., Nickels, B., and Ebright, R.H. (2016) Sequence-specific interactions between RNA polymerase and the core recognition element (CRE). Meeting on Molecular Genetics of Bacteria and Phages. Madison, Wisconsin, August 8-12, 2016.
- Bird, J., Zhang, Y., Tian, Y., Greene, L., Liu, M., Buckley, B., Krasny, L., Lee, J., Kaplan, C., Ebright, R., and Nickels, B. (2016) The mechanism of RNA 5' capping with NAD⁺, NADH, and desphosphoCoA. Meeting on Molecular Genetics of Bacteria and Phages. Madison, Wisconsin, August 8-12, 2016.
- Mazumder, A., Lin, M., Malinen, A., Kapanidis, A., and Ebright, R.H. (2016) Closing and opening of the RNA polymerase "trigger loop": analysis using unnatural-amino-acid mutagenesis labelling and ensemble and single molecule fluorescence resonance energy transfer. Gordon Research Conference on Genetic Code Expansion, Corvallis, Oregon, August 11-14, 2016.
- Nova, I., Mazumder, A., Laszlo, A., Derrington, I., Gundlach, J., and Ebright, R.H. (2016) Repurposing nanopore DNA sequencing to analyze translocation of RNA polymerase on DNA with sub-nucleotide, sub millisecond spatiotemporal resolution. Meeting on Post-Initiation Activities of RNA Polymerases, Mt. Lake, Virginia, October 27-30, 2016.
- Nova, I., Mazumder, A., Laszlo, A., Derrington, I., Gundlach, J., and Ebright, R.H. (2016) "Repurposing nanopore DNA sequencing to analyze translocation of RNA polymerase on DNA at sub-nanometer, sub millisecond spatiotemporal resolution." Zing Nucleic Acids Conference, Tampa, Florida, 2016., December 2-5, 2016.
- Nova, I., Mazumder, A., Laszlo, A., Derrington, I., Gundlach, J., and Ebright, R.H. (2017) Using nanopore tweezers to analyze translocation of RNA polymerase on DNA with sequence registration and sub-nucleotide, sub-millisecond spatiotemporal resolution. Single-Molecule Biophysics 2017, Aspen, Colorado, January 8-13, 2017.
- Gabizon, R., Lee, A., Vahedian-Mohaved, H., Ebright, R.H., and Bustamante, C. (2017) RNA polymerase translocation in processive transcription elongation and pausing: dynamics, force-dependence, and modulation by sequence-specific
- RNAP-DNA interactions. Single-Molecule Biophysics 2017, Aspen, Colorado, January 8-13, 2017.
- Gundlach, J., Derrington, I., Laszlo, A., Craig, J., Nova, I., Brinkerhoff, H., Noakes, M., Ebright, R.H., and Mazumder, A. (2017) Single-molecule Picometer Resolution Nanopore Tweezers (SPRNT). Biophysical Society Annual Meeting, New Orleans, Louisiana, February 11-15, 2017.
- Lee, A., Gabizon, R., Vahedian-Mohaved, H., Ebright, R.H., and Bustamante, C. (2017) RNA polymerase translocation in processive transcription elongation and pausing: dynamics, force-dependence, and modulation by sequence-specific
- RNAP-DNA interactions. Biophysical Society Annual Meeting, New Orleans, Louisiana, February 11-15, 2017.
- 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. Saxtons River, Vermont, June 25-30, 2017.
- Liu, Y., Winkelman, J., Yu, L., Zhang, Y., and Ebright, R.H. (2017) Structural and mechanistic basis of reiterative initial transcription. Saxtons River, Vermont, June 25-30, 2017.
- Winkelman, J., Pukhrambam, C., Yu, L., Vahedian-Mohaved, H., Nickels, B., and Ebright, R.H. (2017) RNA polymerase (RNAP) pausing in initial transcription: occurrence, and position, sequence, and RNAP-structure determinants. Saxtons River, Vermont, June 25-30, 2017.
- Lin, W., Mandal, S., Degen, D., Liu, Y., Ebright, Y., Li, S., Feng, Y., Zhang, Y., Mandal, S., Jiang, Y., Liu, S., Gigliotti, M., Talaue, M., Connell, N., Das, K., Arnold, E., and Ebright, R.H. (2017) Crystal structures of Mycobacterium tuberculosis transcription initiation complexes. Saxtons River, Vermont, June 25-30, 2017.
- Degen, D., Zhang, Y., Maffioli, S., Carzaniga, T., Del Gatto, G., Serina, S., Monciardini, P., Mazzetti, C., Gugliera, P., Candiani, G., Chiriac, A.I., Facchetti, G., Kaltofen, P., Sahl, H.-G., Dehò, G., Donadio, S., and Ebright, R.H. (2017) Target, mechanism, and structural basis of transcription inhibition by the antibiotic pseudouridimycin--a selective nucleoside-analog inhibitor of bacterial RNA polymerase. Saxtons River, Vermont, June 25-30, 2017.
- Yu, L., Winkelman, J., Pukhrambam, C., Strick, T., Nickels, B., and Ebright, R.H. (2017) The mechanism of transcription start site selection. FASEB Meeting on Mechanism and Regulation of Prokaryotic Transcription. Saxtons River, Vermont, June 25-30, 2017.
- Mazumder, A., Winkelman, J., Yu, L., Liu, Y., Zhang, Y., Nova, I., Laszlo, A., Strick, T., Kapanidis, A., Gundlach, J., Nickels, B., and Ebright, R.H. (2017) Structural biology and structural biophysics of transcription. FASEB Meeting on Mechanism and Regulation of Prokaryotic Transcription. Saxtons River, Vermont, June 25-30, 2017.

Gallavotti Lab

- Gallavotti, A. The DNA binding landscape of maize AUXIN RESPONSE FACTORS. Maize Improvement Center, Chinese Academy of Agricultural Sciences, Beijing, China, October 27, 2016.
- Gallavotti, A. Genetic and genomic approaches to

understanding maize inflorescence architecture. Maize Improvement Center, China Agricultural University, Beijing, China, October 26, 2016.

- Gallavotti, A., Galli, M., Sidharth, S., O'Malley, R., Ecker, J., Joshi, T. The DNA binding landscape of maize ARFs. Auxin 2016: 5th International Conference for Auxin Research, Haitang Bay, Sanya, Hainan, China, October 20-25, 2016.
- Gallavotti, A. Genetic and genomic approaches to understanding maize inflorescence architecture. Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China, October 19, 2016.
- Gallavotti, A., Liu, Q., Sidharth, S., Joshi, T., Lu, Z., Schmitz, R., Galli, M. The DNA binding landscape of maize auxin response factors. Maize Genetics Conference Abstract 59:P228. St. Louis, MO, March 9-12, 2017.
- Liu, X., Camehl, I., Galli, M., Gallavotti, A. The transcriptional co-repressor REL2 regulates meristem initiation, determinacy and maintenance in maize inflorescences. Maize Genetics Conference Abstract 59:P240. St. Louis, MO, March 9-12, 2017.
- Chen, Z., Li, W., Menello, C., Galli, M., Gallavotti, A. The function of Barren inflorescence3 in meristem initiation and maintenance in maize inflorescences. Maize Genetics Conference Abstract 59:P231. St. Louis, MO, March 9-12, 2017.
- Liu, Q., Federici, S., Galli, M., Gallavotti, A. Characterization and cloning of a temperature sensitive maize mutant affecting inflorescence development. Maize Genetics Conference Abstract 59:P181. St. Louis, MO, March 9-12, 2017.
- Struttman, J., Marshall, K., Coats, D., Withee, J., Malcomber, S., Gallavotti, A., McSteen, P. Reverse genetic approaches to understanding the role of auxin in maize development. Maize Genetics Conference Abstract 59:P225. St. Louis, MO, March 9-12, 2017.
- Sidharth, S., Galli, M., Gallavotti, A., Joshi, T. Mining auxin response factor binding sites in maize using informatics approaches. Maize Genetics Conference Abstract 59:P46. St. Louis, MO, March 9-12, 2017.
- Taylor-Teeples, M., Moss, B., Gallavotti, A., Nemhauser, J. Making a spiral: the role of Aux/IAA degradation rate in developmental patterning. Auxin 2016: 5th International Conference for Auxin Research, Haitang Bay, Sanya, Hainan, China, October 20-25, 2016.

• **Irvine Lab**

- Cell Contact and Adhesion Gordon Conference. Proctor, NH, June 18-23, 2017.
- Seminar at Northwestern University, Chicago IL, May 11, 2017
- Seminar at UMass, Worcester, Jan 30, 2017
- Seminar at Crick Institute, London, UK, Jan 16, 2017,
- Physical Biology of Tissue Morphogenesis: Mechanics, Metabolism and Signaling. Dresden, Germany, October 17-21, 2016

- KITP Workshop "From Genes to Growth and Form", UCSB, CA, August 1-19, 2016

Maliga Lab

- Maliga, P. "Cell-To-Cell Movement of Organellar DNA in Plants". Mid Atlantic Plant Molecular Biology Society Meeting, Patuxent Research Refuge, Laurel, MD 20708. August 15 -16, 2016.
- Maliga, P. "Cell-To-Cell Movement of Organellar DNA in Plants". Cornell University, Department of Plant Biology, Ithaca, NY, September 30, 2016
- Maliga, P. "The Promise of Synthetic Biology in Plants". New York Hungarian Scientific Society, New York, NY, October 4, 2016.
- Maliga, P. "Transgenic Approach to Probe Gene Regulation by PPR10 RNA Binding Protein in Chloroplasts ". 2016 International Symposium on Plant Transformation Biotechnologies, Academia Sinica, Taipei, Taiwan, October 17-19, 2016.
- Maliga, P. "Transgenic Approach to Probe Gene Regulation by PPR10 RNA Binding Protein in Chloroplasts". 9th Tripartite Meeting, The Tripartite Institute of the Americas: Chemistry, Biochemistry and Molecular Biology for Wellness, Maresias, Brazil, October 30 - November 2, 2016.
- Maliga, P. "Progress in Implementing Plastid Transformation in New Crops". Gordon Research Conference on Chloroplast Biotechnology, Ventura Beach, CA, January 8-13, 2017.
- Zhang, Y., Ananyev, G., G. Dismukes, G.C. and Maliga, P. "Photosystem II engineering enhances photosynthetic efficiency and boosts crop yield". Gordon Research Conference on Chloroplast Biotechnology, Ventura Beach, CA, January 8-13, 2017.
- Maliga, P. "Cell-To-Cell Movement of Organellar DNA in Plants". University of California, Riverside, Institute for Integrative Genome Biology, January 13, 2017.
- Maliga, P. "Cell-To-Cell Movement of Organellar DNA in Plants". Plant & Animal Genome XXV, January 14, 2017, San Diego, CA. Speaker.
- Qiguo Yu, Alexander Ioannou, Tarinee Tung-suchat-Huang, Alice Barkan and Pal Maliga, "Transgenic approach to probe gene regulation by PPR10 RNA binding protein in chloroplasts". Gordon Research Conference on Chloroplast Biotechnology, Ventura Beach, CA, January 8-13, 2017.
- Lisa LaManna, Megan Kelly, Qiguo Yu, Pal Maliga, "Developing an Efficient System for Chloroplast Transformation in Arabidopsis thaliana". Gordon Research Conference on Chloroplast Biotechnology, Ventura Beach, CA, January 8-13, 2017.

McKim Lab

- "Bringing them together and pulling them apart: chromosome segregation in Drosophila meiosis" November 9, 2016, University of Texas, Southwestern.

Messing Lab

- Dong, J., Ge, F, and Messing, J. (2017). Investigation of the redundancy of transcription factors expressed during seed development. 59th Annual Maize Genetics Conference, St. Louis, MO, March 2017.
- Planta, J. and Messing, J. (2017). Increased lysine and methionine accumulation in single seeds by different mechanisms. 59th Annual Maize Genetics Conference, St. Louis, MO, March 2017.
- Nelson, G., Li, Y., Dooner, H., and Messing, J. (2017). The maize dek34-DSG encodes Tti2, a member of the TTT complex essential for PIKK stability. 59th Annual Maize Genetics Conference, St. Louis, MO, March 2017.
- Zhang, Z., Zhang, W., and Messing, J. (2017). Functional studies of divergent prolamins. 59th Annual Maize Genetics Conference, St. Louis, MO, March 2017.
- American Society of Microbiology, Annual Meeting, Symposium on “Foundation to Frontiers” Boston, June 2016.
- International Center for Public Health, Newark, NJ, November 2016.
- ISU Research Symposium: Future Directions in Maize Kernel Biology, June 2017
- Rutgers Board of Trustees Meeting, June 2017

Padgett Lab

- “Dissecting the function of the SMA-10 protein” poster presentation at the International C. elegans Conference, June 21, 2017, by K. Liao, M. Vora, N. Kane, and R.W. Padgett.
- “Some Disease-Associated Mutations of TGF β Receptors Result in Aberrant trafficking”, poster presentation at CINJ Annual Retreat, by J. Lin, M. Vora, R. Gleason, N. Kane, and R.W. Padgett, May 25, 2017.

Rongo Lab

- Christopher Rongo “Uncovering Novel Mechanisms of Neuronal Stress Response Using C. elegans.” UConn Health Department of Neuroscience. May 8, 2016

Singson Lab

- “Fertility Gene Discovery from Worms to Humans” Singson, A. Summit Old Guard Meeting 2016

PATENTS & PUBLICATIONS

Patents

Dismukes Lab

- RU Patent PCT filed October 2016: Dismukes, G. C., Greenblatt, M., Laursen, A., Calvino, K. "Transition metal phosphides as a new type of catalyst for direct electrochemical CO₂ reduction to hydrocarbons"

Ebright Lab

- Ebright, R., Zhang, Y., Degen, D., and Ebright, Y. (2016) Bipartite inhibitors of bacterial RNA polymerase: Sal-target-inhibitor/nucleoside-analog-inhibitor conjugates. US Patent 9243039.
- Ebright, R., Ebright, Y., Shen, J., Bacci, J., Hiebel, A.-C., Solvibile, W., Self, C., and Olson, G. (2016) Antibacterial agents: aryl myxopyronin derivatives. US Patent 9315495.
- Ebright, R., Degen, D., Zhang, Y., Ebright, Y. (2016) Bipartite inhibitors of bacterial RNA polymerase. US Patent 9415112.
- Ebright, R. and Shen, J. (2016) Antibacterial agents: phloroglucinol derivatives. US Patent 9517994.
- Ebright, R., Ebright, Y., Shen, J., Bacci, J., Hiebel, A.-C., Solvibile, W., Self, C., and Olson, G. (2017) Antibacterial agents: aryl myxopyronin derivatives. US Patent 9592221.
- Ebright, R., Ebright, Y., Feng, Y., and Degen, D. (2017) Antibacterial agents: salinamide derivatives. US Patent 9,605,028.

Maliga Lab

- P. Maliga (2016) Selectable marker gene and methods of use thereof in transplastomic plants. US Patent 9,434,952

Severinov Lab

- Methods for identifying Class 2 CRISPR-Cas systems. (2016) Prov. Appln. No. 114203-0200. Shmakov, S., Makarova, K. S., Wolf, Y. I., Smargon, S., Pyzocha, N., Cox, D., Scott, D., Severinov, K., Zhang, F., Koonin, E. V.
- Novel CRISPR enzymes and systems. (2016) Prov. Appln. No. 62/296,522. Severinov, K., Zhang, F., Wolf, Y. I., Shmakov, S., Semenova, E., Minakhin, L., Makarova, K. S., Koonin, E. V., Konermann, S., Joung, J., Gootenberg, J. S., Abudayyeh, O. O.

Publications

Barr Lab

- Barr, M.M. (2016) Middle age has its advantages. *PLoS Genetics*, 2(12):e1006426.
- Lockhead, D., Schwarz, E.M., O'Hagan, R., Bellotti, S., Krieg, M., Barr, M.M., Dunn, A.R., Sternberg, P.W., and Goodman, M.B. (2016). "The tubulin repertoire of *C. elegans* sensory neurons and its context-dependent role in

process outgrowth." *Molecular Biology of the Cell*. Sep 21. pii: mbc.E16-06-0473.

- O'Hagan, R. and Barr, M.M. (2016) Kymographic Analysis of Transport in an Individual Neuronal Sensory Cilium in *Caenorhabditis elegans*. *Methods Mol Biol*. 2016;1454:107-22.
- Silva, M., Morsci, N., Nguyen, K.C.Q., Rizvi, A., Rongo, C., Hall, D.H., and Barr, M.M. (2017) "Cell-specific α -tubulin isotype regulates ciliary microtubule ultrastructure, intraflagellar transport, and extracellular vesicle biology." *Current Biology* 27: 968-980.

Dismukes Lab

- Ananyev, G, C. Gates, G.C. Dismukes, The Multiplicity of Roles for (Bi)carbonate in Photosystem II Operation in the Hypercarbonate-requiring Cyanobacterium *Arthrospira maxima*. *Photosynthetica*, in press.
- Ananyev, G., C. Gates, H. Treves, and G.C. Dismukes, Record Growth Rate and Exceptional Photoprotection Powered by an Adaptable Light Conversion Mechanism in the Microalga *Chlorella ohadii*. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* in press..
- X Qian, MK Kim, GK Kumaraswamy, A Agarwal, DS Lun, GC Dismukes, Flux balance analysis of photoautotrophic metabolism: Uncovering new biological details of subsystems involved in cyanobacterial photosynthesis. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* 1858 (4), 276- 287.
- G Ananyev, C Gates, GC Dismukes, The Oxygen quantum yield in diverse algae and cyanobacteria is controlled by partitioning of flux between linear and cyclic electron flow within photosystem II. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* 1857 (9), 1380-1391.
- Vinyard, D.J., J.S. Sun, J. Gimpel, G.M. Ananyev, S.P. Mayfield and G. Charles Dismukes, Natural isoforms of the Photosystem II D1 subunit differ in photoassembly efficiency of the water-oxidizing complex. *Photosynth Res*, 2016. 128(2): p. 141-50.
- Shuyi Zhang, Xiao Qian, Shannon Chang, G.C. Dismukes and D.A. Bryant, Natural and synthetic variants of the tricarboxylic acid cycle in cyanobacteria: introduction of the GABA shunt into *Synechococcus* sp. PCC 7002 *Frontiers in microbiology*. 2016. 7,
- Qian, X., G.K. Kumaraswamy, S. Zhang, C. Gates, G.M. Ananyev, D.A. Bryant and G.C. Dismukes, Inactivation of nitrate reductase alters metabolic branching of carbohydrate fermentation in the cyanobacterium *Synechococcus* sp. strain PCC 7002. *Biotechnol Bioeng*, 2016. 113(5): p. 979-88.
- Krishnan, A., S. Zhang, Y. Liu, K.A. Tadmori, D.A. Bryant and C.G. Dismukes, Consequences of *ccmR* deletion on respiration, fermentation and H₂ metabolism in cya-

nobacterium *Synechococcus* sp. PCC 7002. *Biotechnol Bioeng*, 2016. 113(7): p. 1448-59.

- Gates, C., G. Ananyev and G.C. Dismukes, Strontium substitution for calcium in the water oxidizing complex of PSII increases water oxidation efficiency in cyanobacteria in vivo. 2016. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* 1857 (9), 1550-1560.
- Foflonker, F., G. Ananyev, H. Qiu, A. Morrison, B. Palenik, G.C. Dismukes and D. Bhattacharya, The unexpected extremophile: Tolerance to fluctuating salinity in the green alga *Picochlorum*. *Algal Research-Biomass Biofuels and Bioproducts*, 2016. 16: p. 465-472.
- Vinyard, D.J., J.S. Sun, G. Ananyev, J. Gimpel, S.P. Mayfield and G.C. Dismukes, Natural isoforms of the D1 reaction center subunit of oxygenic Photosystem II differ in photoassembly efficiency of the water oxidizing complex. *Photosynthesis Research*, 2016 128 (2), 141-150.
- Porter, S.H., S. Hwang, V. Amarasinghe, E. Taghaddos, V. Manichev, M. Li, G. Gardner, A. Safari, E. Garfunkel, M. Greenblatt and G.C. Dismukes, Optimizing the "Artificial Leaf" Photoanode- Photocathode-Catalyst Interface Systems for Solar Water Splitting. *Electrochemical Society Transactions*, 2016. 72 (37), 1-19
- S Hwang, SH Porter, G Gardner, AB Laursen, H Wang, M Li, M. Greenblatt, E. Garfunkel, GC Dismukes, 7. *Electrochemical Society Transactions*, 2016. 72 (23), 31-51
- Smith, P.F., B.J. Deibert, S. Kaushik, G. Gardner, S. Hwang, H. Wang, J.F. Al-Sharab, E. Garfunkel, L. Fabris, J. Li and G.C. Dismukes, Coordination Geometry and Oxidation State Requirements of Corner Sharing MnO₆ Octahedra for Water Oxidation Catalysis: An Investigation of Manganite (γ - MnOOH). *ACS Catalysis*, 2016. 6(3): p. 2089–2099.
- Gardner, G., J. Al-Sharab, N. Danilovic, Y.B. Go, K. Ayer, M. Greenblatt and G.C. Dismukes, Structural basis for differing electrocatalytic water oxidation by the cubic, layered and spinel forms of lithium cobalt oxides. *Energy Environ. Sci.*, 2016. 9: p. 184--192.
- Davis, K.M., M.C. Palenik, L.F. Yan, P.F. Smith, G.T. Seidler, G.C. Dismukes and Y.N. Pushkar, X-ray Emission Spectroscopy of Mn Coordination Complexes Toward Interpreting the Electronic Structure of the Oxygen-Evolving Complex of Photosystem II. *Journal of Physical Chemistry C*, 2016. 120(6): p. 3326-3333.

Dong Lab

- 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.
- Wang Y, Xue X, Zhu J-K#, and Dong J# (2016) Demethylation of *ERECTA* receptor genes by IBM1 histone

demethylase affects stomatal development. *Development*. pii: dev.129932.

- Zhang Y, Guo X and Dong J# (2016) Phosphorylation of the polarity protein BASL differentiates asymmetric cell fate through MAPKs and SPCH. *Curr. Biol.* 26(21):2957-2965. doi: 10.1016/j.cub.2016.08.066.
- Hou Y, Guo X, Cyprys P, Zhang Y, Bleckmann A, Cai L, Huang Q, Luo Y, Gu H, Dresselhaus T, Dong J, Qu L.J. (2016) Maternal ENDOLs are required for pollen tube reception in *Arabidopsis*. *Curr. Biol.* pii: S0960-9822(16)30694-7. doi: 10.1016/j.cub.2016.06.053.
- Shao W and Dong J# (2016) Polarity in plant asymmetric cell division: Division orientation and cell fate differentiation. *Dev. Biol.* pii: S0012-1606(16)30268-8. doi: 10.1016/j.ydbio.2016.07.020.
- Zhang Y, Bergmann DC# and Dong J# (2016) Fine-scale dissection of the subdomains of polarity protein BASL in stomatal asymmetric cell division. *J. Exp. Bot.* doi: 10.1093/jxb/erw274
- Pillitteri LJ, Guo X and Dong J# (2016) Asymmetric cell division in plants: mechanisms of symmetry breaking and cell fate determination. *Cell Mol. Life Sci.* doi:10.1007/s00018-016-2290-2
- Zhang Y, and Dong J# (2016) Imaging spatial reorganization of a MAPK signaling pathway using the tobacco transient expression system. *JoVE J.* (109). doi: 10.3791/53790.
- Xue X and Dong J# (2016) The MAPK substrate MASS2 regulates stomatal patterning in *Arabidopsis*. *Mol. Reprod. Dev.* 83(2):93. doi: 10.1002/mrd.22612.

Dooner Lab

- García, N., Y. Li, H. K. Dooner and J. Messing, 2017 Maize defective kernel mutant generated by insertion of a Ds element in a gene encoding a highly conserved TTI2 cochaperone. *Proc Natl Acad Sci U S A*: doi: 10.1073/pnas.1703498114.
- Xiong, W., H.K. Dooner, C. Du. 2016, Rolling circle amplification of centromeric Helitrons in plant genomes. *Plant Journal* (2016) 88, 1038–1045.

Ebright Lab

- Bird, J., Zhang, Y., Tian, Y., Greene, L., Liu, M., Buckley, B., Krasny, L., Lee, J., Kaplan, C., Ebright, R., and Nickels, B. (2016) The mechanism of RNA 5' capping with NAD⁺, NADH, and desphosphoCoA. *Nature* 535, 444-447.
- Lin, W., Mandal, S., Degen, D., Liu, Y., Ebright, Y., Li, S., Feng, Y., Zhang, Y., Mandal, S., Jiang, Y., Liu, S., Gigliotti, M., Talaue, M., Connell, N., Das, K., Arnold, E., Ebright, R. (2017) Structural basis of *Mycobacterium tuberculosis* transcription and transcription inhibition. *Mol. Cell* 166, 169-179.
- Walker, S, Degen, D., Nickbarg, E., Carr, D., Soriano, A., Mandal, M., Painter, R., Sheth, P., Xiao, Li., Sher, X., Murgolo, N., Su, J., Olsen, D., Ebright, R., Young,

K. (2017) Affinity selection-mass spectrometry identifies a novel antibacterial RNA polymerase inhibitor. *ACS Chem. Biol.* 12, 1346-1352.

- Maffioli, S., Zhang, Y., Degen, D., Carzaniga, T., Del Gatto, G., Serina, S., Monciardini, P., Mazzetti, C., Guglielame, P., Candiani, G., Chiriack, A.I., Facchetti, G., Kaltofen, P., Sahl, H.-G., Dehò, G., Donadio, S., and Ebright, R.H. (2017) Antibacterial nucleoside-analog inhibitor of bacterial RNA polymerase: pseudouridimycin. *Cell* (in press).
- Boucher, H., Ambrose, P., Chambers, H., Ebright, R.H., Jezek, A., Murray, B., Newland, J., Ostrowsky, B., Rex, J. (2017) Developing antimicrobial drugs for resistant pathogens and unmet needs. *J. Infect. Dis.* (in press).
- Bird, J., Nickels, B., Ebright, R.H. (2017) “Ab initio” RNA 5’ capping by transcription initiation with non-canonical initiating nucleotides (NCINs): determination of relative efficiencies of transcription initiation with NCINs and NTPs. *Bio-Protocol* (in press).

Gallavotti Lab

- Chatterjee, M, Liu Q, Menello C, Galli M, Gallavotti A. 2017. The Combined Action of Duplicated Boron Transporters Is Required for Maize Growth in Boron Deficient Conditions. *Genetics* 2017, Early Online <https://doi.org/10.1534/genetics.116.198275>.
- 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 (in press).

Genomics Core Facility

- Xie G, Chastain-Gross RP, Bélanger M, Kumar D, Whitlock JA, Liu L, Farmerie WG, Zeng CL, Daligault HE, Han CS, Brettin TS, Progulske-Fox A. Genome Sequence of *Porphyromonas gingivalis* Strain A7A1-28. *Genome Announc.* 2017 Mar 9;5(10). pii: e00021-17. doi: 10.1128/genomeA.00021-17. PMID: PMC5347233.
- Pareek CS, Błaszczyk P, Dziuba P, Czarnik U, Fraser L, Sobiech P, Pierzchała M, Feng Y, Kadarmideen HN, Kumar D. Single nucleotide polymorphism discovery in bovine liver using RNA-seq technology. *PLoS One.* 2017 Feb 24;12(2):e0172687. doi: 10.1371/journal.pone.0172687 PMID: 28234981
- Chastain-Gross RP, Xie G, Bélanger M, Kumar D, Whitlock JA, Liu L, Raines SM, Farmerie WG, Daligault HE, Han CS, Brettin TS, Progulske-Fox A. Genome Sequence of *Porphyromonas gingivalis* Strain 381. *Genome Announc.* 2017 Jan 12;5(2). pii: e01467-16. doi: 10.1128/genomeA.01467-16. PubMed PMID: 28082501
- Pareek CS, Smoczyński R, Kadarmideen HN, Dziuba P, Błaszczyk P, Sikora M, Walenzik P, Grzybowski T, Pierzchała M, Horbańczuk J, Szostak A, Ogluszka M, Zwierzchowski L, Czarnik U, Fraser L, Sobiech P, Wąsowicz K, Gelfand B, Feng Y, Kumar D. Single Nucleotide Polymorphism Discovery in Bovine Pituitary Gland Using RNA-Seq Technology. *PLoS One.* 2016 Sep

8;11(9):e0161370. doi: 10.1371/journal.pone.0161370.

Irvine Lab

- Park G.-S., Oh, H., Kim, M., Kim, T. Irvine, K.D., Lim, D.-S. An evolutionarily conserved negative feedback mechanism in the Hippo pathway reflects functional difference between LATS1 and LATS2. 2016. *Oncotarget*, 7, 24063-24075.
- Kuta, A., Mao, Y., Whiting, D., Zakaria, S., Ferreira de Sousa, C., Balczerski, B., Irvine, K.D., and Francis-West, P. H. 2016. Fat4-Dchs1 controls cell proliferation in the developing vertebrae. *Development* 143, 2367-2375.
- Sun, S. and Irvine, K.D. 2016. Cellular Organization and Cytoskeletal Regulation of the Hippo Signaling Network. *Trends in Cell Biology*, 26, 694-704.
- Misra, J.R. and Irvine, K.D. 2016. Vamana couples Fat signaling to the Hippo pathway. *Developmental Cell* 39, 254-266.
- Pan, Y., Heemskerk, I., Ibar, C., Shraiman, B. I. and Irvine, K.D. 2016. Differential growth triggers mechanical feedback that elevates Hippo signaling. *Proc. Nat. Acad. Sci. USA* 113, E6974-E6983
- Bilder, D. and Irvine, K.D. 2017. Taking Stock of the *Drosophila* Research Ecosystem. *Genetics* in press

Maliga Lab

- Yu, Q., Lutz, K.A. and Maliga, P. (2017) Efficient Plastid Transformation *Arabidopsis*. *Plant Physiol.* In press

McKim Lab

- Radford, S.J, Go A.M., McKim K.S., Cooperation Between Kinesin Motors Promotes Spindle Symmetry and Chromosome Organization in Oocytes. *Genetics.* 2017 Feb;205(2):517-527
- Radford S.J., Nguyen A.L., Schindler K., McKim K.S., The chromosomal basis of meiotic acentrosomal spindle assembly and function in oocytes. *Chromosoma.* 2016 Nov 11. In press
- Radford, S. J., and K. S. McKim, 2016 Techniques for Imaging Prometaphase and Metaphase of Meiosis I in Fixed *Drosophila* Oocytes. *J Vis Exp* 116: e54666.
- Gyuricza MR, Manheimer KB, Apte V, Krishnan B, Joyce EF, McKee BD, McKim KS. Dynamic and Stable Cohesins Regulate Synaptonemal Complex Assembly and Chromosome Segregation. *Curr Biol.* 2016 Jul 11;26(13):1688-98.

Messing Lab

- Zhang, W., Xu, J-H., Bennetzen, J. L., and Messing, J. (2016). Teff, an orphan cereal in the Chloridoideae, provides insights into the evolution of storage proteins in grasses. *Genome Biology and Evolution* 8, 1712-1721.
- Dong, J., Feng, Y., Kumar, D., Zhang, W., Zhu, T., Luo, M-C., and Messing, J. (2016). Analysis of tandem gene copies in maize chromosomal regions reconstructed from long sequence reads. *Proc. Natl. Acad. Sci. USA.* 113,

7949-7956.

- Wang, W., Wu, Y., and Messing, J. (2016). Genome-wide analysis of pentatricopeptide-repeat proteins of an aquatic plant. *Planta* 244, 893-899.
- Zhang, Z, Zheng, X., Yang, J, Messing, J., and Wu, Y. (2016). Maize endosperm-specific transcription factors O2 and PBF network the regulation of protein and starch synthesis. *Proc. Natl. Acad. Sci. USA.* 113, 10842-10847.
- Zhang, W. and Messing, J. (2016). PacBio RS for gene family studies. *Methods in Molecular Biology* 1551, 61-71.
- Garcia, N., Li, Y., Dooner, H.K., and Messing, J. (2017). A maize defective kernel mutant generated by insertion of a Ds element in a gene encoding a highly conserved TTI2 cochaperone. *Proc. Natl. Acad. Sci. USA.* 114, 5165-5170.
- Messing, J. (2017). Does Investment in Research Always Pay Off? *Am Acad Arts & Sci Bulletin* 70 (3), 45-47.
- Wu, Y. and Messing, J. (2017). Understanding and improving protein traits in maize seeds. In *Achieving Sustainable Maize Cultivation*, Watson, D., ed., Burleigh Dodds, Cambridge, UK, pp.115-128.

Padgett Lab

- Kane, N.S., M. Vora, K.J. Varre, and R.W. Padgett (2016). Efficient Screening of CRISPR/Cas9-Induced Events in *Drosophila* using a co-CRISPR Strategy, *G3*, 7(1):87-93 PMID: 2779397; PMCID:PMC5217126.
- Savage-Dunn, C. and R.W. Padgett (2017) The TGF- β family in *Caenorhabditis elegans*, Cold Spring Harbor Press Perspectives in Biology, The Biology of the TGF- β Family. 2017. PMID:28096268.
- Gleason, R.J., M. Vora, Y. Li, N.S. Kane, K. Liao, and R.W. Padgett (2017). *C. elegans* SMA-10 regulates BMP receptor trafficking, accepted, PLoS ONE.

Rongo Lab

- Cell-Specific α -Tubulin Isoform Regulates Ciliary Microtubule Ultrastructure, Intraflagellar Transport, and Extracellular Vesicle Biology. Silva M, Morsci N, Nguyen KC, Rizvi A, Rongo C, Hall DH, Barr MM. *Curr Biol.* 2017 Apr 3;27(7):968-980. doi: 10.1016/j.cub.2017.02.039. Epub 2017 Mar 16. PMID: 28318980
- Dopamine signaling promotes the xenobiotic stress response and protein homeostasis. Joshi KK, Matlack TL, Rongo C. *EMBO J.* 2016 Jun 3. pii: e201592524. [Epub ahead of print] PMID: 27261197

Severinov Lab

- Abudayyeh, O.O., Gootenberg, J. S., Konermann, S., Joung, J., Slaymaker, I. M., Cox, D. B.T., Shmakov, S., Makarova, K. S., Semenova, E., Minakhin, L., Severinov, K., Regev, A., Lander, E. S., Koonin, E. V., and Zhang, F. (2016) C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. *Science*, 353, aaf5573

- Kuznedelov, K., Mekler, V., Lemak, S., Tokmina-Lukaszewska, M., Datsenko, D. A., Jain, I., Savitskaya, E., Mallon, M., Shmakov, S., Bothner, B., Bailey, B., Yakunin, A., Severinov, K., and Semenova, E. (2016) Altered stoichiometry *Escherichia coli* Cascade complexes with shortened CRISPR RNA spacers are capable of interference and primed adaptation. *Nucleic Acids Res.*, 44, 10849-10861
- Serebryakova, M., Tsibulskaya, D., Mokina, O., Kulikovskiy, A., Nautiyal, M., Van Aerschot, A., Severinov, K., and Dubiley, S. (2016) A Trojan-horse peptide-carboxymethyl-cytidine antibiotic from *Bacillus amyloliquifaciens*. *J. Am. Chem. Soc.*, 138, 15690-15698
- Severinov, K., Ispolatov, I., and Semenova, E. (2016) The influence of copy number maintenance of targeted extrachromosomal genetic elements on the outcome of CRISPR-Cas defense. *Front. Mol. Biosci.*, 3, 45
- Phadtare, S., and Severinov K. (2016) Gene regulation by cold shock proteins via transcription antitermination, in *Stress and Environmental control of Gene Expression in Bacteria* (F. J. de Bruijn, ed) Wiley/Blackwell, 829-836
- Semenova, E. and Severinov, K. (2016) Come together: CRISPR-Cas immunity senses the quorum. *Mol. Cell*, 64, 1013-1015
- Phadtare, S., and Severinov K. (2016) Gene regulation by cold shock proteins via transcription antitermination, in *Stress and Environmental control of Gene Expression in Bacteria* (F. J. de Bruijn, ed) Wiley/Blackwell, 829-836
- Shmakov, S., Makarova, K. S., Wolf, Y. I., Smargon, A., Pyzocha, N., Cox, D., Yan, W., Scott, D., Severinov, K., Zhang, F., and Koonin, E. V. (2017) Diversity and evolution of Class 2 CRISPR-Cas systems. *Nat. Rev. Microbiol.*, 15, 169-182
- Zetsche, B., Mohanraju, P., Fedorova, Y., Winblad, N., Abudayyeh, O.O., Gootenberg, J. S., Cartigny, B., van den Bogaerde, J., Severinov, K., van der Oost, J., and Zhang, F. (2017) Multiplex gene editing by CRISPR-Cpf1 through autonomous processing of a single crRNA array. *Nature Biotechnol.*, 35, 31-34
- Xu, R.-G., Jenkins, H. T., Chechik, M., Blagova, E. V., Minakhin, L., Klimuk, E., Severinov, K., Greive, S. J., and Antson, A. A. (2017) Viral large terminases cleave DNA using the canonical RuvC-like two-metal catalysis mechanism. *Nucleic Acids Res.*, 45, 3580-3590
- Savitskaya, E., Lopatina, A., Kapustin, K., Medvedeva, S., Shmakov, S., Tikhonov, A., Artamonova, I., and Severinov, K. (2017) Dynamics of *Escherichia coli* type I-E CRISPR spacers over 42,000 years. *Mol. Evol.*, 26, 2019-2026
- Lavysch, D., Sokolova, M., Slashcheva, M., Förstner, K., and Severinov, K. (2017) Transcription profiling of *Bacillus subtilis* cells infected with AR9, a giant phage encoding two multisubunit RNA polymerases. *Mbio*, 8,

e02041-16

- Strotskaya, A., Savitskaya, E., Metlitskaya, A., Semenova, E., Datsenko, K. A., Morozova, N., and Severinov, K. (2017) The action of *Escherichia coli* CRISPR-Cas system on lytic bacteriophages with different lifestyles and development strategies. *Nucleic Acids Res.*, 45, 1946-1957
- Metelev, M., Arseniev, A., Bushin, L. B., Kuznedelov, K., Artamonova, T., Kondratenko, R., Khodorkovskii, M., Seyedsayamdost, M. R., and Severinov, K. (2017) Acinetodin and klebsidin, RNA polymerase targeting lasso peptides produced by human isolates of *Acinetobacter gyllenbergii* and *Klebsiella pneumoniae*. *ACS Chem. Biol.*, 12, 814-824
- Piskunova, J., Maisonneuve, E., Germain, E., Gerdes, K., and Severinov, K. (2017) Peptide-nucleotide antibiotic microcin C is a potent inducer of stringent response and persistence in both sensitive and producing cells. *Mol. Microbiol.*, 104, 463-471
- Musharova, O., Klimuk, E., Datsenko, K.A., Logacheva, M., Semenova, E., Severinov, K., and Savitskaya, E. (2017) Non-double stranded spacer-length DNA intermediates are bound to Cas1 in cells undergoing primed CRISPR adaptation. *Nucleic Acids Res.*, 45, 3297-3307
- Sokolova, M., Borukhov, S., Lavysh, D., Artamonova, T., Khodorkovskii, M., and Severinov, K. (2017) A non-canonical multisubunit RNA polymerase encoded by the AR9 phage recognizes the template strand of its uracil-containing promoters. *Nucleic Acids Res.*, 45, 5958-5967
- Mekler, V., Minakhin, L., and Severinov, K. (2017) Mechanism of duplex DNA destabilization by RNA-guided Cas9 nuclease during target interrogation. *Proc. Natl. Acad. Sci. USA*, 114, 5443-5448
- Tabib-Salazar, A., Liu, B., Shadrin, A., Burchell, L., Wang, Z., Wang, Z., Goren, M., Yosef, I., Qimron, U., Severinov, K., Matthews, S., and Wigneshweraraj, S. (2017) Optimal growth of T7 phage requires the specific repression of *Escherichia coli* RNA polymerase activity on early viral promoters by the small DNA-binding T7 protein Gp5.7. *Nucleic Acids Res.*, doi: 10.1093/nar/gkx370
- Popova, A., Lavysh, D., Klimuk, E., Edelstein, M., Bogun, A., Schneider, M., Goncharov, A., Leonov, S., and Severinov, K. (2017) Novel FriI-like viruses infecting *Acinetobacter baumannii* – vB_AbaP_AS11 and vB_AbaP_AS12 – characterization, comparative genomic analysis, and host-recognition strategy. *Viruses*, 9, E188
- 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.*, in press
- 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, in press
- 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*, in press

Singson Lab

- A. Krauchunas and A. Singson (2016) Marriage shrines and worms impacting our understanding of mammalian fertilization. *Worm* (In Press).
- A. Krauchunas, M. Marcello, and A. Singson (2016) The molecular complexities of fertilization: Introducing the concept of a fertilization synapse. *Molecular Reproduction and Development* 83: 376-386.