



WAKSMAN INSTITUTE

of Microbiology

ANNUAL REPORT
2015-2016

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 the State of New Jersey, 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.



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Rutgers Research and Educational Foundation

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

RREF Committee Members 2015-2016

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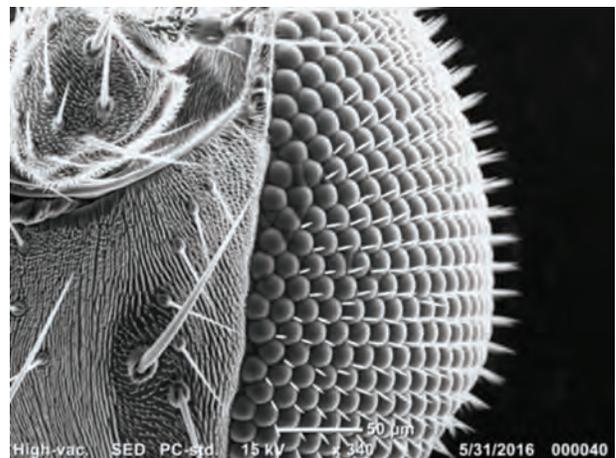
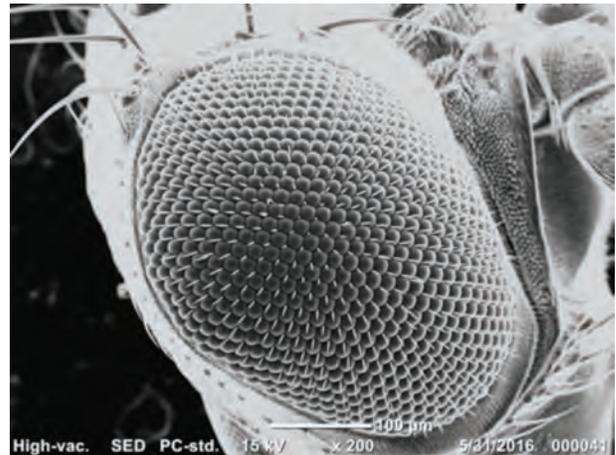
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Above: SEM images of *Drosophila melanogaster* eyes, Andrea Gallavotti and Nanci Kane
On the Cover: SEM image of *Arabidopsis* trichomes, Andrea Gallavotti

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 state to support the recruitment and appointment of faculty on regular lines that are split with lines from the 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 600TB 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 in order 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) 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 two new sequencers. It has added the Illumina NextSeq500 and Ion Proton systems to its force of NGS sequencers while retiring the SOLiD 5500XL systems. Together, WGCF now has three sequencers covering broad range of NGS requirements. The equipment and services provided by the core are aimed at reducing the startup time and degree of expertise necessary for an individual user to design and execute experiments requiring sequencing.

The throughput of Next Generation Sequencer (NGS) is much more than a traditional DNA sequencer making it an ideal choice for genome sequencing applications such as whole-genome and targeted re-sequencing for deep SNP discovery, structural variation (SNV) and copy number variation (CNV). In addition, NGS is gaining in popularity over microarrays for others genomic applications as well. NGS offers better resolution and independence from prior genome knowledge and proves to be a much better platform for whole genome transcriptome experiments including alternative splice site discovery, gene fusion detection, small RNAs, novel transcript finding, and digital gene expression as well as epigenome (ChIP-seq and bisulfite-seq) studies.

With three state of the art NGS sequencers on board, WGCF can produce up to 150 GB of data per day in fragments of 50 bp, 75 bp, 150 bp, 200 bp, and paired-ends of 2x75, 2x150, 2x250, 2x300 sizes. The combination of instruments gives faculty flexibility to pick one that fits their needs and budgets. The throughput of the NextSeq500 is best suited for 'tag and count' experiments including RNA-seq, small RNA and Chip-Seq. Up to 20 transcriptome or one human genome at 30x coverage can be sequenced on a single flowcell in 30 hours at a cost of ~\$5000. The benefits of Ion Proton semiconductor sequencing lies

in its avoidance of modified nucleotides and optical measurements. Ion Proton offers fastest turnout time at low operating costs delivering data in just four hours. It is most suitable for mid throughput need including metagenome- and amplicon-sequencing applications. 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. These new instruments decreased turnout time drastically by half. In addition, our genomics facility has access to HiSeq and PacBio instrumentation in collaborating facilities, instruments that extend the throughput of NextSeq500 for larger projects and in the latter case the length of sequencing reads.

It has become clear 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 assistance of Brian Gelfand. 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 2015/2016, the Institute consisted of fifteen resident, two non-resident, and seven emeriti faculty members. The Institute accommodates four assistant research professors, thirteen visiting researchers, eleven research associates, twenty-one postdoctoral researchers, twenty-one technical assistants, thirty-two graduate students, and two visiting students. The Waksman Institute's total resident population is currently 135, which does not include the 52 undergraduate students that did independent research during the last year. The latter number doubled compared to last year reflecting the attraction that the Institute enjoys among life sciences majors.

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 and Pathology, two in the Department of Chemistry, one in the Department of Ecology, Evolution, and Natural Resources, and one in the Department of Biochemistry and Microbiology (Dr. Dismukes has two departmental affiliations). Of the fifteen resident and two non-resident members, two are Assistant Professors, one is Associate Professor, eight 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 seven 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 five Fellows of AAAS. One faculty member is Howard Hughes Institute Investigator.

It is with great sadness to report the passing of two faculty members and one research faculty member. Hubert Lechevalier, who as a graduate in Selman Waksman's laboratory, discovered the antibiotics candicidin and neomycin; the latter is still used today in topological applications and as a selectable marker in genetic engineering. He died at the age of 89 last November in his home in Morrisville, Vermont. Remarkably, his wife followed him a few weeks later at the age of 87. She was research faculty at the Institute and best known for her work on actinomycetes and their products. She and her husband won in 1982 the Charles Thom Award of the Society for Industrial

Microbiology. On May 9th this year Karl Maramorosch passed away at the age of 101 while visiting Poland. He lived the last year with his daughter Lydia Ann in Los Angeles. Karl was best known for his work on insect viruses and plant pathogenesis, which brought him the Wolf Prize in Agriculture in 1980. He also was a member of the German National Academy of Sciences Leopoldina. After his retirement at the Institute he worked as an emeritus at the Department of Entomology. All three are missed and expanded versions of their careers have been published elsewhere. While we lost three emeriti faculty, we gained one. Hugo Dooner still works at the Institute and kept his laboratory to work on a major genetic resource for functional genomics in maize, which should be completed by the end of next academic year. We then will seek to replace this highly visible faculty member.

Lectures

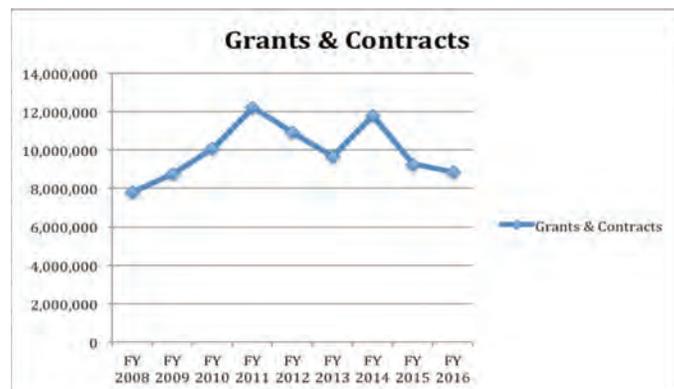
Because there are so many lecture series in the life sciences on our and the Cook campus, the Institute conducts mainly ad hoc seminars of visitors of our faculty that are listed at the end of the Report. We also list there the program of our Annual Retreat from September 10th, 2015. In addition, the Institute sponsored The Microbiology Symposium, in New Brunswick, NJ, in January 2016 at Traves Hall, Douglass College.

Recruitment and Funding

Although the Board of Governors had approved our plans for expansion of laboratory space, realization of these plans have been delayed in the design phase. We do not anticipate construction to begin before spring of 2017, which will delay recruitment for another year.

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. Last year, the university changed its budget to the Responsibility Center Management or RCM system. The Institute has been designated RCM under Chancellor Richard Edwards. One of the new features of RCM is that new resources are based on tuition and indirect cost received. As one can see at the end of this report, Institute members have a teaching load comparable to full-time research-active department members. However, there is no formula yet of how to split tuition income between decanal units and Institutes. Furthermore, total external grants

fluctuate from year to year and one cannot predict with certainty grant income from the preceding year. To illustrate this, I show a graph of this fluctuation over the last 9 years. Based on these historical values, our grant income can fluctuate by \$2 million annually. Given the significant changes in external grants from year to year, there is no way that we can manage responsibly budget



changes of 12% either as a deficit or a surplus, which would also contradict the current concept of RCM. In particular, if services (Cost Centers) are not subject to these fluctuations, it creates a budget imbalance between faculty and support structure. Under the new RCM model of budgeting the Waksman Institute has a budget reduction of over \$550,000 or 12%. The Unrestricted Revenues have remained relatively the same but the University has increased the Allocated Costs resulting in a significant budget reduction.

Awards/Honors

I am pleased to report several awards/honors of our students and faculty this year. As a major recognition for the Institute, Evelyn Witkin won the Lasker Award in Basic Medical Research for her groundbreaking work in DNA repair. Pal Maliga won the 2016 Lawrence Bogorad Award for Excellence in Plant Biology Research of the American Society of Plant Biologists for his pioneering work in plastid genetics of higher plants. Richard Ebright and I got elected as members of the American Academy of Arts and Sciences. I also was awarded the 2016 Academician Hsien-Wen Li Lectureship of Academia Sinica. Ken Irvine won the 2016 Rutgers Board of Trustees Award for Excellence in Research. Juan Dong won the Women's Young Investigator Award for ASPB Plant Biology 2016, Austin, TX. Margaret Morash (BS 2016), performing undergraduate research in the Barr laboratory, received the 2015-2016 Goldwater Scholarship. 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 of the kidney and cilia. 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 (EVs), and stress-induced neuronal restructuring.

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Malan Silva, Graduate Student

Gloria Androwski, Research Associate

Sebastian Belotti, Undergraduate Student

Maggie Morash, Undergraduate Student

Alina Rashid, Undergraduate Student

Anza Rivzi, Undergraduate Student



IRVINE LAB

Developmental Biology



Dr. Kenneth Irvine
Molecular Biology & Biochemistry

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 forces. Much of our research takes advantage of the

powerful genetic, molecular, and cellular techniques available in *Drosophila melanogaster*, which facilitate both gene discovery and the analysis of gene function.

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. We have also identified a link between Hippo signaling and JNK signaling, which is particularly important for promoting regenerative growth after tissue damage.

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.

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McKIM LAB

Molecular Genetics of Meiotic Recombination and Chromosome Segregation



Dr. Kim McKim
Genetics

Summary

During the reductional division of meiosis I, pairs of homologous chromosomes segregate from each other. Initially, the homologs are brought together in an elaborate pairing process that culminates with synapsis, where bivalents are held together along their entire length by the synaptonemal complex (SC). Within this context, recombination is initiated with double strand breaks (DSB), some of which are repaired as crossovers. Chiasmata, the result of meiotic crossing over between homologs, facilitate homolog orientation and segregation on the meiosis I spindle.

Chromosome segregation depends on the formation of a bipolar spindle and on the homologous centromeres arranging themselves towards opposite poles, known as bi-orientation. Errors during meiosis I lead to an abnormal number of chromosomes in the gametes (aneuploidy). The frequency of these errors is particularly high in oocytes and the most frequent cause of infertility in human females. A striking feature of the meiotic spindles in the oocytes of many animals is that they lack centrosomes. With no microtubules growing from preexisting poles in acentrosomal oocytes, the nature of the chromosome-based signals that initiate microtubule recruitment and the mechanisms that organize a bipolar spindle are poorly understood.

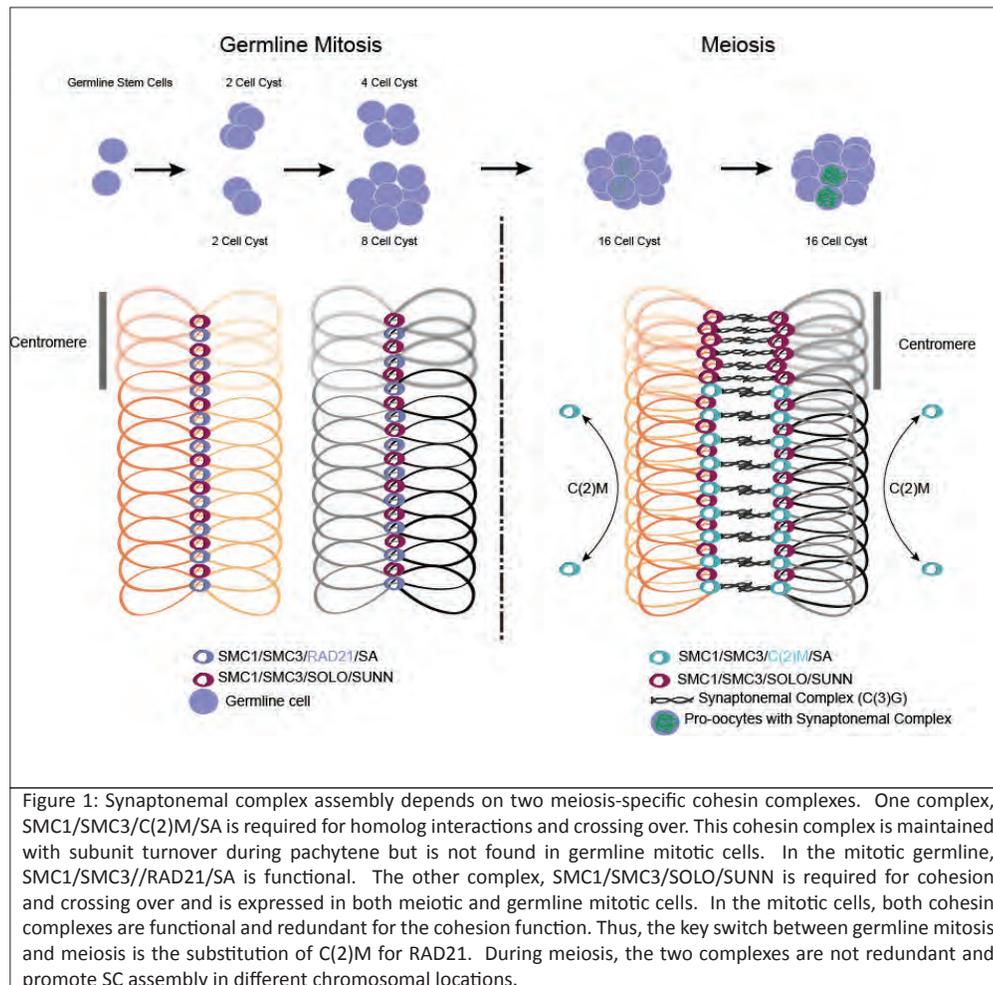
Research in my laboratory uses genetic, biochemical and cytological approaches to study and understand meiosis in the model organism *Drosophila melanogaster*. We are interested in identifying the “weak points” that make the oocyte susceptible to nondisjunction. Characterizing these weak points is important to understand how meiosis works, and also important to understand why errors occur that lead to infertility.

Multiple Cohesin complexes are required during meiosis (Figure 1)

Cohesion is what holds sister chromatids together after DNA replication but prior to cell division. In mitosis, the cohesin complex, composed of SMC₁, SMC₃, Stromalin (SA) and a kleisin Rad21, required to hold sister chromatids together until anaphase. Meiotic Cohesin proteins, however, have multiple roles including being required for SC assembly. Because there are two divisions in meiosis, cohesion must be regulated based on its position on the chromosome. In meiosis I, cohesion along the chromosome arms is lost. In meiosis II, centromere cohesion is lost. A third type of cohesion fuses the sister centromeres together during meiosis I, so that they segregate to the same pole at anaphase I (co-orientation). We have found that there are multiple cohesin complexes, some of which are required for homolog interactions, others for cohesion.

C(2)M was the first of a subgroup of kleisins that appear to function in synapsis and are required for homolog but not sister chromatid interactions. This pathway also includes SA, the mitotic stromalin subunit of cohesion, which with the kleisin, forms a bridge between the two SMC subunits. In addition, the cohesin loader Nipped-B is a positive regulator required to load the other proteins. Surprisingly, SA and Nipped-B are only required for SC assembly and not cohesion during meiosis. Cohesion depends on a second pathway with three highly diverged meiosis-specific subunits, SOLO, SUNN and ORD, instead of C(2)M, SA and Nipped-B. While SOLO maybe a kleisin subunit and SUNN maybe a stromalin subunit, the role of ORD is less clear and may be a positive regulator. Mutant analysis in *Drosophila* has shown that SC assembly depends on these two cohesin pathway. In the absence of SMC₁ and SMC₃, all SC assembly is eliminated. In the absence of C(2)M or SA, SC assembly occurs at the centromeres and a few euchromatic sites. SC assembly at these sites depends on the second cohesin pathway with SOLO, SUNN and ORD.

Cohesion is a possible weak part of meiosis because cohesion is thought to be established only during S-phase. Because cohesion is not established at other times of the cell-cycle, cohesin molecules that are displaced from the



chromosomes cannot be replaced. This is a particular problem for oocytes because they usually undergo a prolonged prophase; the time between S-phase and the meiotic divisions can be days in *Drosophila* and years in humans. The dissociation of cohesin complexes from meiotic chromosomes has been proposed to be a major contributing factor to the increase in aneuploidy and reduced fertility observed in older human mothers (the “aging cohesion” hypothesis). Consistent with this, we have found that SOLO/SUNN is only loaded at the centromeres during premeiotic S-phase. This is the cohesion required to hold sister chromatids together and, consistent with the aging cohesion hypothesis, cannot be replaced if lost during prophase.

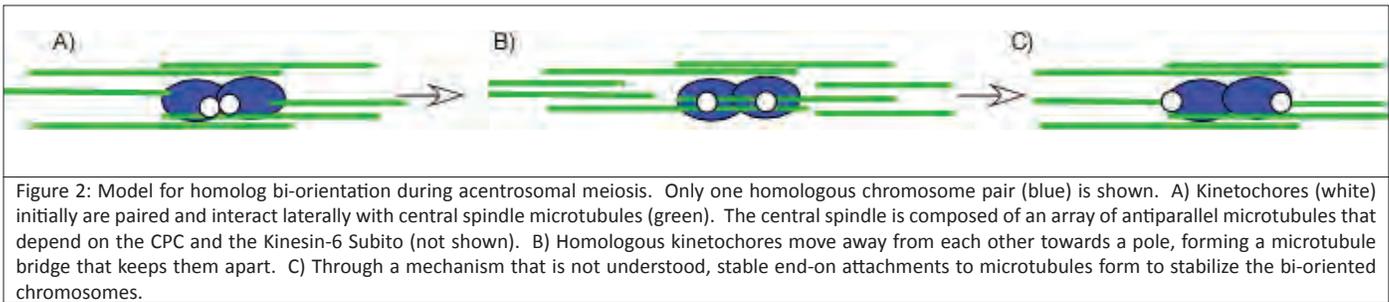
In contrast, we have discovered that the C(2)M-containing cohesin is dynamic and can be replaced if it dissociates from the chromosomes during meiotic prophase. Thus, the two cohesin complexes differ in their capacity to be replenished during prophase. Specifically, the C(2)M/SA cohesin complex is dynamic whereas centromeric SUNN/SOLO is not. Since it is clear that cohesins can be loaded during prophase, it remains to be determined why cohesion can only be

established during S-phase.

One clue has come from the expression pattern of c(2)M. The ovary contains stem cells, which generate cystoblasts. These cells divide mitotically 4 times, which generates an oocyte and 15 nurse cells. The c(2)M transcript is induced precisely when the oocyte is formed and is not expressed in the mitotic cells. Thus, the switch from mitosis to meiosis involves an important substitution within one cohesin complex, from a RAD21/SA/SMC1/SMC3 complex to a C(2)M/SA/SMC1/SMC3 complex. We tested this model by inducing c(2)M in the mitotic germline cells and found this induces SC assembly. Thus, C(2)M is sufficient to induce SC assembly, and its expression may be repressed until after S-phase when the oocyte enters meiotic prophase.

An interaction between the central spindle and the kinetochores is required for chromosome segregation (Figure 2)

During meiosis I, pairs of homologous chromosomes segregate from each other during the reductional division. This depends on the formation of a bipolar



spindle and that the homologous centromeres arrange towards opposite poles, also known as bi-orientation. Bi-orientation is a critical part of metaphase I since it establishes how homologous chromosome pairs will segregate at anaphase I. Our previous work revealed three features which are key to the acentrosomal spindle: the Chromosome Passenger Complex (CPC), a metaphase central spindle, and multiple modes of microtubule-kinetochore interactions. The CPC is required for the accumulation of microtubules around the chromosomes, organizing a bipolar spindle, and bi-orientation of homologous chromosomes, suggesting that the CPC initiates all aspects of acentrosomal spindle assembly. The CPC is composed of four proteins: Incenp, Aurora B kinase, Survivin and Borealin. The chromatin recruits the CPC following nuclear envelope breakdown and the CPC subsequently interacts with microtubules and promotes organization of a bipolar spindle and orientation of the homologs.

The central spindle is composed of the overlap of antiparallel microtubules and several proteins including the CPC and the kinesin-6 microtubule motor protein Subito. The importance of the central spindle is exhibited by the subito mutant phenotype. In the absence of Subito, tripolar and monopolar spindles are observed and the central spindle bundles of microtubules are absent. Perhaps as a consequence of these problems, errors in the bi-orientation of homologs are frequent. Interestingly, a central microtubule array that acts as a backbone or scaffold for organizing acentrosomal spindle is conserved in the oocytes of mammals and *C. elegans*. A robust central spindle may be a conserved element required for acentrosomal meiosis. During prometaphase in mouse meiotic cells, the chromosomes congress to a “prometaphase belt”, which is probably a region of the mouse spindle which is similar to the *Drosophila* central spindle. It has been proposed that this migration may enhance the rate of bi-orientation by bringing kinetochores into the vicinity of a high density of microtubule plus ends present in the central spindle. How the central spindle promotes bi-

orientation, however, is not known.

The kinetochore includes the proteins that interact with the microtubules of the spindle. A core component of the kinetochore is the KMN network, which in *Drosophila* includes the Mis12 (Mis12, Nnfi, Nsl1) and Ndc80 complexes (Ndc80, Nuf2, Spc25) and Spc105R/Knl1. Interactions involving the kinetochores laterally along microtubules is mediated by SPC105R. More stable end-on kinetochore-microtubule attachments requires NDC80 and are essential to maintaining the bi-orientation of centromeres and probably chromosome movement at anaphase. The lateral interactions leading to homolog bi-orientation may occur with microtubules of the central spindle. We envision two non-mutually exclusive models for how homologous chromosomes on the acentrosomal spindle achieve bi-orientation. First, the central spindle organizes the bipolar spindle, and independently, bi-orientation occurs. Alternatively, the central spindle may have a direct role in promoting bi-orientation. This could occur if the lateral interactions of the kinetochores involve central spindle microtubules.

A critical component of the anaphase midzone is the Centralspindlin complex, which in *Drosophila* is composed of RacGap50c and the Kinesin 6 Pavarotti. RacGap50C colocalizes with Subito and the CPC on the meiotic spindle and is required for bi-orientation. Similarly, we found knockdowns of midzone proteins Citron kinase (sticky) and Rho have a similar phenotype. None of these genes are required for spindle bipolarity, suggesting they are required for central spindle function but not structure. We propose these genes are required for interactions between the kinetochores and the central spindle prior to making end-on kinetochore attachments. We are currently testing a specific model that “bridging fibers” connect homologous centromeres have role in establishing or maintaining bi-orientation. In this model, the bi-orientation process begins with lateral interactions between the kinetochores and the central spindle microtubules. The central spindle has a direct role in

promoting bi-orientation by mediating separation of homologous centromeres towards opposite spindle poles. The lateral interactions are then converted to stable end-on interactions once the homologs are bi-oriented. How the cell determines the homologous centromeres are properly bi-oriented is being investigated.

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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.

As a foundation for further SMA-10 studies, we examined the normal trafficking of TGF β receptors in the

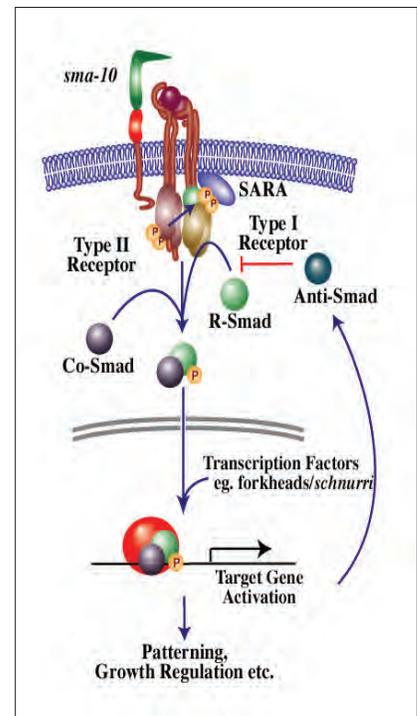


Figure 1: Model for TGF β Signaling

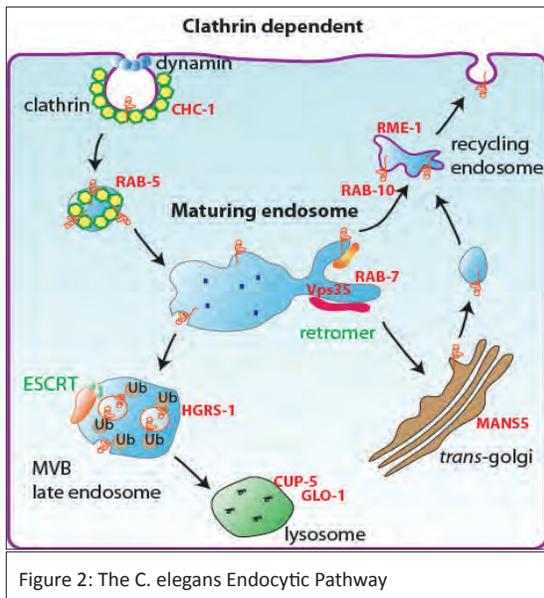


Figure 2: The *C. elegans* Endocytic Pathway

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. In preliminary work, we show that these mutations alter the intracellular localization of the receptors in *C. elegans*, supporting our hypothesis.

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.

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.

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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.

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SINGSON LAB

Reproductive Biology, Cell-Cell Interactions



Dr. Andrew Singson
Genetics

Summary

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

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

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

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

Sperm function

We characterized the first *C. elegans* gene (*spe-9*) that encoded a protein required for sperm function at fertilization. All other genes with a similar mutant phenotype are now 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

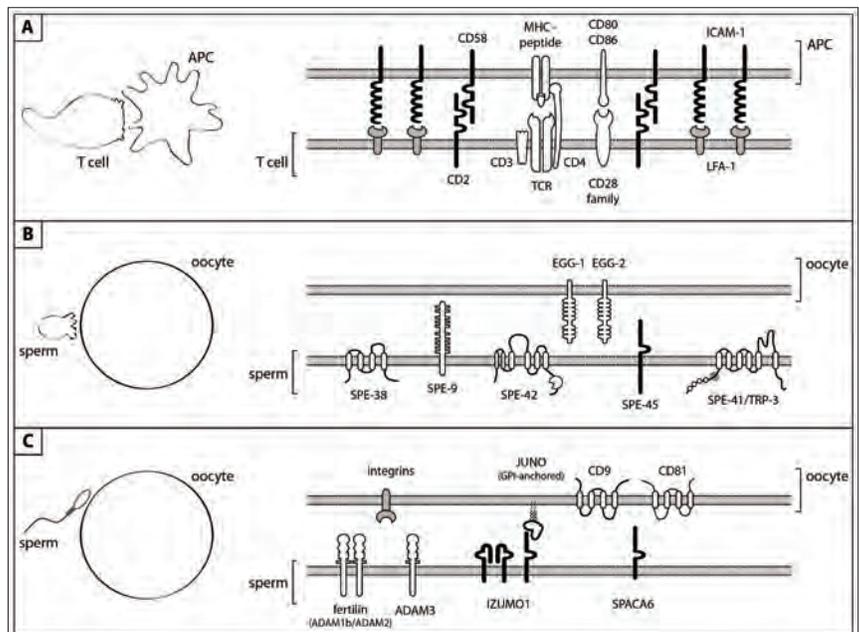


Figure 1: Molecular components of the immune and fertilization synapses. A) Components and organization of a stable immune synapse. The TCR is centrally clustered in the cSMAC, along with coreceptors and costimulatory molecules such as CD3, CD4 and CD28. This is surrounded by the pSMAC containing LFA-1. B) Proteins on the surface of sperm and oocyte genetically verified to be essential for fertilization in *C. elegans*. At present, no known binding pairs between sperm and oocyte have been identified in this system. The spatial organization of these proteins at the site of fertilization also requires further investigation. C) Proteins involved in mammalian fertilization. Izumo1 and Juno are the one sperm-oocyte binding pair identified in mammals to date. Genetic data also shows essential roles for CD9 on the oocyte and Spaca6 on the sperm. (Figure and legend modified from Krauchunas et al. 2016).

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.

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 (Figure 1).

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 a novel gene (spe-43) that is required for *C. elegans* spermiogenesis. 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.

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STEWARD LAB

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



Dr. Ruth Steward
Molecular Biology & Biochemistry

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.

To investigate if the requirement of *Zfrp8* is restricted to *Drosophila* hematopoiesis and to obtain insight into the molecular function of the gene we studied the *Zfrp8* phenotype in ovaries and found that that *Zfrp8* is essential in stem cells because both somatic and germline mutant stem cells stop dividing and are ultimately lost. We established that *Zfrp8* complexes with *Mael*, a piRNA pathway protein, and is required for *Mael* localization within GSCs. While *Zfrp8* has weaker effects on transposon expression than *mael* it causes stronger phenotypes especially in stem cells, suggesting that *Zfrp8* has functions in addition to the regulation of transposons.

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.

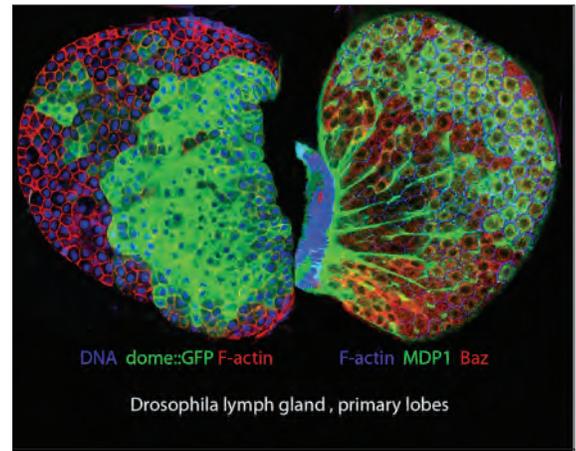


Figure: The *Drosophila* lymph gland is a symmetrical multi lobed organ. Each gland contains two primary lobes that comprise a medulla, containing undifferentiated cells (green, on the left), and a cortex, where the differentiated cells reside (red on the left and green on the right). Individual hemocytes are shown in red on the right side. Two independently stained primary lobes are shown.

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.

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 binds 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 containing the enzyme catalytic domain, with 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, Tetnull genetically interacts with Zfrp8. Tetnull 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 yield 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 shown 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 on 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 gene is expressed primarily in embryonic and larval nerve cells and Tet mutant larvae have a smaller, disorganized brain. Studying the behavior of normal and Tet mutant larvae showed that the locomotion is strongly affected in the mutants, while the muscles look indistinguishable in the two groups. It is thus likely that the gene has an essential function in the function of nerve cells.

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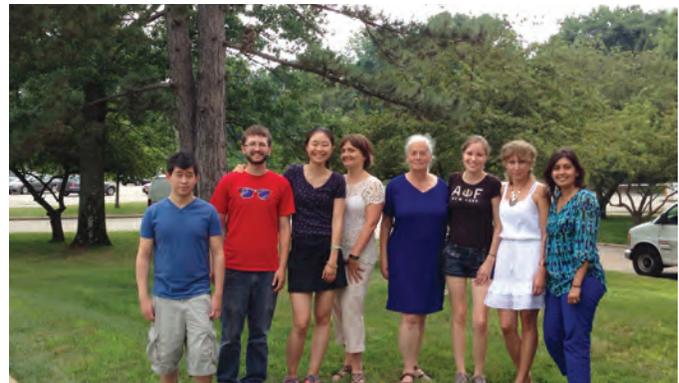
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EBRIGHT LAB

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



Dr. Richard Ebright
Chemistry & Chemical Biology

Summary

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

Structures of Transcription Complexes

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

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

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

Mechanism of Transcription

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

- (1) RNA polymerase (RNAP) binds to promoter DNA, to yield an RNAP-promoter closed complex.
- (2) RNAP unwinds ~14 base pairs of promoter DNA surrounding the transcription start site, rendering accessible the genetic information in the template strand of DNA, and yielding an RNAP-promoter open complex.
- (3) RNAP begins synthesis of RNA as an RNAP-promoter initial transcribing complex. During initial transcription, RNAP uses a "scrunching" mechanism, in which RNAP remains stationary on promoter DNA and unwinds and pulls downstream DNA into itself and past its active center in each nucleotide-addition cycle, resulting in generation of a stressed intermediate.
- (4) After RNAP synthesizes an RNA product ~10-15 nucleotides in length, RNAP breaks its interactions with promoter DNA, breaks at least some of its interactions with sigma, escapes the promoter, and begins transcription elongation as a transcription elongation complex. Energy stored in the stressed intermediate generated by scrunching during initial transcription is used to drive breakage of interactions with promoter DNA and interactions with sigma during promoter escape.

During transcription elongation, RNAP uses a "stepping" mechanism, in which RNAP translocates relative to DNA in each nucleotide-addition step. Each nucleotide-addition cycle during initial transcription and transcription

elongation can be subdivided into four sub-steps: (1) translocation of the RNAP active center relative to DNA (by scrunching in initial transcription; by stepping in transcription elongation); (2) binding of the incoming nucleotide; (3) formation of the phosphodiester bond; and (4) release of pyrophosphate.

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

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

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

Regulation of Transcription

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.

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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 410 (~1,000,000) barcoded sequences. For MASTER experiments RNA transcripts are produced from the template library *in vitro* or *in vivo* and analyzed by high-throughput sequencing to determine the sequence of transcript ends and the relative transcript yields. For MASTER-XL experiments unnatural amino acid-mediated protein-DNA photo-cross-linking (or other forms of protein-DNA cross-linking) is performed and high-throughput sequencing is used to define the location of specific regions of RNAP on each member of the template library. For MASTER-FP experiments chemical reagents that detect changes in DNA accessibility (e.g. KMnO₄, chloroacetaldehyde, DMS, hydroxyl radicals) are used to perform protein-DNA footprinting and high-throughput sequencing is used to

detect RNAP-dependent changes in DNA accessibility on each member of the template library.

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

Defining the role of endoribonuclease toxins in bacterial pathogenesis

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

Toxin/antitoxin (TA) systems are widespread in pathogens and have been implicated in virulence, survival during stress, and in promoting formation of a dormant state that is refractory to antibiotic treatment. In *M. tuberculosis*, there are greater than 80 TA systems. Furthermore, the majority of the toxins associated with these TA systems are homologues of sequence-specific 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.

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SEVERINOV LAB

Mechanisms of Transcription in Microorganisms



Dr. Konstantin Severinov
Molecular Biology & Biochemistry

Summary

Our laboratory studies transcription the central step, and a major regulatory checkpoint of gene expression. Defective transcription regulation can cause aberrant growth and development and may result in malignant transformation. Transcription is carried out by DNA-dependent RNA polymerases. Understanding RNA polymerase (RNAP) structure, function, and regulation holds the key to understanding of gene expression in molecular detail. We study the molecular basis of transcription mechanism and regulation through structure-functional analysis of bacterial RNAP, its transcription factors, and low-molecular weight inhibitors. We also study the perpetual arms race between bacteriophages and their bacterial hosts to uncover novel mechanisms of transcription regulation and various defense mechanisms (CRISPR-Cas, restriction-modification and abortive infection systems) used by the host to counter viral attacks. Finally, we study bioactive peptides that inhibit bacterial growth and may be developed as antibiotics. The

following research projects were actively pursued during the last year.

Studies of CRISPR-Cas bacterial adaptive immunity

A novel mechanism of bacterial resistance to phages -- through the action of CRISPR-Cas (Clusters of Regularly Interspersed Palindromic Repeats/CRISPR associated sequences) system -- is being studied using *Escherichia coli*, *Pseudomonas aeruginosa*, *Thermus thermophilus*, and human pathogen *Clostridium difficile* as model organisms. Highly efficient model systems of CRISPR-Cas-mediated interference with development of various bacteriophages and maintenance of plasmids have been created in our laboratory and are being used to study fine mechanistic details of CRISPR-Cas function, including the enigmatic process of CRISPR adaptation, during which new spacers derived from foreign DNA are acquired into host CRISPR-Cas arrays. Powerful in vitro methods, including fluorescent beacon assays inspired by our work with RNA polymerases are being developed to study on-site and off-site activities of CRISPR effector complexes during target selection and target destruction. We also developed, in collaboration with Eugene Koonin laboratory from NIH, a bioinformatics pipeline that allows one to predict novel, previously uncharacterized CRISPR-Cas system types. The predicted systems are then experimentally validated in collaboration with Feng Zhang laboratory from MIT. Implementation of the pipeline approach led to discovery of several new CRISPR-Cas system types, some of which have high potential for applications in genome editing and may be superior to currently used Cas9-based systems.

Structure-activity analyses of antibacterial microcins

Microcin C is a peptide-adenosine antibiotic produced by some *E. coli* strains. It is a potent inhibitor of growth of some Gram-negative bacteria. Upon entry into sensitive cells the peptide part of microcin C is processed with the release of a modified non-hydrolyzable aspartyl-adenylate that inhibits aspartyl-tRNA synthetase. During the past year a large family of bioactive microcin C-like compounds encoded by various bacteria have been identified bioinformatically and validated in vitro and in vivo. Unusual cases of microcin C-like molecules containing peptides modified with cytosine rather than adenosine were discovered. The potential of these new compounds and their genetically engineered variants to inhibit various medically significant bacteria is being investigated. The details of microcin C-like compounds biosynthesis are investigated by means of X-ray crystallography and biochemical analysis.

Microcin J is a lasso peptide that inhibits bacterial RNAP, a validated drug target. Using bioinformatics searches, operons encoding various lasso peptides have been predicted and several have been validated, leading to identification of novel antibacterial compounds with excellent potential for future development as antibiotics.

Microcin B is an oxazole-thiazole containing peptide that targets DNA gyrase. Several novel operons encoding enzymes homologous to microcin B biosynthetic enzymes have been bioinformatically predicted and validated.

The structures of new microcin-like molecules have been determined and their targets, which, unexpectedly, include bacterial ribosome, have been identified.

Structure-functional analysis of novel RNAPs and transcription factors

Genomic sequences of several novel bacteriophages have been determined and annotated. Two families of unique multisubunit RNAPs were identified in giant phages, a poorly characterized group of viruses with very large genomes. Phages of the family rely solely on viral RNAPs for transcription of their genes. Such independence from host transcription may have contributed to success of these phages, allowing them to infect a wide range of phylogenetically distant bacteria. Structural and comparative functional analysis of novel phage RNAPs is underway. Some of the new enzymes are, unexpectedly, related to RNA-dependent RNA polymerases involved in gene silencing in eukaryotes, providing an evolutionary link between gene transcription and gene silencing.

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VERSHON LAB

Transcriptional Regulation in Yeast



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Summary

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

The yeast $\alpha 2$ 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.

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DISMUKES LAB

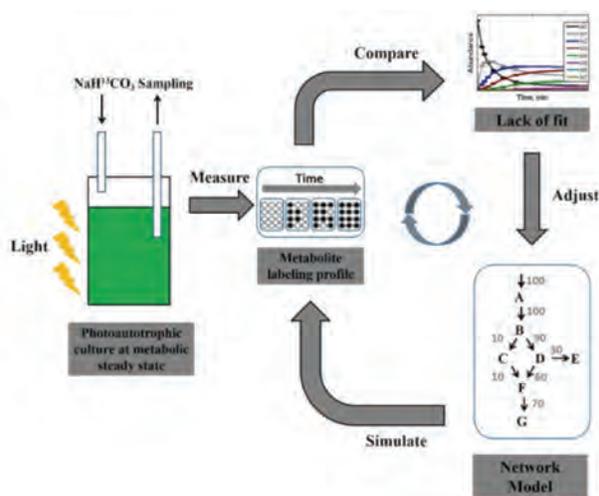
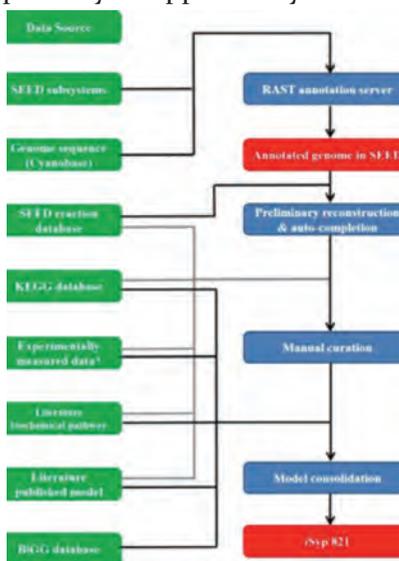
Biological and Chemical Approaches to Renewable Energy Research



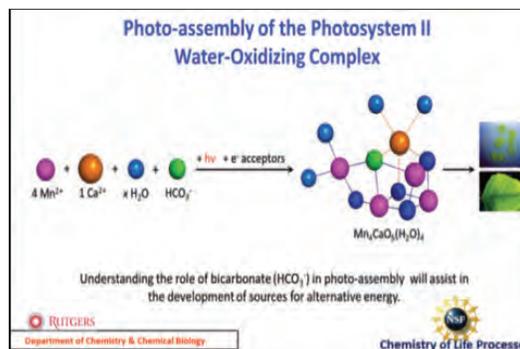
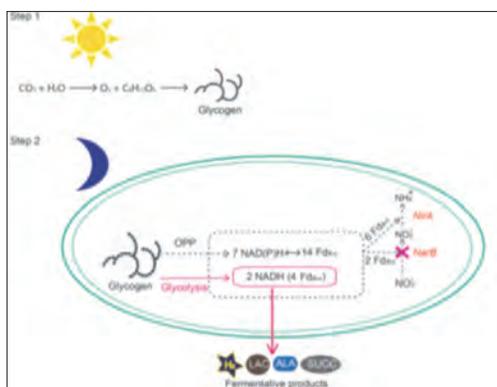
Dr. G. Charles Dismukes
Chemistry & Chemical Biology
Microbiology & Biochemistry

The Dismukes research group conducts fundamental and applied research in the areas of renewable energy production via biological and chemical systems. 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 2015-June 2016 period the group was comprised of 28 researchers (listed below).

1) **Photoautotrophic Carbon Fluxomics.** Our goal is to use flux balance analysis (FBA, left figure) and isotopically nonstationary metabolic flux analysis (INST-MFA, right 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.



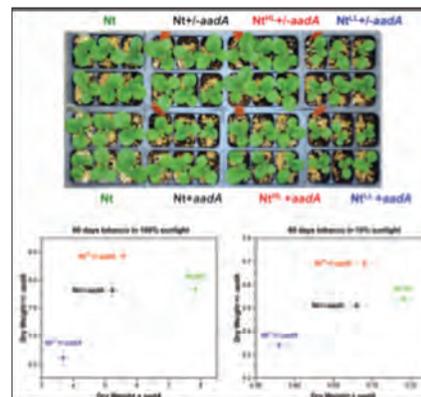
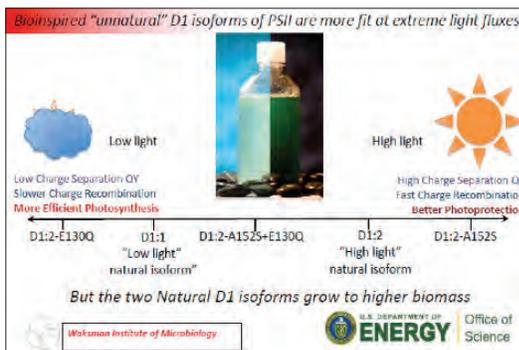
2) **Sustainable BioH₂.** Our goal is to metabolically engineer *Synechococcus* sp. PCC 7002 to reroute electrons towards the synthesis of valuable fermentative products, away from nitrate reduction in the presence of nitrate. By eliminating the narB-encoded nitrate reductase, we have significantly elevated the fermentative H₂ yield in *Synechococcus* 7002. This work is supported by DOE-MCB.



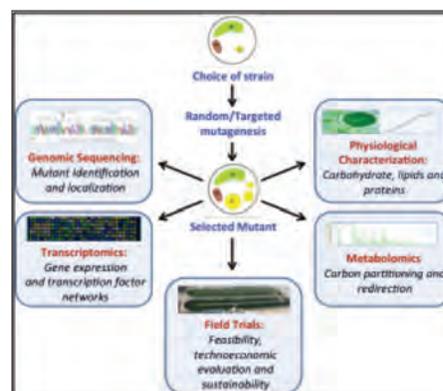
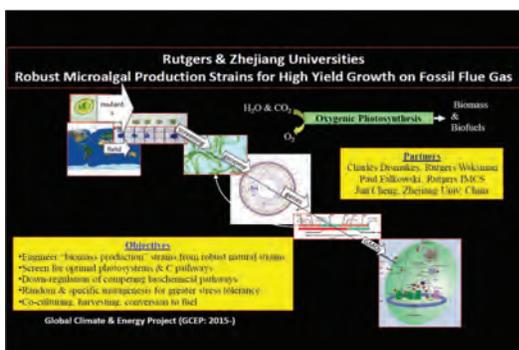
3) PSII-WOC Photo-Assembly & Inorganic Mutants. This project aims to understand the biogenesis of the oxygenic reaction center (PSII) and the functions of the inorganic components comprising its catalytic site (WOC). We do so by substitution of the inorganic cofactors (Mn^{2+} , Ca^{2+} , Cl^- , CO_3H^- , H_2O) 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.

4) Diversity of Photosynthetic Water Oxidation. 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) Optimizing PSII Photoconversion by D1 Subunit Engineering. From the two differentially expressed cyanobacterial isoforms of the D_1 -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 D_1 -PSII to incorporate the cyanobacterial phenotype for high light tolerance (graphic 2). Supported by DOE-BES.



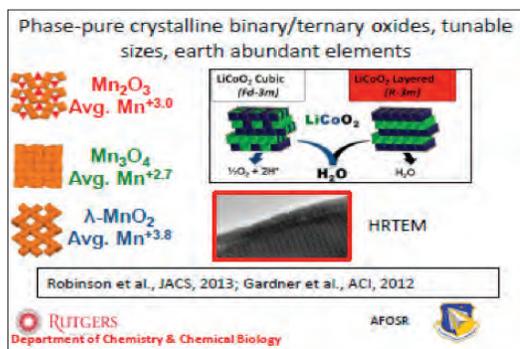
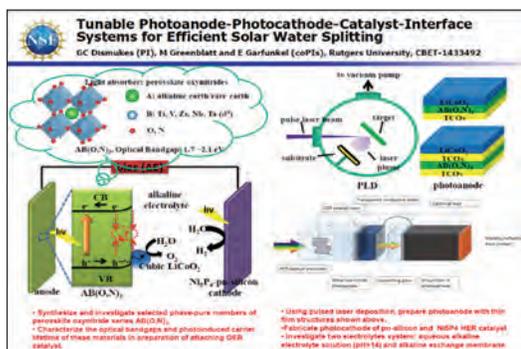
6) Sustainable Biofuels. The goal of this program is to control the metabolism of microbial photosynthetic organisms to efficiently produce biomass and fuels from sunlight and CO_2 . We apply genetic and environmental methods to modify the metabolic pathways to reroute the flux of fixed carbon and extract H_2 or carbon fuel precursors. We are collaborating with Christoph Benning's lab (Michigan State U.) to generate potential CO_2 -tolerant strains of *Nannochloropsis* by random mutagenesis combined with high throughput screening, as well as targeted genetic engineering of selected genes involved in fatty acid biosynthesis, TAG assembly and TAG catabolism pathways (graphic 4 & 5). Supported by the Global Climate & Energy Project.



7) Electrocatalysts for Water Splitting and CO_2 Reduction. Our goal is to apply the principles learned from enzymes to synthesize better catalysts for the generation of H_2 and O_2 from water and CO_2 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. Supported by the DOE-EERE-SBIR program (see graphic 6) and by the Pray Family Fund.

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



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DONG LAB

Cell Polarity and Asymmetric Division in Plants



Dr. Juan Dong
Plant Biology & Pathology

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. Extensive studies in animal systems have revealed a set of conserved proteins that trigger cell polarization and differentiate daughter cell fates. 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.

The formation and patterning of stomata (pores on the plant epidermis that regulate CO₂ and H₂O exchange with the atmosphere) proceeds via a series of asymmetric divisions. These divisions are required for stem cell maintenance, cell fate specification, and overall patterning. BASL was first demonstrated to regulate asymmetric cell divisions by its mutant phenotype; BASL's striking sub-cellular polarization in the stomatal lineage cells (Figure 1), however, was the key data that suggested a molecular mechanism for the creation of plant cell polarity. 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.

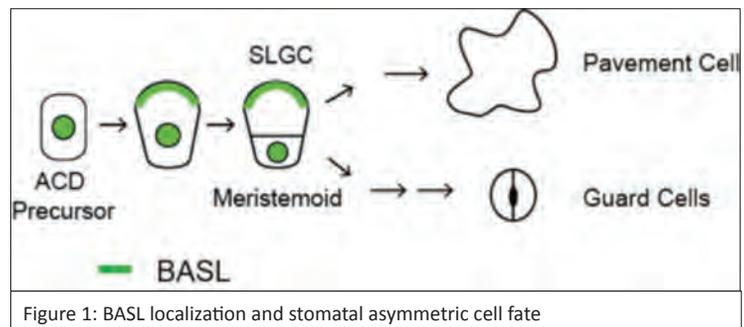


Figure 1: BASL localization and stomatal asymmetric cell fate

Regulatory mechanisms for BASL polar trafficking

The molecular mechanisms for protein polarization in plants have been extensively studied in two systems: 1) vesicular trafficking-based polarization of PIN auxin efflux carriers (membrane embedded proteins) and 2) cytoskeleton-dependent and -independent positive feedback loop-based ROP polarization (small Rho-like GTPases from plants). 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

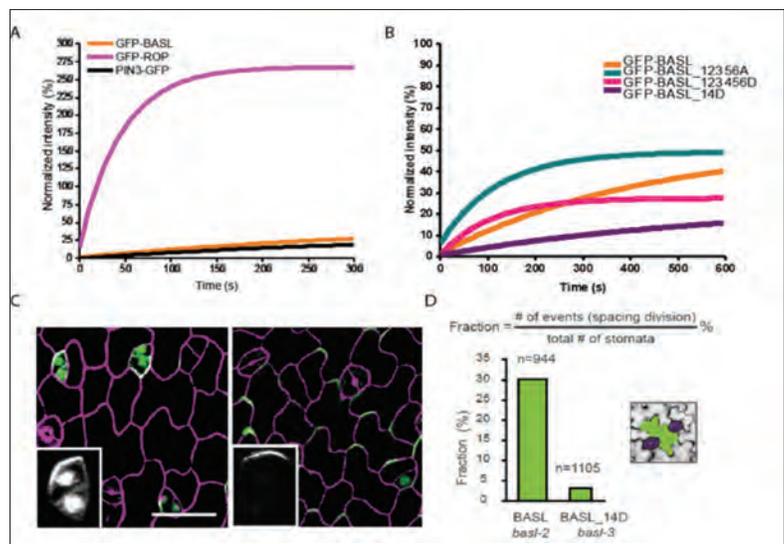


Figure 2: Fluorescence recovery of BASL at the polarity crescent. (A) Normalized FRAP curves for GFP-BASL, GFP-ROP2 and PIN3-GFP in the stomatal lineage cells. (B) Comparison of FRAP curves of BASL and phospho-variants. BASL_12356A indicates 5 Ser sites mutated to Ala (phospho-deficient). BASL_123456D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

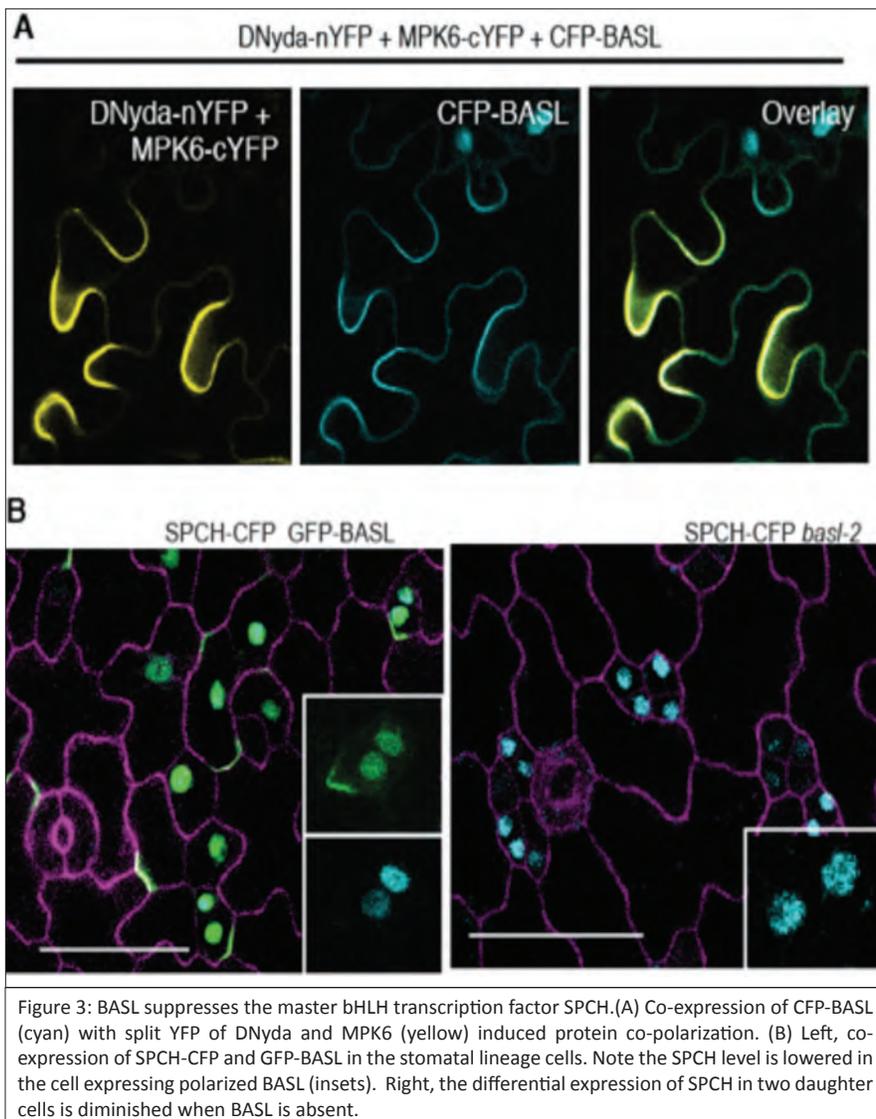


Figure 3: BASL suppresses the master bHLH transcription factor SPCH. (A) Co-expression of CFP-BASL (cyan) with split YFP of DNya and MPK6 (yellow) induced protein co-polarization. (B) Left, co-expression of SPCH-CFP and GFP-BASL in the stomatal lineage cells. Note the SPCH level is lowered in the cell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

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 expression in the nucleus

Previous, we demonstrated that a canonical MAPK signaling pathway, including the MAPKKK YODA (YDA) and MAPK₃ and 6 (MPK_{3/6}), is spatially concentrated by BASL polarization (Figure 3A). As SPCH is a direct target of MPK_{3/6}, phosphorylated SPCH is subject to protein degradation and low activity. When GFP-BASL was crossed with SPCH-CFP, we found that the cells expressing polarized BASL showed reduced accumulation of nuclear SPCH, likely due to the enriched MAPK cascade in the polarized cells (Figure 3B). Conversely, in the absence of BASL, the differential expression of SPCH in two daughter cells was abolished (Figure 3B). Therefore, we provided a direct link between BASL and SPCH to interpret daughter fate differentiation in stomatal ACD.

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

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DOONER LAB

Plant Genetics



Dr. Hugo Dooner
Plant Biology & Pathology

A sequence-indexed reverse genetics resource for maize

This past year, we continued our NSF-funded long term work to develop a user-friendly reverse genetics resource for maize based on the transposable elements Ac and Ds. Such a resource is highly desirable 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. Over 6000 Dsg insertions have been mapped to the reference genome with Insertion Mapper, a publicly available pipeline developed by our collaborators Drs. Charles Du and Wenwei Xiong at Montclair State University. 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.

Figure 1: Access to our Dsg resources at <http://acdsinsertions.org>

We are also creating a genome-wide gene knockout resource for the community consisting of 120 roughly equidistant Ds* launching platforms carrying GFP. 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. Eighty-six platforms have already been mapped to all 20 chromosome arms of the maize reference genome.

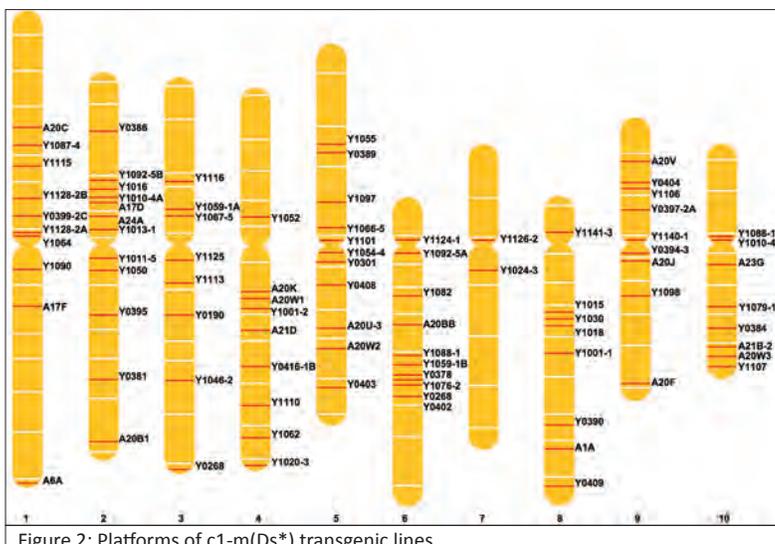


Figure 2: Platforms of c1-m(Ds*) transgenic lines.

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. Eighty-six platforms have already been mapped to all 20 chromosome arms of the maize reference genome.

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GALLAVOTTI LAB

Mechanisms of Plant Development



Dr. Andrea Gallavotti
Plant Biology & Pathology

Summary

In stark contrast to animal development, plant development mainly occurs after embryogenesis. This ability is provided by small groups of stem cells called meristems that are continuously formed and maintained throughout vegetative and reproductive 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 and activity of meristems, focusing in particular on a class of meristems, called axillary meristems, that are responsible for the formation of branches and flowers in plants (Figure 1). 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) co-repressors. The auxin-dependent degradation of Aux/IAs frees interacting activating ARF transcription factors from TPL repression, allowing them to activate downstream genes. Aux/IAs and ARFs belong to large families of transcriptional regulators whose combinatorial interaction is believed to trigger specific developmental responses.

We are part of a 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 degree of functional conservation of genes and pathways regulating auxin function among different plant species (auxinevodevo.org).

In maize disruption of the biosynthesis, transport or signaling of auxin causes several developmental defects, most notably the absence of branches and flowers in tassels and ears. Using forward genetic screens, we have identified and characterized several mutants affected in inflorescence development that are impaired in auxin function. Among these, Barren inflorescence₁ (Bif₁) and Barren inflorescence₄ (Bif₄) are two novel semi-dominant maize mutants that are severely impaired in reproductive organogenesis. BIF₁ and BIF₄ encode Aux/IAA proteins that function synergistically to initiate the many specialized types of reproductive axillary meristems that form the highly complex inflorescences of maize. Our findings showed that both genes are core components of the auxin signaling pathway for the initiation of reproductive primordia. We are also characterizing several ARF genes expressed in maize inflorescences by a combination of expression and molecular analysis, reverse genetic and genomic approaches.

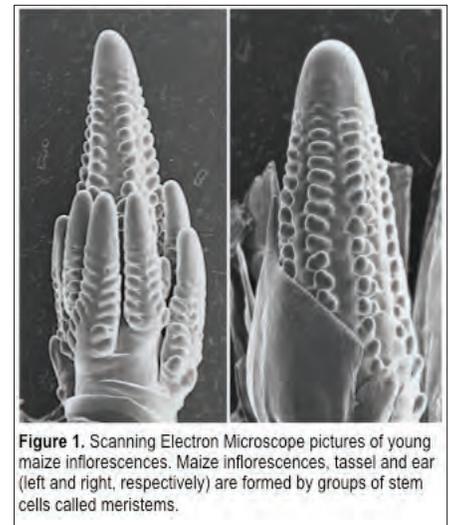


Figure 1. Scanning Electron Microscope pictures of young maize inflorescences. Maize inflorescences, tassel and ear (left and right, respectively) are formed by groups of stem cells called meristems.

Transcriptional repression in maize 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 co-repressor 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 new grant from the Developmental Systems cluster of the National Science Foundation.

REL2 is recruited by an array of transcription factors containing a specific repressor motif (EAR motif) to repress the transcription of their target genes. Using protein-protein interaction screens we uncovered a large number of interacting partners that form excellent candidates for REL2-mediated repression in different developmental pathways. We are currently characterizing a number of these pathways involved in inflorescence development, as well as the downstream targets of these REL2-containing repressor complexes by using several complementary genomic 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 recently 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 six additional boron transporters in the maize genome (RTE2-RTE7). By a combination of molecular and genetic approaches we are further investigating their role in the uptake of boron from the soil and its distribution to the shoot. 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.

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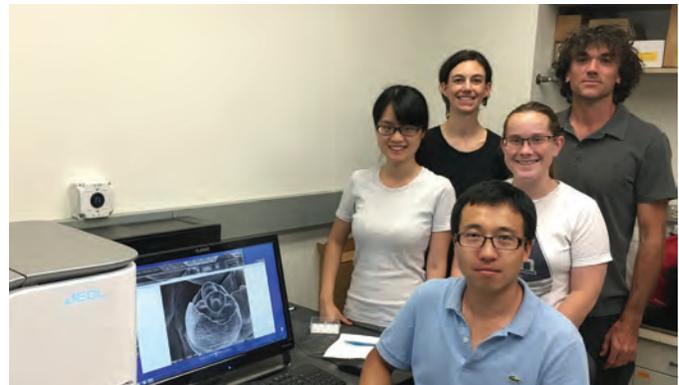
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MALIGA LAB

Plastid Molecular Genetics



Dr. Pal Maliga
Plant Biology & Pathology

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.

Lincosamide resistance genes for efficient transformation of the plant nucleus

The feasibility of selecting plastid-encoded 23S rRNA mutants by lincosamide resistance in *Nicotiana plumbaginifolia* suggests that genes detoxifying lincosamide antibiotics may be suitable markers for plastid transformation. To identify a suitable detoxifying enzyme, we expressed seven lincosamide resistance genes from the nuclear genomes of tobacco (*Nicotiana tabacum*). The *lnu* genes were cloned into a constitutive nuclear expression cassette in the T-DNA region of the pPZP200A *Agrobacterium* binary vector. Cocultivation of *Agrobacterium* with tobacco leaves, followed by plant regeneration on a medium containing lincomycin and clindamycin yielded transgenic events at a high efficiency. The transgenic events were recognized as green shoots and callus on leaves bleached by the antibiotic treatment. Nuclear integration of the resistance genes was confirmed by GFP accumulation in chloroplasts from a linked, plastid-targeted *gfp* gene and Mendelian segregation of antibiotic resistance in the seed progeny. The lincosamide resistance genes also yielded transgenic events in *Arabidopsis thaliana* using the floral dip transformation protocol and cocultivation of potato leaf disks with *Agrobacterium* carrying the binary vectors. The new lincosamide resistance marker genes will be useful additions to the toolkit of plant biologists. We are now exploring the feasibility of using lincosamide resistance as a selective plastid marker.

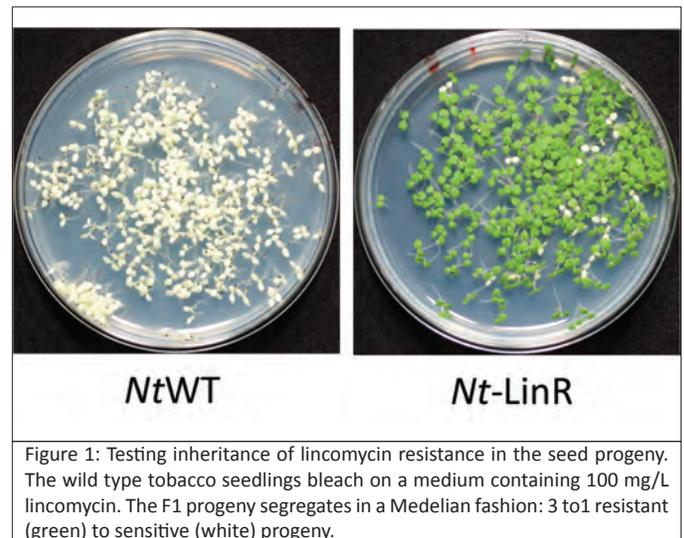


Figure 1: Testing inheritance of lincomycin resistance in the seed progeny. The wild type tobacco seedlings bleach on a medium containing 100 mg/L lincomycin. The F1 progeny segregates in a Mendelian fashion: 3 to 1 resistant (green) to sensitive (white) progeny.

Progress in implementing plastid transformation in *Arabidopsis thaliana*

Arabidopsis thaliana, an important model plant species, is recalcitrant to plastid transformation. To enable early identification of transplastomic events, we developed a novel marker system that is selectively expressed in chloroplasts. The new marker system has been successfully deployed in *Arabidopsis* that is now as efficient in plastid transformation as tobacco. The availability of plastid genome engineering will open up the unique genomic resources available in *Arabidopsis* for studies of plastid-nucleus interactions and improving crop productivity by engineering the photosynthetic machinery.

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

We used 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 (>20x) dynamic range between baseline and induced rates can be obtained by the combination of mRNA stabilization and enhanced translation mediated by PPR₁₀ binding. These experiments are carried out in collaboration with Prof. Alice Barkan, Oregon State University, Eugene, OR.

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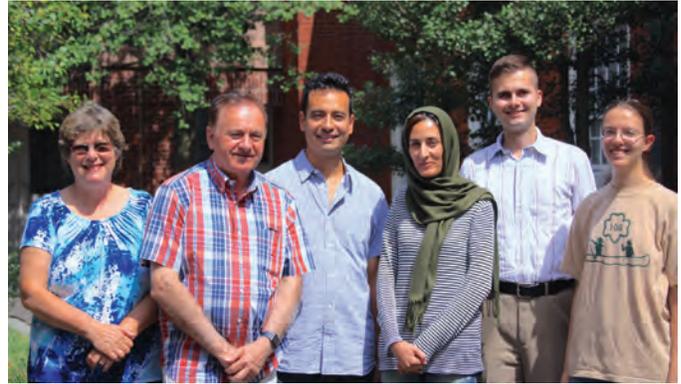
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MESSING LAB

Molecular Biology of Plant Development



Dr. Joachim Messing
Molecular Genetics

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, (see institute's web site). Publications during the last academic year reported in most of these categories.

Bioenergy, Shotgun DNA Sequencing, Genome Evolution, Genome Structure

We have taken a genomics approach to study how we can sustain a healthy environment. Plants over time have shown a remarkable adaptation to a changing environment, which is reflected in its genome structure. One example is a group of aquatic plants, generally known as duckweeds.

This family of species have many ecotypes that grow in many geographically locations on slow streaming waters and ponds. We had selected *Spirodela polyrhiza* for generating the sequence of a reference duckweed genome with support of the Joint Genome Institute (JGI) of the Department of Energy a year before because it had the smallest genome size of duckweeds with 158 Mb. In the absence of a genetic map, 32 pseudomolecules were constructed from a whole genome shotgun sequencing experiment using mainly the older "454" sequencing instruments. However, chromosomal spreads confirmed that there are only 20 chromosomes. Therefore, we undertook last year the alignment of the assembled sequences with these 20 chromosomes in collaboration with the Gatersleben Cytogenetics group in Germany. We selected bacterial artificial chromosomes (BACs) from a genomic library of *Spirodela polyrhiza* that was sequenced at the ends of the inserted DNA. These BAC ends were aligned with the assembled sequence to reconstruct their full-length sequence. The purpose of this was to select 100 BACs that were low in DNA sequences repeated throughout the genome. These filtered clones were then used in multi-color Fluorescence-In-Situ-Hybridization (mcFISH) to identify junctions in the 32 pseudomolecules of incorrectly joined sequence scaffolds. This resulted in an improved reference genome for duckweeds aligned with its chromosomes. Such data will be critical in studying polyploidy and segmental duplications in this family of species and how this could have been the result of adaptation.

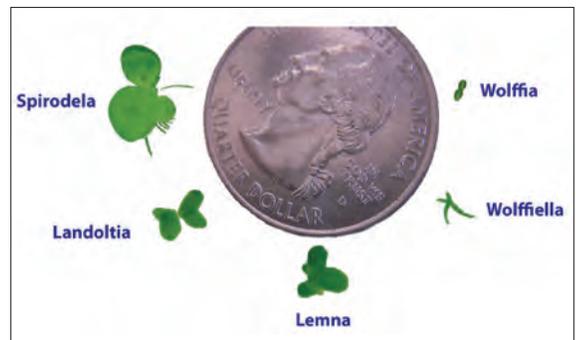


Figure: The Lemnoideae (duckweeds) consists of five genera that differ in their size compared to the American quarter. Their compact structures are called a frond that propagates by budding, enabling the plant to grow very rapidly.

Another important genomics character of the species is the expansion and contraction of gene families. As an example, we studied the genes responsible for RNA editing. Some transcripts that are produced in the organelles of plants cannot be directly translated and have to be edited by substituting single nucleotides so that the correct codon is restored. This editing function, however, is not carried out with proteins encoded in the organelle but the nuclear genome. These proteins are known as Pentatricopeptide-repeat proteins (PPRs). Taking advantage of the sensitivity of next generation sequencing of cytoplasmic and organellar cDNA permitted us to identify variable levels of RNA editing efficiencies. *Spirodela* appears to have an expansion of the PPR gene family, whereas the total gene number is reduced by 50% compared to rice, another monocot species, indicating that the RNA editing function has expanded in this family of species that represent the fastest growing plants on earth.

Gene expression and evolution of seed proteins

An important aspect of gene expression is DNA modification and chromatin structure. We have taken advantage

of next generation sequencing methods to study at the genome-wide level the acetylation of histone 3 at lysine residue #27 of maize because it has been implied in the activation of gene expression. Maize seems to be in particular suited for this purpose because the portion of the genome representing active genes is rather small. The maize genome has an even higher percentage of repeat elements than the human genome, 85% versus 50%. Therefore, detection of such epigenetic marks in maize had to be highly enriched for a fraction of the genome. Indeed, this specific histone acetylation correlated well with transcribed genes and could be verified with many known examples of genetic loci.

A more specific study was concerned with the maize seed storage proteins. The genes encoding a particular subgroup, known as the alpha zeins, are located in six chromosomal locations. In five of them, they are organized as tandem gene clusters. These genes are expressed during seed development and serve as a reservoir of reduced nitrogen during dormancy. For the plant life cycle these proteins are important for germination of the seed and early plant development. For agriculture, they are an important source of nutrition. Transcriptional analysis of seed development has shown that many of them are not expressed despite a normal gene structure. That has prompted us to investigate how epigenetic silencing could play a role in repressing gene expression. We applied bisulfite DNA sequencing, which can detect the methylation of DNA sequences. The methylation pattern of specific gene clusters can then be related to the transcriptional activity of them during seed development. There were several findings. 1) Genes have a higher degree of methylation in non-expressing tissues, which is

consistent with the observation that active genes are hypomethylated. 2) The difference in methylation, however, can differ between chromosomal locations. 3) Culturing endosperm, which keeps cells dividing, not only reduces DNA methylation, but also can lead to transcription of genes that were previously inactive.

Physical properties of storage proteins are critical for an important food item, bread. Cereal seeds are milled to make flour and the flour quality makes the dough rise and gives bread its well-known property. The type of food that is consumed has adapted to the evolution of these proteins because not all cereals can be used to make bread, giving wheat a special position in agriculture. To better understand the divergent properties of these proteins, we investigated a species that takes an intermediate position in the phylogeny between wheat and maize, which cannot substitute for bread making quality of wheat. This species is called teff and is mainly consumed in Ethiopia. Although it is closer to maize than to wheat because it also has alpha prolamins that are present in maize but not in wheat, it has more ancient proteins that can restore the bread making properties lost in maize. The type of bread baked in Ethiopian restaurants is called injera.

Overviews

Several subjects have been reviewed in the last year. In a book for agricultural applications we have reviewed the status of what we know and do not know about the protein traits in maize. We also wrote up a protocol for the use of long single molecule sequencing of cDNAs. In a minireview, I described how bacteriophage M13 accidentally emerged as a potential remedy of Alzheimer and Parkinson disease.

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CORE FACILITIES

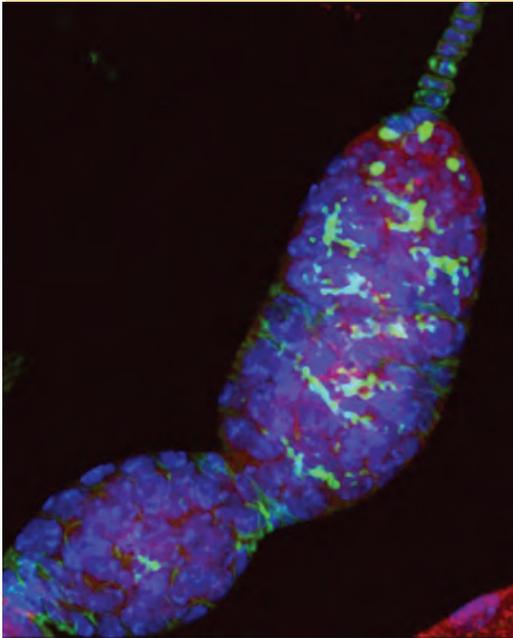


Image: McKim

Waksman Confocal Imaging Core Facility

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

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

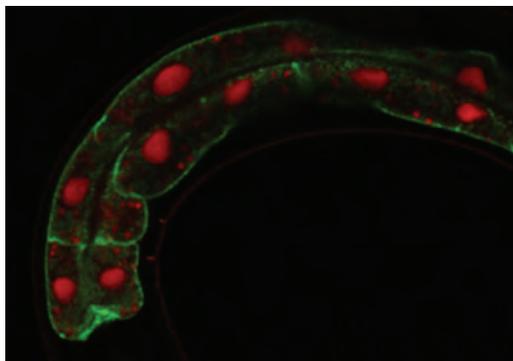


Image: Lin

The Waksman Core Facility has approximately 40 trained users, primarily Waksman researchers, from twelve laboratories and is used an average of 65 hours per week. The Confocal Manager provides training, troubleshooting, and consultation on the use of our confocal microscopes. The future aim of the facility is to continue to provide exceptional imaging capabilities to Waksman researchers.

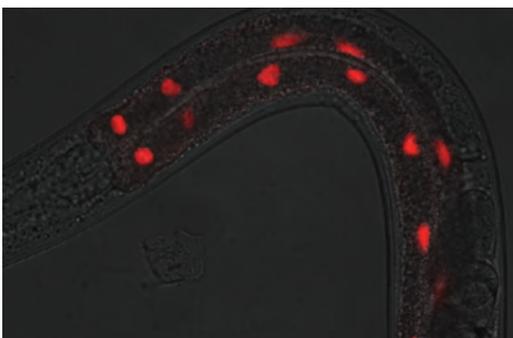


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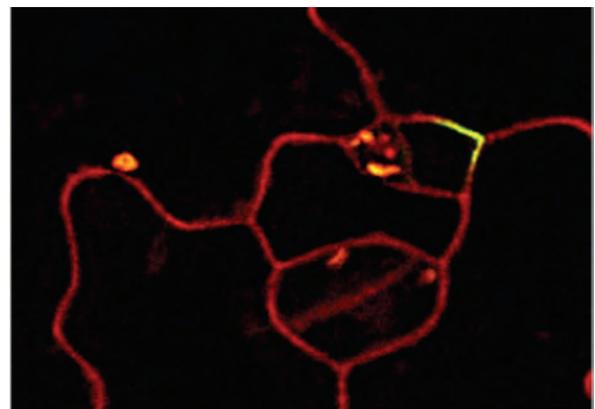


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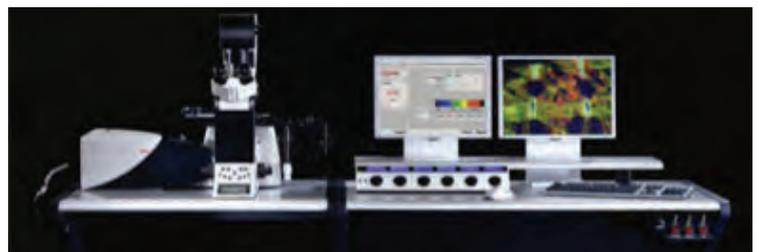
Nanci S. Kane, Manager

Laboratory Researcher IV

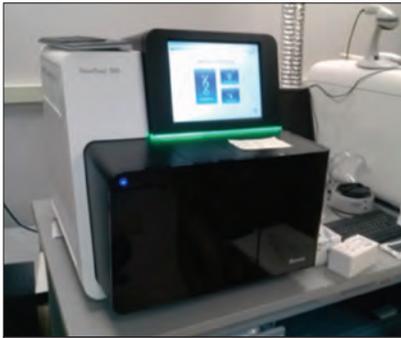
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Waksman Genomics Core Facility



NextSeq500



MiSeq



IonProton

Waksman Genomics Core Facility (WGCF) is a state-of-the-art laboratory facility, providing high-throughput next generation sequencing services to the Rutgers research community and to the broader scientific community. Waksman institute is one of the earliest adopters of sequencing technology with extensive experience in NGS sequencing. Since 2008, core offers single read, paired-end, and multiplex sequencing using various Next Generation Sequencers. One of the main driving force is to provide latest sequencing service across the entire spectrum of user ranging from highest throughput sequencing to small sequencing need. WGCF has three sequencers covering broad range of NGS requirements. The first one is NextSeq500, Illumina's newest 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 upto 16 exomes in a single run. Whereas, our second instrument, 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. Semiconductor-based sequencer Ion Proton is latest and third gem to our facility. Ion Proton does not use fluorescence or chemiluminescence as with other platforms; Instead, it measures the H⁺ ions released during base incorporation. The absence of optics has allowed the machine to use inexpensive consumables thus reducing sequencing cost. Further, Proton's short runs time of four hours allowed us to reduce the turnaround time drastically.

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

contracts cost and consumable reagents expense. WGCF accepts raw samples as well as prepared libraries, giving researcher's flexibility to try their own protocols. In addition to sequencing, WGCF also offers Real-Time PCR on Thermo Fisher's StepOnePlus system, DNA shearing services using Covaris, as well as DNA qualification services using fluorometer Qubit, NanoDrop and Agilent bioanalyzer.

In addition to the wet-lab services, Waksman Genomics Core Facility also provides powerful, accessible and intuitive analysis tools through its bioinformatics consulting and data analysis services. This allows researcher to bypass learning curve and focus on the result that are both statistically and biologically meaningful. 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	Ion Proton	MiSeq	Pacbio RS II*
Whole Genome Resequencing	✓✓✓✓	✓✓	✓	✓✓✓✓
De novo sequencing	✓✓✓✓	✓✓✓	✓	✓✓✓✓
Human Exome Sequencing	✓✓✓✓	✓✓✓		
Target Resequencing	✓✓	✓✓✓	✓	
mRNA Sequencing	✓✓✓✓	✓✓✓		✓✓
Small RNA Sequencing	✓✓✓	✓✓✓		
ChIP Sequencing	✓✓✓✓	✓✓✓		
Metagenome analysis	✓	✓✓	✓	

**PacBio RSII is offered through collaborative agreements with other sequencing units*

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

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Dr. Brian Gelfand, Laboratory Researcher
 Dr. Yaping Feng, Bioinformatics Research Scientist/
 Research Associate
 Dr. Min Tu, Bioinformatics Research Scientist/
 Postdoctoral Fellow



Waksman Greenhouse



Our state of the art Greenhouse facility features 4,200 square feet of growing space, divided into two rooms topped by a roof structure with a height of 14 feet at the truss to better enable the proper growth of corn plants. This facility replaced the earlier Waksman greenhouse, which was originally constructed in 1986.

Two identical, yet independently controlled (heating, cooling, shade curtain, growth lights, roof vent) greenhouse bays are connected by an adjoining headhouse, which includes an outdoor weather station (providing sensor inputs for precipitation detection, solar readings, temperature, humidity, wind speed, wind direction) make up our state of the art Greenhouse facility. The facility, completed in 2006, features a total of 4,200 square feet of growing space to better enable the proper growth of corn plants. Insect Netting, a cost-effective and environmental-friendly alternative to the use of pesticides, is used as a physical barrier to block out insects from the facility to maintain a purely organic environment. This newer construction replaced the original, smaller Waksman greenhouse constructed back in 1986, featuring 3,600 square feet of growing space divided in six rooms and a roof structure only 7.5 feet at the truss.

Marc Probasco, Greenhouse Supervisor

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Cell and Cell Products Fermentation Facility

The Cell and Cell Products Fermentation Facility is dedicated to providing affordable fermentation services to our highly diverse client base through, “fee for service” agreements.

The facility staff conduct research and development primarily; process scale-up, optimization, characterization and purification of biologics for; academic institutions, biotech firms and companies representing the pharmaceutical, food-flavor and cosmetic industries.

The facility also serves as a production unit and an incubator for virtual companies. All materials are produced or purified via cGLP/cGMP guidelines for the production of ‘Preclinical Biologics’. We do not ferment, ‘gray area or opportunistic’ organisms.

Individual projects are conducted under CDA/NDA agreement and therefore confidential.

The facility’s bioreactors range from 40 to 1000 liters. New purchases include two Eppendorf BioFlo 510 bioreactor systems, which have a maximum working volume equal to 30 liters.



125 Liter Bioreactor



1000 Liter Tank



23 Liter Bioreactor

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TRAINING FUTURE LEADERS

Postdoctoral Fellowships

Predocctoral Fellowships

Graduate & Undergraduate Courses

High School Outreach

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. We showed that a promoter element upstream of the TSS region, the “discriminator element,” participates in TSS selection. To define the mechanism, we combined transcription start site mapping of transcripts generated on a high-complexity promoter library containing over 4^{10} promoter variants (MASTER-N₁₀) with unnatural-amino-acid-mediated protein-DNA crosslinking (MASTER N₁₀-XL), enabling us to determine simultaneously, for the library of 4^{10} promoter sequences, the TSS, the position of the RNA polymerase (RNAP) leading-edge, and the position of the RNAP trailing-edge. We observed that, as the position of the TSS changed, the position of the RNAP leading edge changed in lock-step register, but the position of the RNAP trailing edge did not change. We proposed that changes in TSS selection result from changes in DNA scrunching.

CHARLES AND JOHANNA BUSCH FELLOWS

Predoctoral Research



Massimo Bosacchi
Maliga Lab

Plastid Genotyping Reveals the Uniformity of Cytoplasmic Male Sterile-T Maize Cytoplasm

Cytoplasmic male-sterile (CMS) lines in maize (*Zea mays*) have been classified by their response to specific restorer genes into three categories: cms-C, cms-S, and cms-T. A mitochondrial genome representing each of the CMS cytotypes has been sequenced, and male sterility in the cms-S and cms-T cytotypes is linked to chimeric mitochondrial genes. To identify markers for plastid genotyping, we sequenced the plastid genomes of three fertile maize lines (B37, B73, and A188) and the B37 cms-C, cms-S, and cms-T cytoplasmic substitution lines. We found that the plastid genomes of B37 and B73 lines are identical. Furthermore, the fertile and CMS plastid genomes are conserved, differing only by zero to three single-nucleotide polymorphisms (SNPs) in coding regions and by eight to 22 SNPs and 10 to 21 short insertions/deletions in noncoding regions. To gain insight into the origin and transmission of the cms-T trait, we identified three SNPs unique to the cms-T

plastids and tested the three diagnostic SNPs in 27 cms-T lines, representing the HA, I, Q, RS, and T male-sterile cytoplasm. We report that each of the tested 27 cms-T group accessions have the same three diagnostic plastid SNPs, indicating a single origin and maternal cotransmission of the cms-T mitochondria and plastids to the seed progeny. Our data exclude exceptional pollen transmission of organelles or multiple horizontal gene transfer events as the source of the mitochondrial *urf13-T* (unidentified reading frame encoding 13-kD cms-T protein) gene in the cms-T cytoplasm. Plastid genotyping enables a reassessment of the evolutionary relationships of cytoplasm in cultivated maize.



Jing Lin
Padgett Lab

Research Summary

Transforming growth factor- β (TGF- β), which is the prototype of TGF- β super family, plays a pivotal role in cellular proliferation, differentiation and apoptosis. The over 30 years' research on this super family makes it very clear that TGF- β signal transduction activation depends on its kinase receptors, type I and type II, which will form a heteromeric receptor complex upon ligand binding. Many diseases are associated with the abnormal function of TGF- β signaling pathway, including the autosomal heritable connective tissue disorder Marfan syndrome (MFS) and MFS-like diseases. It is found that one single missense mutation in either TGF- β type I or type II receptor can result in MFS/MFS-like disease and a large quantity of missense mutations are screened from patients. Since many mutations are located in the kinase domain, it was first thought these mutations inactivate the kinase activity in receptors causing the above diseases. However, recent studies have shown that mutant TGF- β receptors still retain kinase activity.

This gives rise to the question, how do these mutations cause MFS/MFS-like diseases? TGF- β signaling also depends on the endocytosis of receptors. There are endocytosis motifs defined in kinase domain in both type I and type II receptors. Interestingly, many MFS/MFS-like disease-associated mutations reside in these endocytosis motifs. With all information collected, we hypothesize that the abnormal function of TGF- β signaling in these mutants is caused by the abnormal endocytosis of the TGF- β receptors. To test this model we examine the genes, *sma-6* and *daf-4*, which encode type I and type II receptors in *C. elegans* respectively. In every DAF-4 mutant line, the mutant receptors are delivered to the apical surface of intestine cells. This phenotype demonstrates that these MFS-associated mutations in endocytosis motif disrupt the distribution pattern of receptors, which may have an effect on TGF- β signaling.



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 particularly for neurons, which have high-energy demands and do not store glycolytic reserves, relying instead on mitochondrial oxidative phosphorylation to meet these demands. In addition to energy production, mitochondria mediate processes as diverse as sugar and fatty acid metabolism, steroid and lipid synthesis, calcium homeostasis, and apoptosis. Given their critical role in cellular physiology, mitochondrial dysfunction contributes to the etiology of multiple diseases and disorders.

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. 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, I am performing 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, from this screen I have identified several mutants displaying either elongated mitochondria in neuronal axons, indicative of a defect in mitochondria fission, or fewer mitochondria present in the neuronal axons, suggesting a transport defect. I am in the process of mapping and characterizing these mutants. Once we identify the underlying genes of these mutations we can further understand what cellular process they are involved in and if how these gene mutations regulate mitochondria in neurons.



Wanchen Shao
Dong Lab

*The microtubule-associated protein YIP1 confers a negative regulation on the polarity positive feedback loop in *Arabidopsis* stomatal development*

Cell polarization is a fundamental feature of almost all cellular organisms. The asymmetric distribution of intracellular structures and functions within a cell plays critical roles in plant development, growth and responses to environmental stimuli. Our previous work established a positive feedback loop between the polarity protein BASL (BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE) and the YODA MAPK pathway, the components of which constitute a novel polarity complex at the cell cortex to regulate stomatal asymmetric cell division (Zhang et al., 2015). But how this positive feedback loop is balanced by restriction signals to maintain a proper level remains unknown. Here we identified a novel YODA Interacting Protein 1 (YIP1) that exerts a negative regulation on the BASL-YODA polarity module. Phenotypic analyses of the loss-of-function *yip1* and overexpression of YIP1 suggested that YIP1 mediate protein turnover of the cortical

polarity complex of BASL-YODA. Furthermore, the microtubule association of YIP1 is required for its function. Recent progress also suggest that YIP1 interacts with the components functioning in endomembrane trafficking. This set of data for the first time suggested that the membrane associated BASL-YODA polarity complex is negatively modulated by the endomembrane system.



Malan Silva
Barr Lab

α -tubulin isotype orchestrates ciliary microtubule architecture, IFT kinesin coordination, and extracellular vesicle biogenesis

Microtubules are crucial for many essential cellular processes including cell division and migration, intracellular transport, neuronal morphogenesis, and ciliogenesis¹⁻³. Microtubules are comprised of α - and β -tubulin heterodimers and can form several types of architectural conformations: singlets, doublets and triplets. In mammals, there are 10 α -tubulin isotypes and 9 β -tubulin isotypes known to date. The multi-tubulin hypothesis and the tubulin code - combinatorial use of tubulin isotypes and tubulin post translational modifications - propose that utilization of particular tubulin isotypes specifies microtubule structure, motor-based transport, and cellular function⁵. This work demonstrate our discovery of a third type of microtubule architecture: a splayed doublet, and show that it requires a specific α -tubulin and plays a critical deterministic role in defining the shape and function of a conserved eukaryotic organelle, the cilium.

Cilia are a cell's sensory and, sometimes also motile, organelles that are conserved and omnipresent, but diverse in appearance⁶. All cilia contain a microtubule core that consists of circularly arranged microtubules that are doublets at the base of the cilia and form singlets at the distal end. Ciliary morphological and functional diversity is in part achieved by modification of the conserved microtubule core: the axoneme. Thus, cilium presents a good model to investigate how combinatorial use of tubulins and posttranslational modifications of tubulins generate microtubules with unique structure and function.

We discovered that *C. elegans* cephalic male (CEM) cilia contain novel microtubule architecture in which both A- and B- tubules of the doublet microtubules splay to form singlets. B-tubules in the splayed doublets display "C"-shaped architecture previously only observed *in vitro*. We found that α -tubulin TBA-6 is required for this microtubule architecture. In *tba-6* mutant background, microtubule doublets do not splay to form A- and B-tubule singlets. Instead, as doublet microtubules become singlets in the distal cilium, only A-tubules continue. Thus, the microtubule architecture of CEM cilia in *tba-6* animals resemble that of *C. elegans* amphid channel cilia^{7,8}. Loss of *tba-6* also alters CEM cilia curvature, ciliary shape, and localization of some ciliary proteins. We found that *tba-6* is required for the wild type velocity and coordination microtubule motors that transport ciliary cargo, as well as for the composition and bioactivity of extracellular vesicles released by CEM cilia.

We conclude that α -tubulin TBA-6 is a structural component of ciliary microtubule architecture and is required for the specialized structural and functional identity of the CEM cilium.

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Fei Wang
Steward Lab

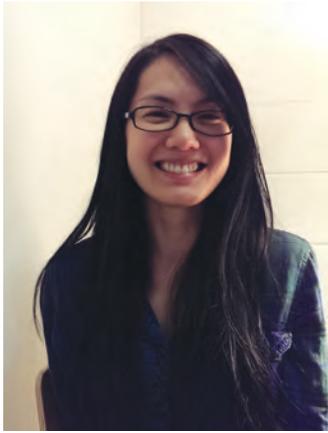
Research Summary

The three vertebrate Tet (*Tet1/2/3*) genes encode 5-methylcytosine (5mC) hydroxylases that catalyze the conversion of 5mC to 5hmC, resulting in the elimination of the methyl mark on DNA. *Drosophila* has only one Tet gene encoding several alternatively spliced isoforms from two distinct promoters. I have induced a Tet null allele that produces no transcripts by deleting both promoters. This allele is lethal at the pupal stage, indicating that Tet is essential in *Drosophila*. It has been shown that the *Drosophila* genome does not contain significant levels of 5mC and we determined that it also lacks 5hmC. Interestingly, it has been shown recently that the vertebrate Tet3 protein can convert 5mC to 5hmC in RNA. Together with our collaborator Dr. F. Fuks (Universite Libre, Brussels) we have determined that 5hmC also exists in *Drosophila*. In addition, we found that the 5hmC was mostly enriched in polyA+ RNA. Since Tet is a DNA binding protein that can also modify RNA, I propose that Tet binds to specific regions on DNA, and then catalyzes

the transition from 5mC into 5hmC on adjacent nascent transcripts. This modification may control RNA maturation, transport to the cytoplasm or even loading of RNAs onto polysomes.

Hence, the newly discovered 5hmC mark may represent a new level of control of gene expression. The functional importance of 5hmC is largely unknown. This modification has likely been overlooked because of the high levels of 5mC and 5hmC present in vertebrate DNA. *Drosophila* is an excellent model to study 5hmC because it lacks DNA 5mC and 5hmC. Because of the conservation of fly and vertebrate Tet proteins, it is likely that my discoveries will also apply to vertebrates.

Tet mutants displayed the most severe defect in pupal ovaries. Oogenesis was arrested at early stages and germline cells overpopulated the germarium. Clonal analysis shows that Tet has essential oogenic functions at the embryonic stage. Tet mutants also displayed significant locomotion phenotypes in the measure of body contractions, matching distance, turns and wiggles per minute. We also investigate the influence of Tet knockdown and overexpression on circadian rhythm of adult fly. Under the control of clock cell driver, *tim-gal4* and *pdf-gal4*, we knockdown and over express Tet gene respectively and monitor their daily activity. The preliminary results showed Tet KD and OE flies lose their circadian rhythm after 2 days in Dark-Dark (DD) as well as showing the abnormalities in Light-Dark (LD) training time.



Lin-Ing Wang
McKim Lab

Research Summary

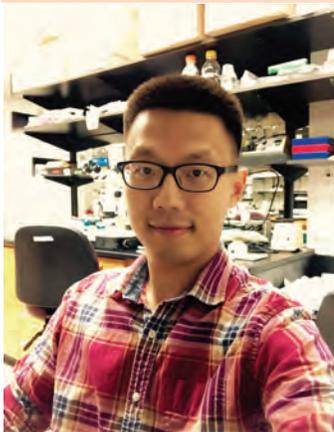
Cohesin complex is the ring-shaped protein complex that holds sister chromatids together after DNA duplication. In meiosis, two distinct rounds of cell division generate haploid gametes. Meiosis I is inherently different from mitosis and meiosis II in that homologous chromosomes need to be bi-oriented and sister chromatids need to be co-oriented for proper segregation. Therefore, several things need to be precisely achieved in meiosis I: 1) timely release of the cohesion along the chromosome arms to segregate homologous chromosomes, which are held together by a chiasma, 2) centromere cohesion must be maintained in pericentric chromosomes for sister chromatid co-segregation, and 3) a mechanism needs to be generated between sister kinetochores for sister chromatid co-orientation.

In *Drosophila melanogaster*, our results show that there are two cohesin complexes active in meiosis. Both complexes share SMC₁ and SMC₃ but one complex is dynamic and contains the proteins C(2)M and SA, whereas the other complex is static and contains the proteins SUNN and SOLO. Our recent publication has shown that the static complex plays an important role in meiotic sister-chromatid cohesion; whereas, the dynamic complex has only a minor effect on cohesion. However, the processes responsible for regulating these two complexes in meiosis have yet to be described.

We are interested in several cohesin regulators to see how cohesions are regulated to have chromosomes properly segregate in meiosis. Two known negative regulators, Wapl and Separase, have been characterized in *Drosophila* mitosis, and we extend their study to their meiotic functions. From our preliminary results, it is shown that compared to Separase, which is important for meiotic release of sister-chromatid cohesion, Wapl does not show an obvious impact on cohesion. This might suggest that Wapl is more likely to be involved in regulating the dynamic complex than the static one. To understand whether or not the dynamic cohesin complex is affected by Wapl, we are planning to test the dynamics of fluorescently tagged C(2)M has any defect. Furthermore, to clarify whether the co-orientation mechanism is cohesin complex-dependent, we tested both negative cohesin regulators in SPC_{105R} RNAi oocytes, where loss of this kinetochore protein causes defects in co-orientation. In our preliminary result, it is shown that loss of Separase can suppress the phenotype of SPC_{105R} knockdown in metaphase I. This result indicates that co-orientation in *Drosophila* female meiosis is dependent on a cohesin complex. To further understand the mechanism of Separase-mediated co-orientation, characterizations of the target cohesin complex and the regulation of Separase in SPC_{105R} RNAi oocytes will be included in my future experiments.

BENEDICT MICHAEL FELLOW

Predoctoral Research



Yuanwang Pan
Irvine Lab

Research Summary

Growth regulation is needed to form organs of correct size and proportion, yet the mechanisms are still poorly understood. Recently, mechanical forces have emerged to be an important regulator of tissue growth. For example, high cytoskeletal tension enhances tissue growth while low cytoskeletal tension decreases tissue growth. However, how mechanical forces are modulated and experienced by cells within developing tissues is not clear. One way cells could experience mechanical forces in a growing organ was provided by the mechanical feedback model: 1) differential growth rates could lead to local tissue compression as faster-growing cells push against surrounding slower-growing cells, and 2) that this local tissue compression would then decrease growth, thereby restoring even growth rates and minimizing further compression.

We tested the mechanical feedback hypothesis by inducing differential growth in *Drosophila* wing disc epithelia through distinct approaches. We showed that differential growth triggers a mechanical response that lowers cytoskeletal tension along apical cell junctions within faster-growing cells. This reduced tension modulates a biomechanical Hippo pathway, decreasing recruitment of Ajuba LIM protein and the Hippo pathway kinase Warts to junctions, and reducing the activity of the growth-promoting transcription factor Yorkie. This provides the experimental support and a molecular mechanism for lowering growth rates within faster-growing cells by mechanical feedback. We also proposed a theoretical model to explain the observed reduction of tension within faster-growing clones, supported through simulations using a modified vertex model. Finally, we found that bypassing mechanical feedback induces tissue distortions and inhomogeneous growth. Thus our research further identifies the roles of mechanical feedback in maintaining tissue shape and controlling patterned growth rates during development.

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 2015-2016

- Advanced Inorganic Chemistry
- Core Seminars in Plant Biology I
- Core Seminars in Plant Biology II
- Developmental Genetics
- Essential Skills I
- Essential Skills II
- Experimental Methods in Molecular Biosciences
- Fundamentals of Molecular Biosciences
- Genetic Analysis II
- Genetics and Cell Biology of Fertilization
- Harnessing Solar Energy
- Honors Introduction to Molecular Biology and Biochemistry Research
- Honors Thesis Seminar
- Introduction to Molecular Biology and Biochemistry Research
- Introduction to Research
- Microbial Biochemistry
- Microbiology
- Molecular Biology and Biochemistry
- Molecular Biology of Gene Regulation & Development
- Molecular Biosciences
- Mutant Isolation and Analysis
- Plant Molecular Biology
- Research in Biochemistry
- Research in Chemistry
- Structural Biology, Structural Biophysics and Chemical Biology of Transcription

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 will change the way we live and how the world operates in the next few decades. To compete successfully in the global economy, the United States needs to be at the forefront of technology. 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 over 20 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. Our current project that received funding from several sources, including the National Science Foundation, is titled: Conducting Authentic Molecular Biology and Genomics Research in High Schools (MBGR), and is under the umbrella of the Waksman Student Scholars Program (WSSP).

WSSP

The 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 2015 WSSP consisted of two interrelated parts: a Summer Institute (SI) and an Academic Year Program (AYP). In July 2015, 26 high school teams consisting of one or two students and their teachers 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 Merck Foundation to purchase additional equipment for BELL.

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.

The Research Question

The 2015 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 2015-2016 SI and AYP, over 2600 plasmid clones were purified and 2280 were sequenced. To date, 2054 DNA sequences have been analyzed by the students and 1360 have been or will be submitted for publication on the National Center for Biotechnology Information (NCBI) DNA sequence database with the students' names as contributing authors.

Conducting Authentic Molecular Biology and Genomics Research in High Schools (MBGR)

MBGR provided high school teachers with an intense and rigorous professional development experience in the emerging field of bioinformatics. This program enabled them to acquire the technological skills and experiences that characterize the cyberinfrastructure of molecular biology. Also, with funding from the NSF, we extended our programs to teachers from outside the central New Jersey region. A two-week summer Institute for 9 teachers and 19 students was conducted by Drs. Forrest Spencer and Kirby Smith at John Hopkins University, in Baltimore MD. 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 1303 students participated in the program this past year.

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.

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Dr. Janet Mead, Laboratory Director

John Brick, Laboratory Assistant



SHARING OUR DISCOVERIES

Waksman Annual Retreat

Presentations & Meeting Abstracts

Patents & Publications

WAKSMAN ANNUAL RETREAT

September 10, 2015

Rutgers University Inn & Conference Center

Presentations

- Mehul Vora and R. Padgett, “SMA-10 / LRIG1 is a novel regulator of TGF β receptor trafficking”
- Nelson Garcia and J. Messing, “The maize dek34-Dsg1 gene encodes a putative Tel2-interacting protein 2 important for plant development”
- Amber Krauchunas and A. Singson, “Characterization of the spe-43 gene that is required for sperm activation in *C. elegans*”
- Konstantin Severinov, “Searching for new CRISPRs (and finding them)”
- Stephanie Pyonteck and C. Rongo, “Identifying Transcriptional Targets Of The Hypoxia Response Pathway”
- Xueyi Xue and J. Dong, “MASS proteins, novel regulators in stomatal development”
- Jeremy Bird and B. Nickels, “The mechanism of RNA 5' capping with NAD⁺, NADH, and CoA”
- Yuanwang Pan and K. Irvine, “Differential growth triggers mechanical feedback that elevates Hippo signaling”
- Andrea Gallavotti, “Auxin signaling modules regulate maize inflorescence architecture”
- Sarah Radford and K. McKim, “Cooperation between kinesin motors promotes spindle symmetry and chromosome organization in oocytes”
- Csanad Gurdon and P. Maliga, “Identification of a cytoplasmic male sterility-associated gene in recombinant tobacco mitochondria”
- Fei Wang and R. Steward, “Tet function in *Drosophila* development”
- Malan Silva and M. Barr, “How To Specialize A Cilium: Alpha-Tubulin 6 Regulates Axonemal Ultrastructure”
- Hanif Vahedian-Movahed and Richard Ebright, “Sequence-specific interactions between RNA polymerase and the core recognition element”
- Xiao Qian and G.C. Dismukes, “Inactivation of nitrate reductase alters metabolic branching of carbohydrate fermentation in the cyanobacterium *Synechococcus* sp. strain PCC 7002”
- Yaping Feng and D. Kumar, “NGS solutions at Waksman Genomics Core”

Poster Session

-lead presenters listed below, all authors listed on individual posters-

Barr Lab

- Extracellular matrix genes regulate ciliary integrity and ciliary receptor localization, presented by Deanna De Vore.

Dismukes Lab

- Dynamics of S-states Cycling of the Water-Oxidizing Complex in Microalgae at Different Redox Conditions, presented by Gennady Ananyev.
- The carbon decision tree of *Chlamydomonas reinhardtii*: How starchless mutant strains redirect carbon metabolism, presented by Anagha Krishnan.
- Physiological Responses to Strontium Substitution in the Water-Oxidizing Complex of PSII in vivo, presented by Colin Gates.
- Engineering Photosystem II Subunit D1 in *Nicotiana tabacum* to Improve Photochemical Efficiency, presented by Yuan Zhang.
- Investigating the role of Transhydrogenase in autofermenting *Synechococcus* 7002, presented by Kumara Swamy Gollarahally Kenchappa.

Dong Lab

- Phosphorylation status affects BASL localization and function in stomatal asymmetric cell division, presented by Ying Zhang.
- Microtubule Associated Proteins Regulate Stomatal Development and Patterning in Arabidopsis, presented by Wanchen Shao.
- BASL as a scaffold protein promotes MAPK kinase pathway, presented by Xiaoyu Guo.
- Untangling the asymmetry: functional characterization of BIP2 in Arabidopsis, presented by Dongmeng Li.

Dooner Lab

- A portal to sequence-indexed single-gene knockout resources for maize, presented by Wenwei Xiong.

Ebright Lab

- Structural basis of transcription activation and transcription initiation, presented by Yu Feng.
- Target, Mechanism, and Structural Basis of Transcription Inhibition by the Antibiotic Pseudouridimycin—A Selective Nucleoside-analog Inhibitor of Bacterial RNA Polymerase, presented by David Degen.
- Bacterial RNA polymerase core recognition element (CRE): sequence determinants, recognition mechanism, and functional role, presented by Hanif Vahedian-Movahed.
- Structural basis of transcription inhibition by the nucleoside-analog inhibitor thuringiensin, presented by Ruiheng Yin.
- Novel Small-Molecule Inhibitors of Bacterial RNA Polymerase: Arylpropionyl-phloroglucinol (APPs), presented by Juan Shen.
- Crystal Structures of Mycobacterium Tuberculosis Transcription Initiation Complexes, presented by Wei Lin.
- Closing and opening of the RNA polymerase “trigger loop”: detection and analysis by single-molecule fluorescence resonance energy transfer, presented by Abhisek Mazumder.
- Crystallographic and single-molecule-FRET studies of DNA scrunching: determining the path of scrunched nontemplate-strand DNA, presented by Adam Hasemeyer.

Gallavotti Lab

- The *bif173* gene controls maize inflorescence development, presented by Qiujie Liu.
- Coordinated action of boron transport in maize development, presented by Mithu Chatterjee.

Genomics Core Facility

- Cleaving, Cutting, and Splicing: Identifying Genomic Features, presented by Brian Gelfand.

Irvine Lab

- Vamana and early girl, two novel regulators of Fat-Hippo signaling, presented by Jyoti Misra.
- Localization of Hippo Signaling complexes and Warts activation in vivo, presented by Shuguo Sun.

Maliga Lab

- Genotyping Reveals Uniformity of Plastids in Independently Isolated cms-T Cytoplasms in Maize, presented by Massimo Bosacchi.
- Transgenic approach to probe gene regulation by PPR₁₀ RNA binding protein in chloroplasts, presented by Qiguo Yu.

McKim Lab

- Centromere protein cenp-c reloads during meiosis, presented by Aashka Parikh.
- PP1-87B antagonises Aurora B for chromosome structure, centromere cohesion and kinetochore localisation in *Drosophila* oocytes, presented by Arunika Das.
- Non-canonical cohesin complexes in *Drosophila* female meiosis, presented by Mercedes Gyuricza.
- Effects in Mitotic Cohesion Regulators in *Drosophila* Meiotic Chromosome Segregation, presented by Lin-Ing Wang.

Messing Lab

- MicroRNA expression and activity in *Spirodela polyrhiza* under various stress and hormonal conditions, presented by Paul Fourounjian.
- Transgenic maize seeds with enhanced accumulation of the essential amino acids methionine and cysteine, presented by Jose Planta.
- Teff grass, a gluten-free baking alternative, presented by Wei Zhang.

Nickels Lab

- A conserved pattern of primer-dependent transcription initiation in *Escherichia coli* and *Vibrio cholerae* revealed by 5 RNA-seq, presented by Kyle Skalenko.
- Mechanistic studies of transcription start site selection and initial transcription, presented by Jared Winkelman (also in Ebright Lab).

Padgett Lab

- Marfan syndrome-like is caused by mutations in the TGFB receptors, presented by Jing Lin.
- SMA-10 / LRIG1 is a novel regulator of TGF β /BMP receptor trafficking, presented by Mehul Vora.

Rongo Lab

- Dopamine Signaling Regulates Protein Homeostasis Through The Ubiquitin Proteasome System, presented by Kishore K. Joshi.
- Modulation of the Hypoxia Response Pathway by PMK-1 p38 MAP kinase Signaling, presented by Eunchan Park.

Severinov Lab

- Role of 3'-terminal segment of guide RNA in assembling the guide RNA-Cas9 complex, presented by Vladimir Mekler.

Steward Lab

- Tet is Essential for Normal Larval Locomotion in *Drosophila*, presented by Yoon Mi Kim.

PRESENTATIONS & MEETING ABSTRACTS

Waksman Institute Hosted Seminars

- Blake Meyers, Danforth Plant Science Center, St. Louis, MO. "Phased siRNAs in Plant Reproductive Organs." January 29, 2016.
- David Meinke, Department of Botany, Oklahoma State University, Stillwater, OK. "Natural Variation in Sensitivity to a Loss of Chloroplast Translation in Arabidopsis." February 12, 2016.
- Ralph Bock, MPI for Molecular Plant Physiology, Potsdam, Germany. "Taming Plastids for Biotechnology: Pathway Engineering, Resistance Engineering and Molecular Farming." March 11, 2016.
- Keiko Torii, Molecular and Cellular Biology, University of Washington. "Opening New Pores: Communication and Fate Specification during Stomatal Development." March 25, 2016.
- Bo Liu, Department of Plant Biology, UC Davis, Davis, CA. "Centrosome-independent Microtubule Organization in Plant Cells." April 1, 2016.
- James Birchler, Division of Biological Sciences, University of Missouri, Columbia, MO. "Construction and Applications of Engineered Minichromosomes in Plants." April 8, 2016.

Waksman Student Scholars Programs

- Waksman Student Scholars Summer Institute, Waksman Institute, July 6- July 24, 2015.
- Upward Bound Math-Science DNA Workshop, Waksman Institute, June 29-July 2, 2015.
- 2015 National Science Teachers Association (NSTA) Area Conference, Using the Cyberinfrastructure to Conduct Bioinformatics Research in Your Classroom, Philadelphia, PA, November 13, 2015.

Barr Lab

- Barr, M. FASEB Conference on the Biology of Cilia and Flagella, Colorado, July 2015.
- Barr, M. Department of Genetics and Development 30th Annual Retreat, Columbia University, September 2015.
- Barr, M. UT Southwestern O'Brien Kidney Center, January 2016.
- Barr, M. Institut du Cerveau et de la Moelle epiniere, Paris, France, February 2016
- Barr, M. Department of Genetics, Washington University, March 2016.
- Barr, M. Department of Biology, Johns Hopkins, April 2016.
- Barr, M. Department of Molecular Biology and Biophysics, University of Connecticut Health Center, May 2016.

Dismukes Lab

- Dismukes, G.C. Catalysis Society of Metropolitan New York, Annual Conference, Stevens Institute of Technology, Newark, NJ, March 2015.
- Dismukes, G.C. Eastern Regional Photosynthesis Conference, Wood Hole, MA, April 2015.
- Dismukes, G.C. invited speaker, Electrochemical Society National Meeting, Chicago, convenor Eric Miller, May 2015.
- Dismukes, G.C. invited speaker, NERM Northeast Regional Meeting American Chemical Society, Cornell Univ. convenor: Nikolay Dimitrov, June 11-12, 2015.
- Dismukes, G.C. GRC Photosynthesis, Bentley Univ. Waltham, MA, July 2015.
- Dismukes, G.C. Microbiology Symposium, Rutgers SEBS, Feb 26, 2016.
- Dismukes, G.C. hosted visiting scholar Dr. Dmitry Shevela, Umea University, Sweden, Mar 10-24, 2016.
- Dismukes, G.C. New York Metro Catalysis Symposium at Rutgers, New Brunswick, March 23, 2016.
- Dismukes, G.C. DOE-EERE, 2016.
- Dismukes, G.C. Rutgers LSM Symposium, April 4, 2016.
- Dismukes, G.C. Eastern Regional Photosynthesis Meeting, Marine Biological Lab, Woods Hole, MA, April 8, 2016.
- Dismukes, G.C. National Renewable Energy Laboratory, Golden, CO, consultation with Andriy Zakutayev, April 22, 2016.
- Dismukes, G.C. Rutgers Energy Institute Symposium, May 4, 2016.
- Dismukes, G.C. International Hydrogen Production Conf., Hanzhou, China; paper & chaired session, May 8-12, 2016.
- Dismukes, G.C. launch of the Rutgers-Zhejiang joint project for Global Climate and Energy Project and tour of the Yantai bioreactor facility. Zhejiang University, Hanzhou, China, May 13-14, 2016.
- Dismukes, G.C. 229th Electrochemical Society Meeting. 2 oral papers and 1 poster in three divisions, May 29 - June 3, 2016.

Dong Lab

- Dong, J. "Phospho-status of BASL regulates protein polar trafficking and coordinates cell fates in stomatal ACD." Gordon Research Conference on Posttranslational Modification Networks, Hong Kong, China, 2015.
- Dong, J. "Re-organized MAPK signaling in asymmetric cell division." Seminar at Institute of Botany, Chinese Academy of Sciences, Beijing, China, 2015.
- Dong, J. "A universal stress signaling pathway feeds into plant development - about fate decision."

International Symposium on Plant Responses to Stress at China Agriculture University, Beijing, China, 2015.

- Dong, J. "BASL differentiates asymmetric cell fate through MAPKs and SPCH in stomatal development." International Conference on Arabidopsis Research, Gyeongju, Korea, 2016.
- Dong, J. "Cell polarity and MAPK signaling in plant asymmetric cell division." Plant Biology 2016 Meeting, American Society of Plant Physiology, Austin, TX, 2016.
- Dong, J. "Cell polarity and MAPK signaling in plant asymmetric cell division." Seminar at the Department of Molecular Biology, Princeton University, Princeton, NJ, 2016.
- Dong, J. "Cell polarity and MAPK signaling in plant asymmetric cell division." Seminar at Delaware Biotechnology Institute, University of Delaware, Newark, DE, 2016.
- Dong, J. "Cell polarity and MAPK signaling in plant asymmetric cell division." Seminar at the Department of Plant Biology, University of California, Davis, Davis, CA, 2016.
- Dong, J. "Cell polarity and asymmetric cell division in Arabidopsis." Seminar at Center for Plant Cell Biology, University of California, Riverside, Riverside, CA, 2016

Ebright Lab

- Ebright, R. "Structural studies of transcription initiation and activation, and anti-tuberculosis drug discovery targeting transcription." Department of Microbiology and Immunology, Harvard Medical School, Boston, Massachusetts, 2015.
- Ebright, R. "Antibacterial agents: small-molecule inhibitors of bacterial RNA polymerase." Institute for Quantitative Biomedicine Symposium, Antimicrobial Resistance, Rutgers University, Piscataway, New Jersey, 2015.
- Ebright, R. "Therapeutics for drug-resistant bacteria: arylpropionyl phloroglucinols." Centers of Excellence in Translational Research Review Meeting, National Institute of Allergy and Infectious Diseases, Rockville MD, 2016.
- Bird, J., Zhang, Y., Tian, Y., Lee, J., Kaplan, C., Ebright, R.H., and Nickels, B. (2015) NAD and 3'dpCoA capping of RNA 5' ends occurs ab initio during transcription initiation. Meeting on Molecular Genetics of Bacteria and Phages, Madison, Wisconsin, August 4-9, 2015.

Gallavotti Lab

- Gallavotti, A. "Auxin signaling in maize inflorescences." Plant and Animal Genome XXIV Conference, San Diego, CA, January 9, 2016.
- Gallavotti, A. "Molecular mechanisms of maize inflorescence architecture." Chinese Academy of Agricultural Sciences, Beijing, December 10, 2015.
- Gallavotti, A. "Molecular mechanisms of maize

inflorescence architecture." University of Florida Genetics Institute, Gainesville, November 3, 2015.

- Galli, M., Liu, Q., Moss, B., Malcomber, S., Li, W., Gaines, C., Federici, S., Roshkovan, J., Meeley, R., Nemhauser, J., Gallavotti, A. Auxin signaling modules regulate maize inflorescence architecture. Plant Molecular Biology, Gordon Research Conference, poster. Holderness, NH, June 12-17, 2016.
- Gallavotti, A., Camehl, I., Liu, X., Galli, M. The transcriptional co-repressor REL2 is required for maize vegetative and reproductive development. Maize Genetics Conference Abstract 58:P217. Jacksonville, FL, March 17-20, 2016.
- Liu, Q., Federici, S., Gallavotti, A. The bif173 gene controls maize inflorescence development. Maize Genetics Conference Abstract 58:P134. Jacksonville, FL, March 17-20, 2016.
- Kiley, M., Joseph, S., Liu, Q., Robert Coats, D., Withee, J., Malcomber, S., Gallavotti, A., McSteen, P. Auxin evo-devo: reverse genetic approaches to understanding the role of auxin in shoot development. Maize Genetics Conference Abstract 58:P157. Jacksonville, FL, March 17-20, 2016.
- Gallavotti, A. "Auxin signaling modules regulate maize inflorescence architecture." Waksman Institute Annual Retreat, September 10, 2015.
- O'Malley, R.C., Huang, S.C., Song, L., Bartlett, A., Nery, J.R., Galli, M., Gallavotti, A., Ecker, J.R. An atlas of the Arabidopsis transcription factor binding sequence. Plant Genomes & Biotechnology: from Genes to Networks. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, December 2-5, 2015
- Camehl, I., Galli, M., Gallavotti, A. REL2 – an essential transcriptional corepressor for the development of maize inflorescences. From Molecules to the Field, Botanikertagung 2015. Ludwig-Maximilians-Universität & Technische Universität München, München, Germany, August 30-September 3, 2015.
- Gallavotti, A., Camehl, I., Galli, M. The transcriptional corepressor REL2 functions in multiple pathways during maize vegetative and reproductive development. Mechanisms in Plant Development, FASEB Science Research Conferences, poster #13. Saxtons River, VT, August 2-7, 2015.

Irvine Lab

- Irvine, K. Seminar at Cancer Institute of New Jersey, New Brunswick, June 16 2016.
- Irvine, K. Seminar at Neuroscience Dept UT Southwestern, Dallas, TX, May 17, 2016.
- Irvine, K. Seminar Dept of Molecular Physiology Stanford University, Stanford CA, April 26, 2016.
- Irvine, K. Seminar at Dept Biology, U Kentucky, Lexington, KY, April 14, 2016.
- Irvine, K. Seminar at Dept Molecular, Cell and Cancer Biology, UMASS Med School, Worcester, MA, March 22, 2016.

- Irvine, K. co-organizer meeting on *Drosophila* Research Ecosystem, at Janelia Research Campus, VA, Feb 17-19 2016.

Maliga Lab

- Maliga, P. Invited Speaker. “Cell to Cell Movement of Organellar DNA in Plants.” The 3rd Plant Genomics Congress USA, September 13-15 2015, St. Louis, MO.
- Maliga, P. Invited Speaker & Organellar Genetics Workshop Organizer. “Progress in Implementing Plastid Transformation in *Arabidopsis thaliana*.” Plant and Animal Genome Conference XXIV, San Diego, CA, January 9-13, 2016.
- Maliga, P. Invited Speaker. “Progress in Implementing Plastid Transformation in *Arabidopsis thaliana*.” Mitochondria & Chloroplasts Gordon Research Conference, Mount Snow, West Dover, VT, June 19-24, 2016.
- Bosacchi, M., Gurdon, C. and Maliga, P. Plastid Genotyping Reveals the Uniformity of Cytoplasmic Male Sterile-T Maize Cytoplasms. The World Congress of the Society of In Vitro Biology, San Diego CA, June 11-15, 2016 and ASPB, Austin TX, July 2016.
- Yu, Q., Ioannou, A., Tungsuchat-Huang, T., Barkan, A. and Maliga, P. Transgenic approach to probe gene regulation by PPR₁₀ RNA binding protein in chloroplasts. Mitochondria & Chloroplasts Gordon Research Conference, Mount Snow, West Dover, VT, June 19-24, 2016.

McKim Lab

- McKim, K. EMBO Meiosis Conference, August 31, 2015.
- McKim, K. Meiosis Gordon Conference, June 26, 2016.

Messing Lab

- Fourounjian, P., Tang, J., Tanyolac, B, Zhang, B-Y, Zhang, Y-Y, Wakim, C., Ma, J., Messing, J. (2015). *Spirodela polyrhiza* post-transcriptional regulation responses to environmental and hormonal stimuli. 3rd International Duckweed Conference, Kyoto, Japan, July 2015.
- Garcia, N. and Messing, J. (2016). A maize kernel mutant that lacks a putative function in cell division. Plant and Animal Genome XXIV, San Diego, California.
- Wang, W., Wu, Y., and Messing, J. (2016). Dormancy without reproduction. Plant and Animal Genome XXIV, San Diego, California.
- Wang, W., Fourounjian, P., and Messing, J. (2016). The ancient genome of an aquatic plant, *Spirodela polyrhiza*, at the root of monocot evolution. Plant and Animal Genome XXIV, San Diego, California.
- Zhang, Z., Zheng, X., Yang, J., Messing, J., and Wu, Y. (2016). Opaquez and Prolamine-Box Binding Factor also control kernel yield. 56th Annual Maize Genetics Conference, Jacksonville, FL, March 2016.
- Dong, J., Zhang, W., Feng, Y., Kumar, D., and Messing,

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