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.
The Waksman Institute is a research unit of the New Brunswick campus of Rutgers, 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 1, 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 Waksman Institute

Our reading room is home to over 20,000 volumes on microbiology, biochemistry, and genetics, although most of our literature has become digital. Therefore, the actual reading room has become a computer classroom, which can also be used for instruction in bioinformatics.

The Waksman computing infrastructure has dedicated space on the fourth floor in the Old Wing. Randy Newman, Daja O’Bryant, and Brian Schubert provide the hands-on maintenance of the Institute’s computing resources. These include a state-of-the-art data center located on the 4th floor, which hosts 35 servers, two multi-node HPC computing clusters, and over 27 terabytes of enterprise class storage with an offsite backup location for disaster recovery. In addition, 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 GPUs, FDR Infiniband, 10Gb Ethernet, and a Lustre filesystem for various research tasks. Additionally, by utilizing Rutgers’ Internet 2 connection, Waksman users have a bandwidth of direct connection to 270 universities and 48 affiliate members of the Internet 2 consortium. The Institute provides its users with many traditional office packages and common molecular biology tools, but also offers a multi-seat site license for the latest version of the reference sequence and Vector NTI software suites. 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 SP8 II and a Leica SP8 - both of which are spectral confocal microscopes equipped for imaging multiple wavelengths, including UV and capable of collecting high resolution optical sections where out-of-focus light is eliminated. Both microscopes have sensitive GaAsP detectors, and the SP5 uses the latest high-speed resonance scanner for rapid image acquisition for real time imaging. Computing software is available for the analysis and deconvolution of three and four dimensional image data, as well as for FRAP, FLIP, and FRET measurements. The core also possesses a Zeiss Axioplan 2, which is a fully motorized immunofluorescence system capable of the automated collection of 3D and 4D image stacks using multiple wavelengths for 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 has 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 retaining the SOLID 5500XL systems. Together, the WGCF now has the capability to cover a 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 Sequencers (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 (SVN) 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 much prior genome knowledge and proves to be a much better platform for whole transcriptome experiments including alternative splice site discovery, gene fusion detection, smallRNA, 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 275, 252x, 232x, 230x 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’ type experiments including epigenome, 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 optical system for target 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 metabonome- 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 of DNA sequencing contribute to a dramatic turnover of our capacity, for which we are very grateful.

The Waksman Institute’s total resident population is currently 130, 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, four 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 sixteen resident and two non-resident members, two are Assistant Professors, one is Associate Professor, eight are Professors, five 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 nine professor emeriti, who are all well, and periodically join us here for events. Three professors are members of the National Academy of Sciences (US), two of the National Academy of Sciences Leopoldina (Germany), one a member of the Hungarian Academy of Sciences, four are Fellows of the American Academy of Microbiology, and five Fellows of AAAS. One faculty member is Howard Hughes Institute Investigator.

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 held on September 19th, 2014. In addition, the Institute sponsored The Microbiology Symposium, in New Brunswick, NJ, in January 2015 at Trayes Hall, Douglass College.

Funding & Recruitment
Clearly, the outside support achieved through competition is the most notable highlight. Congratulations to all faculty that received either new grants or renewals. In particular, I am pleased that our new Assistant Professors won sizeable five-year grants, which shows that recruitment is an important investment for the Institute. Although we will have to wait until construction of the middle wing is completed, we anticipate recruiting again in the following academic year. 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 8 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 responsibly manage 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.

Awards & Honors
I am pleased to report several awards/honors of our students and faculty this year. This year, we had several seniors that have been recognized with the 2015 Rutgers Scholar Award: Rachel A. Battaglia, McKim Lab, Matthew B. Gigliotti, Elbright Lab, Benjamin P. Keepers, Irvine Lab, and Alina Rashid, Barr Lab. In addition, Rachel A. Battaglia and Alina Rashid were also recipients of The Duncan & Nancy Macmillan Award for research excellence. Rick Padgett was appointed to the Advisory Board for the Marie Skłodowska-Curie Innovative Training Network and as the Ombudsman for the Fellows. As major recognition for the Institute, Evelyn Witkin won the international Wiley Prize in Biomedical Sciences for her groundbreaking work in DNA repair. Bryce Nickels was named Rutgers-New Brunswick Chancellor Scholar in recognition of his work on transcription. Congratulations to these accomplishments of our students and members! I am also pleased to have been elected as a Fellow of the American Academy of Microbiology and as a Member of the US National Academy of Sciences.
Cilia protrude from cell surfaces to monitor the surrounding environment. In addition to its role as sensory receptor, the cilium also releases extracellular vesicles (EVs). The release of sub-micron sized EVs is a conserved form of intercellular communication used by all three kingdoms of life. These extracellular organelles play important roles in both short and long range signaling between donor and target cells and may coordinate systemic responses within an organism in normal and diseased states. EV shedding from ciliated cells and EV-cilia interactions are evolutionarily conserved phenomena, yet remarkably little is known about the relationship between the cilium and EVs and the fundamental biology of EVs. Studies model organisms have begun to shed light on ciliary EVs. We found that C. elegans EVs are shed and released by ciliated sensory neurons in an intraflagellar transport (IFT)-dependent manner. C. elegans EVs play a role in modulating animal-to-animal communication, and this EV bioactivity is dependent on EV cargo content. Some ciliary pathologies, or ciliopathies, are associated with abnormal EV shedding or with abnormal cilia-EV interactions, suggest the cilium may be an important organelle as an EV donor or as an EV target. Until the past few decades, both cilia and EVs were ignored as vestigial or cellular junk. As research interest in these two organelles continues to gain momentum, we envision a new field of cell biology emerging. We propose that the cilium is a dedicated organelle for EV biogenesis and vesicle receptor localization. We have continued to exploit C. elegans for new gene discovery and continue to use these 27 EV-releasing ciliated neurons as a springboard to study the fundamental biology of EVs in vivo, which will advance frontiers of knowledge where very little is known [3, 4].

Very recently we showed that the C. elegans cilium is a source of bioactive EVs. EVs are nanometer scale cargo-containing particles that are released from virtually every human cell and function in intercellular communication. In pathological conditions such as cancer, infectious disease, or neurodegenerative disorders, EVs function diabolically to spread toxic cargoes. In studies related to our C. elegans model for ADPKD, we discovered that 21 male-specific neurons and six gender shared IL2 ciliated sensory neurons shed and release GFP-labeled EVs. In pathological conditions such as cancer, infectious disease, or neurodegenerative disorders, EVs function diabolically to spread toxic cargoes. In studies related to our C. elegans model for ADPKD, we discovered that these 27 EV-releasing ciliated neurons can serve as a springboard to study the fundamental biology of EVs in vivo, which will advance frontiers of knowledge where very little is known [3, 4].

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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 Dachsous-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: Dachsous (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 accumulation influences Hippo signaling. Our current understanding is that when Dachs is at the membrane, it cooperates with another protein that we placed in the Fat-Hippo pathway, called Zyxin. Zyxin and Dachs interact with each other and promote degradation and inactivation of the Warts kinase, which is a central component of the Hippo pathway.

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 Jab, at cell-cell junctions is dependent upon cytoskeletal tension. Jab then recruits Warts into junctions; formation of this Jab-Warts complex inhibits Warts activity, thereby promoting growth.

Initial studies of Hippo signaling focused on its roles in controlling growth during development of the wing and eye discs of Drosophila. Our studies have since revealed important roles for Hippo signaling in controlling growth in many other contexts, including during development of neuroepithelial cells within the optic lobe, development of glial cells within the brain and eye, proliferation of intestinal stem cells, and regenerative growth after tissue damage. Each of these studies has revealed unique aspects of Hippo pathway regulation and function.

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, Dchs, and we and our collaborators have characterized it, together with mutations in a murine fat homolog, Fat4. Our analysis indicates that Dchs and Fat4 function as a ligand-receptor pair during mouse development, and we have identified novel requirements for Dchs-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. Ken 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 biorientation. 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 often focus on the "weak points" that make the oocyte susceptible to nondisjunction. We are interested in identifying and characterizing these weak points because they are important to understanding how meiosis works, and also important to understand why errors occur that lead to infertility.

Multiple Cohesin Complexes are Required During Meiosis
Cohesion is what holds sister chromatids together during after DNA replication but prior to cell division. In mitosis, cohesion depends on the cohesin complex, made up of four subunits: SMC1, SMC3, Stromalin (SA) and a Kleisin Rad24. In mitotic cells, this cohesin complex holds 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 type of cohesion fuses the sister centromeres together during meiosis I, so that they segregate to the same pole at anaphase I. With multiple functions, it is not surprising that meiosis is characterized by multiple cohesin complexes. In meiosis, we have found that there are multiple cohesin complexes, some of which are required for homolog interactions, others for cohesion.

Mutant analysis in Drosophila has shown that SC initiation involves two independent pathways defined by two sets of cohesin-related genes. Both pathways depend on SMC3. One pathway depends on C(2)M, a Kleisin family protein that physically interacts with the cohesin SMC3. 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, and Nipped-B, a positive regulator. Surprisingly, SA and Nipped-B are not required for cohesion during meiosis, only in SC assembly. 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 and SUNN maybe Kleisin and stromalin subunits, respectively, the role of ORD is less clear and may be a positive regulator.

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, cohesion molecules that are displaced from the
In contrast to this hypothesis, we have discovered that some cohesion complexes are dynamic and exchanged during prophase. Chromosome bound C(a)M is dynamic during prophase and can be replaced if it dissociates from the chromosomes. The resolution to this problem is that the multiple cohesion complexes differ in their capacity to be replenished during prophase. Specifically, we have found that centromeric SOLO/SUNN is not dynamic. This is the cohesion required to hold sister chromatids together and, consistent with the aging cohesion hypothesis, can only be loaded during pre-meiotic S-phase. Thus, the C(a)M/SA cohesion 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. Answers to this question may be revealed by understanding how these two complexes are regulated.

The Role of the Central Spindle, Centromere and Kinetochore Proteins in Chromosome Segregation (Figure 1: A) 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 subtilo 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 spindled is conserved in the oocytes of mammals and C. elegans.

Like cohesion proteins, centromere proteins are also loaded onto the chromosomes at a specific time in the cell cycle, either G2 or S. Thus, like cohesion, centromere proteins are a potential weak point because they could be susceptible to degradation during a prolonged G2 or prophase. Centromere proteins associate with the chromatins and are a platform upon which the kinetochore are assembled. The kinetochore includes the proteins that interact with the microtubules of the spindle. Our results are consistent with this: centromere proteins CENP-C and C(l)D (a special subunit of the CENP-C family) are loaded prior to meiotic prophase. The kinetochores are not assembled until the nuclear envelope breaks down and the meiotic divisions begin. A core component of the kinetochore is the KMN network, which in Drosophila includes the Mis2 (Mis2, Nfni, Nski) and Ndc80 complexes (Ndc80, Nuf2, Sps25) and Spc24/SKu1.

The kinetochores are critical for the process of chromosome bi-orientation, where each pair of homologous centromeres attaches to microtubules from opposite poles. This process can be broken down into a series of chromosome movements that depend mostly on the kinetochores. First, the centromeres make an attempt at bi-orientation. This results in the directed poleward movement of centromeres that depend on lateral kinetochore-microtubule attachments mediated by SPC40R. These lateral-based chromosome movements required for chromosome orientation are probably mediated by the meiotic central spindle. End-on kinetochore-microtubule attachments via Ndc80, however, are essential to the directed poleward movement of centromeres. Thus, we propose that the initial attempt at bi-orientation occurs during the period when both kinetochores and the central spindle are required for spindle stability. Then, as the oocyte progresses toward metaphase, and the central spindle decreases in importance, this reflects a trend toward the formation of stable end-on kinetochore-microtubule attachments that, in turn, stabilize the bipolar spindle.

SPC40R has another meiosis-specific function: it is required for co-orientation of sister centromeres during meiosis I. Co-orientation is a process that fuses the core centromeres of sister chromatids to ensure that they form a single kinetochore that attaches to microtubules that are attached to the same spindle pole. In mitosis, the sister centromeres are not fused so that they can attach to microtubules from opposite poles. The same occurs in meiosis II. In meiosis I, however, cohesion is stably maintained at the core centromeres. This depends on the Monopolin protein complex in budding or fission yeast but the proteins and mechanisms that mediate this process in animals is not known. Our finding that sister centromeres separate in meiosis I oocytes lacking SPC40R is the first insights into the mechanism of this mysterious process. The mechanism may involve SPC40R recruiting PPI. This is based on our observation that oocytes lacking PPI have separate centromeres at meiosis I like the oocytes without SPC40R, whereas this phenotype is suppressed in oocytes lacking Aurora B kinase. In the future, it will be important to identify if other proteins are recruited by SPC40R, the targets of Aurora B and PPI that regulate centromere bi-orientation, and how these factors interact with centromere proteins like CENP-C.
We find that the two TGFβ receptors, type I and type II, traffic differently from each other. The type I receptor, SMA
As a foundation for further SMA-10 studies, we examined the normal trafficking of TGFβ receptors in the nematode.
process. In mutants of sma-10, we find that the receptors do not traffic properly and accumulate in vesicles. Further
It is known that efficient signaling of pathways requires proper recycling of receptors, so SMA-10 could affect that
physically interacts with SMA-6 and DAF-4, the C. elegans TGFβ receptors, but not with the TGFβ ligand. Its
Given that SMA-10 is a transmembrane protein, we reasoned it could physically interact with either the ligand or
From our genetic screen, we have focused on one locus, sma-10. It encodes a
Studies of TGFβ Receptor Trafficking

MicroRNA (miRNA) genes comprise at least 2% of animal genomes and represent an important aspect of gene regulation. In animals, they attenuate translation of target messages and often affect mRNA levels of most genes (Fig.3). We are exploring their role in regulating growth in the Drosophila brain.

We propose that regulation by bantam is a conserved feature of Dpp/BMP signaling. We have identified two
Accordingly, we propose to name Smad1 and Smad5 as Mad homologs. They are enriched in the brain, two of which are regulated by Dpp. One of them is bantam, and this argues that bantam and Dpp function in a regulatory loop. In the brain, we find that alterations of bantam levels affect glial cell number. Experiments are underway to determine which aspect of cell cycle regulation bantam controls.

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

We propose that regulation by bantam is a conserved feature of Dpp/BMP signaling. We have identified two vertebrate bantam homologs and are determining if they regulate the BMP Smads, which are Mad homologs. Interestingly, Smad 1 is closely related to Smad 5, but does not contain putative bantam binding sites. We propose that bantam may be partially responsible for functional differences between Smad 1 and Smad 5.

To further our studies of Dpp and miRNA action in the brain, we have profiled miRNA expression in the fly brain and compared these changes to those in the mouse, rat, and human brain. We find six conserved miRNAs that are enriched in the brain, two of which are regulated by Dpp. One of them is bantam, and this argues that bantam and Dpp function in a regulatory loop. In the brain, we find that alterations of bantam levels affect glial cell number. Experiments are underway to determine which aspect of cell cycle regulation bantam controls.

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 TGFβ receptors, but not with the TGFβ ligand. Its physical association with the receptor could affect receptor stability or it could affect receptor trafficking (Fig.2).

As a foundation for further SMA-10 studies, we examined the normal trafficking of TGFβ receptors in the nematode. We find that the two TGFβ receptors, type I and type II, traffic differently from each other. The type I receptor, SMA-10, affects the growth of cells and how signaling strength of TGFβ 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 TGFβ-like signal transduction pathways.

MicroRNA Genes Affect TGFβ-like Pathways in Drosophila
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As a foundation for further SMA-10 studies, we examined the normal trafficking of TGFβ receptors in the nematode. We find that the two TGFβ receptors, type I and type II, traffic differently from each other. The type I receptor, SMA-10, affects the growth of cells and how signaling strength of TGFβ 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 TGFβ-like signal transduction pathways.

MicroRNA Genes Affect TGFβ-like Pathways in Drosophila
MicroRNA (miRNA) genes comprise at least 2% of animal genomes and represent an important aspect of gene regulation. In animals, they attenuate translation of target messages and affect mRNA levels of most genes (Fig.3). We are exploring their role in regulating growth in the Drosophila brain.

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

We propose that regulation by bantam is a conserved feature of Dpp/BMP signaling. We have identified two vertebrate bantam homologs and are determining if they regulate the BMP Smads, which are Mad homologs. Interestingly, Smad 1 is closely related to Smad 5, but does not contain putative bantam binding sites. We propose that bantam may be partially responsible for functional differences between Smad 1 and Smad 5.

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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 protein turnover 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 expression relates to the development, 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 show that trafficking 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 endocyotosed receptors, possibly through outgrowth 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 mitochondrial under oxygen deprivation results in an hypoxia-related fusion response. 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/Nrf2 is required for both rapid mitochlondrial refuslusion and rapid functional recovery of the nervous system during reoxygenation. In response to anoxia, SKN-1 promotes the expression of the mitochondrial resident protein Somatatin-like 1 (STL-1), which helps facilitate mitochondrial dynamics following anoxia. This conserved anoxia 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, understanding how 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 how mitochondria fuse and divide in C. elegans neurons using a mitochondria-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 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 polyubiquitination. 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.

C. elegans Offers a Unique Opportunity to Define Sperm and Egg Components Required for Fertilization and Gamete Activation

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

Sperm Function

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

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

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.
In addition to ongoing genetic screens for new sperm function mutants (Figure 1), 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.

**Sperm Activation**

Post meiotic sperm differentiation (spermiogenesis) is required for a haploid spermatozoon to build cellular structures required for motility and interactions with the egg. We recently cloned 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 egg-3, egg-4 and egg-5 genes encode inactive protein tyrosine phosphatases or “antiphosphatase” required for egg activation after sperm entry.

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 (figure 1). 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.

**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 (PDCDs 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 embryonic fibroblasts fail to grow and ultimately die, similar to the phenotype we see in Drosophila ovaries.

**Zfrp8Controls 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, Rps16a, and Rps5a, 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 Zfp8/PDCD2 is required for efficient nuclear export of select transcripts, including some TE-RNAs and endogenous mRNAs.

Based on the predicted chaperone activity of Zfp8/ PDCD2 and its interaction with RNA binding proteins, we propose that Zfp8/assists the assembly of transcript-specific RNPs and facilitates their nuclear export.

Another Zfp8-interactor was NUFAST (Nuclear fragile X mental retardation-interacting protein) and we have been able to show that Zfp8 forms a complex with NUFAST 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 neurodevelopmental and reproductive developmental defects in humans and in flies where it is essential in gonadal stem cell maintenance.

We have identified components of the Zfp8 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 Zfp8 is required in the nucleus and for proper localization and activity of FMRP. Further we showed that Zfp8 genetically interacts with the translational regulators, Fmr1 and tral, in an antagonistic manner. These results suggest that Zfp8 is required for nuclear export of the FMRP complex, and that associated cooperation negatively regulates the activity of FMRP/TraL-dependent translational repression within the cytoplasm.

Zfp8 binds Tet1, a Methylcytosine Dioxygenase

We also identified Tet as a direct interactor of Zfp8/ PDCD2 in flies and human cells. Tet encodes a methylcytosine dioxygenase that transforms 5 methyl cytosine (5mC) into 5-hydroxymethylcytosine (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, Tettnull genetically interacts with Zfp8. Tettnull is pupal lethal and removing one copy of Zfp8 suppresses this lethality; ~40% of animals survive to adulthood but die soon after eclosion. This result supports our protein interaction data and indicates that Zfp8 may have an antagonistic function to Tet.

The Tett gene is expressed primarily in embryonic and larval nerve cells and Tet mutant larvae have a smaller, disorganized brain. It is thus likely that the gene has an essential function in the development or function of nerve cells.

In flies 5hmC 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 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. We hypothesize that Tet modifies specific transcripts and regulates the recruitment of Zfp8 to these RNAs, thus controlling their processing and translation.

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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 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." 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. 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 RNAPII, RNAPIII, 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 infections in the absence of therapy. 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—that is, 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 published bacterial RNAP sequence data, 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 pharmacological studies of new scaffolds to identify new small-molecule inhibitors of bacterial RNAP.

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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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.

Transcription is the first step in gene expression and thus is highly regulated. This regulation occurs 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 Recently it has been shown that certain RNAs in bacteria carry 5'-NAD+, NADH, or 3'-diphospho-coenzyme A (dPcOA) "caps" it has been proposed that NAD+, NADH, and dPcOA caps are added to RNAs post-translationally, in a manner analogous to addition of γ-methylguanlylate caps in eukaryotes. In unpublished work we have established that NAD+, NADH, and dPcOA can be incorporated into RNA 5'-ends during transcription initiation, functioning as non-canonical initiating nucleotides (NCINs). Use of NCINs as initiating nucleotides is a consequence of their structural similarities to the canonical initiating nucleotide ATP. We refer to the incorporation of NAD+, NADH, and dPcOA into RNA 5'-ends during transcription initiation as "ab initio capping." In collaboration with Richard Ebright's lab we have also obtained crystal structures of bacterial transcription initiation complexes containing NAD+ and dPcOA-capped initiation products. Thus, our preliminary results define the mechanism and structural basis of ab initio capping. We further find that both bacterial and eukaryotic RNAP can incorporate NCINs during initiation in vitro, suggesting ab initio capping may occur in all organisms if conditions are favorable. Our current efforts are to define the physiological impact of ab initio capping by: (i) Identifying promoter sequence determinants that affect the efficiency of ab initio capping. (ii) Determining the impact of ab initio capping on gene expression. (iii) Establishing whether or not the presence of a 5'-NCIN recruits specific protein factors to bind RNA and/or affects the localization of RNAs in the cell.
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. Independently, we study the perpetual arms race between bacteriophages and their bacterial hosts to uncover novel mechanisms of transcription regulation and defense mechanisms used by the host to counter viral attacks. We also study microcins, small ribosomally-synthesized inhibitors of bacterial growth. The following research projects were actively pursued during the last year:

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.

Using a powerful fluorescence-based protein beacon method developed in the laboratory, the fine details of RNAP-promoter interactions with promoters and the ways these interactions are affected by various transcription factors and low-molecular weight inhibitors are quantitatively studied. An unusual dual function transcription factor p7 encoded by bacteriophage XP10 infecting Xanthomonas oryzae, an important rice pathogen, was characterized and its mechanism of function uncovered.

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 several bacteriophages and maintenance of plasmids have been created 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-sight activities of CRISPR effector complexes during target selection and target destruction.

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. Microcin J is a lasso peptide that inhibits bacterial RNAP, a validated drug target. Using bioinformatics searches,
The yeast alpha and a proteins, members of the homeodomain family of DNA-binding proteins, bind in combination to specific DNA sites to repress haploid-specific genes in the diploid cell type. To determine which genes are regulated by the a1/alpha2 repressor complex, we searched for potential target sites of the complex in the yeast genome. In addition to finding binding sites in the promoter regions of genes that are repressed by the a1/alpha2 complex, we identified binding sites downstream of two genes that require a1/alpha2 for expression in diploid cells. We have shown that these sites regulate transcription of haploid-specific, antisense ncRNAs that interfere with expression of their respective genes. Repression by these antisense transcripts only works in a cis configuration to the genes that they are regulating, suggesting that that mechanism of repression is not through the formation of double stranded RNA. Chromatin immunoprecipitation (ChIP) assays showed that the repression of the sense transcripts does not interfere with transcription factors binding to the promoters. We have identified regions within the open reading frames 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 1000 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 ncRNAs 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.
s) Sustainable Biofuels

The goal of this program is to modify and control the metabolism of microbial photosynthetic organisms as a means to produce fuels from sunlight and CO2. We apply genetic and environmental methods to modify the metabolic pathways within microbial photoorganisms to reroute the flux of fixed carbon and extract hydrogen.

Translation of research: Rutgers catalysts for water splitting, both an OER and HER catalyst, were submitted manuscripts in peer reviewed journals. to an industrial developer, 8 publications and 8 another patent filed, one compound licensed advances are represented by 1 patent published.

Fundamental scientific and technological advances are represented by 1 patent published, another patent filed, one compound licensed to an industrial developer, 8 publications and 8 another patent filed, one compound licensed.

**Regulatory Mechanisms for Polarity Protein 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). Polar trafficking of BASL, a membrane novel protein, has not been successfully connected to either pathways and might represent an unknown mechanism. Our previous results revealed the significance of BASL to be polarized at the cell cortex region for its function. We started pursuing the polarity determining factors from BASL protein itself. After a fine-scale analysis, including domain swapping, deletion, point mutations and other modifications, we are able to identify a conserved MAPK-docking motif that

**DONG LAB**

**Cell Polarity and Asymmetric Division in Plants**

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.

**Summary**

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 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 highlight specific subsets of polarity defects.

**Figure 1. BASL localization and stomatal asymmetric fate**

**Figure 2. Phosphorylation is important for BASL nuclear exportation**

A) Putative MAPK phosphorylation sites in BASL. (B-C) Phospho-defective BASL and BASL (D) basal phospho-deficient mutant. (E-F) Phospho-deficient BASL and BASL (G) wild type BASL. (H) The MAPK/ERK YODA binds to phospho-mimicking BASL 123456D stronger than the wild type and phospho-deficient BASL in a yeast-two-hybrid assay. (I) A dominant negative version of YODA (DhYODA) is polarized at the cell cortex, similar to where BASL concentrates (arrow).
Remarkably, when tagged with YFP and expressed on the interaction with YODA. Phospho-mimicking MAPKKK YODA, which showed interaction with BASL cortex. We recently isolated a binding protein, the physical partners of BASL, particularly at the cell cortex. One focus of our research has been investigating the trafficking network.

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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Figure 3. BASL reorganizes the YODA MAPK signaling pathway (A) Representative confocal image of CFP-BASL overexpression (cyan) in tobacco epidermal cells. Note, expression in the nucleus and at the cell periphery (not polarized). Images were captured 2-3 days after infiltration. (B) Overexpression of YODA-YFP. (C) Co-expression of CFP-BASL (cyan) and YODA-YFP (yellow). Note the protein distribution change. White arrowheads indicate protein polar accumulation. Red marks cell outline. (D) Co-expression of CFP-BASL (cyan) with split YFP of DNA and MPK6 (yellow). (E) BASL and the YODA MAPK cascade form a feedback loop, which reinforces cell polarity in stomatal ACD precursors. This model does not exclude the possibility of MAPKs or other kinase phosphorylating BASL in the nucleus, cytoplasm and/or at the plasma membrane.

Polarity Protein Complex at the Cell Cortex and Reorganized MAPK Signaling in Stomatal ACD

One focus of our research has been investigating the physical partners of BASL, particularly at the cell cortex. We recently isolated a binding protein, the MAPKKK YODA, which showed interaction with BASL in a yeast-two-hybrid assay (Fig. 2). Interestingly, the phosphorylation status of BASL has differential impacts on the interaction with YODA. Phospho-mimicking BASL (123456D) showed stronger interaction with YODA than the phospho-deficient version (123456A). Remarkably, when tagged with YFP and expressed in the Arabidopsis stomatal lineage, YODA displayed a polarized pattern similar to that of BASL (Fig. 2).

We also established a transient expression assay in tobacco epidermal cells and showed that, when co-expressed, BASL and YODA redistribute and enrich to sub-regions along the plasma membrane (Fig. 3A-C). Furthermore, we demonstrated that the YODA MAPK signaling was spatially reorganized into a highly polarized pattern by the co-expression of BASL. We therefore proposed a novel mechanism for plant cell polarization that involves the feedback regulation between BASL and the YODA MAPK pathway (Fig 3).

DOONER LAB
Dr. Hugo Dooner
Plant Genetics & Pathology
A Sequence-indexed Reverse Genetics Resource for Maize
A sequence-indexed, user-friendly, reverse genetics resource is highly desirable to fully exploit an organism’s 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. With the support of a $2 M grant from the NSF-Plant Genome Program we are continuing to develop such a reverse genetics resource for maize based on the transposable elements Ac and Ds. 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 next-generation sequencing technologies.

Specifically, we are sequencing new collections of insertions of engineered Dsg elements that carry the jellyfish green fluorescent protein (GFP) to facilitate tracking their movements in the genome. As of last spring, more than 3500 Dsg insertions had been mapped to the reference genome with our publicly available pipeline Insertion Mapper, developed by our collaborators Drs. Charles Du and Wenwei Xiong at Montclair State University (Fig. 1). The location of the insertions has been added to the websites of the Maize Genome Database (maizegdb.org) and our project (acdsinsertions.org), where they are cross-referenced to stocks available from the Maize Stock Center.

We 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.

Polarized Recombination at the bz Locus
Meiotic recombination between homologous paternal and maternal chromosomes allows the shuffling of genes that otherwise would be coherently and originates at nonrandom sites termed hotspots. In maize, an organism with 90% repetitive DNA, recombination hotspots correspond to genes.

Gene conversion is a nonreciprocal form of recombination that leads to aberrant allelic segregation ratios and is recognized genetically in most higher organisms by the production of intragenic recombinants having a parental arrangement of flanking markers. To investigate whether or not gene conversion events are evenly distributed within the gene, two large diallel crossing matrices involving 7 mutant sites spread across the bz gene were
performed and over 2500 intragenic recombinants were scored. In both diallels, around 90% of recombinants could be accounted for by gene conversion.

We have found that mutant sites located within 150 bp of the start and stop codons of the bz gene convert to wild-type more frequently than those in the middle. The striking conversion polarity observed argues for a polarized, rather than random, distribution of recombination initiation sites within the gene.

Summary

One of the most distinctive features of plant development is that plants establish a body plan and form the vast majority of organs after embryogenesis. This ability is provided by small groups of pluripotent 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 also provide the ability 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 determination.

In our laboratory we study the formation and activity of axillary meristems, a class of meristems that are responsible for the formation of branches and flowers in plants. We use maize as a model system for our research because of the vast genetic and genomic resources available, and because of its economic 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/IAAs frees interacting activating ARF transcription factors from TPL repression, allowing them to activate downstream genes. Aux/IAAs and ARFs belong to large families of transcriptional regulators whose combinatorial interaction is believed to trigger specific developmental responses.

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 (Figure 1). We are part of a collaborative research project sponsored by the NSF 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). This research focuses both on maize, a monocotyledonous species, and Arabidopsis, a dicotyledonous species.

Using forward genetic screens, we have identified and characterized several mutants affected in inflorescence development that are likely impaired in auxin function. Among these, we are studying two novel semi-dominant maize mutants that are severely impaired in reproductive organogenesis. These genes act synergistically to initiate the many specialized types of reproductive axillary meristems that form the highly complex inflorescences of maize. Our findings show that both genes are core components of the auxin signaling pathway and suggest that maize has specifically co-opted these genes for the initiation of reproductive primordia. As a complementary approach, we are characterizing additional components of the auxin signaling pathway in maize inflorescences by a combination of expression and molecular analysis, as well as reverse genetic approaches.

Figure 1. Defective auxin-signaling impairs organ formation (branches and flowers) in maize tassels. Left panel, normal tassel; right panel, auxin-signaling mutant.

Dr. Andrezza Gallavotti
Plant Biology & Pathology

Mechanisms of Plant Development

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Development and Productivity
Mechanisms of Boron Transport for Maize

In maize, low levels of boron in the soil affect vegetative and in particular reproductive development, eventually causing widespread sterility in its inflorescences. We recently reported the characterization of the maize 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 certain areas of the world.

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 and high quality harvests. The productivity of a variety of crops in nearly 80 countries has been impeded due to deficiency in boron, making it more widespread than deficiencies in any other plant micronutrient. While fertilization is one option to alleviate poor 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.

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Summary
Plastids are semi-autonomous organelles with a relatively small (20-180 kb), highly polyploid genome present in 1,000 to 10,000 copies per cell. Over the past twenty years we have developed protocols for the transformation of the tobacco (Nicotiana tabacum) plastid genome based on integration of the transforming DNA by homologous recombination. We use plastid transformation to study plastid biology, transgene biosafety and biotechnological applications of plastid transgenes. Currently, we pursue research in the following areas.

Plastid Engineering in Maize

Most commercial maize grown in the United States is genetically modified to carry herbicide and/or insect resistance traits. The genes encoding these valuable agronomic traits are localized in the plant nucleus, and capable of spreading via pollen. The plastids in maize are maternally inherited; therefore incorporating the genes in the plastid genome would provide a natural means for transgene containment. Plastid transformation is routine in the dicotyledonous crops, but thus far has not been successful in monocots. Our goal is to develop a routine protocol for plastid transformation in maize, an important crop and a cereal model species.

To facilitate biotechnological applications, we sequenced the plastid genome of three commonly used fertile maize lines and three of the principal cytoplasmic male sterile cytoplasts. Utilizing the new plastid DNA markers we determined that the plastid genome of independently isolated cms-T lines are identical. This implies that the tested 27 cms-T group accessions have a single origin and strict maternal co-transmission of plastids and mitochondria to the progeny. Our data exclude exceptional pollen transmission of mitochondria or multiple horizontal gene transfer events as the source of the urf13-T gene in the cms-T cytoplasts.

Intercellular Movement of DNA-containing Organelles Through Graft Junctions

Horizontal gene transfer is an evolutionary process in which a recipient organism acquires genetic material from a donor organism by asexual means. Although there is evidence for horizontal gene transfer during evolution, cellular DNA is normally assumed to stay associated with its compartment. To address the mechanism of intercellular organelle (or organelar DNA) transfer, we grafted two species of tobacco. Nicotiana tabacum carries a nuclear gentamycin resistance gene and Nicotiana sylvestris a plastid-localized spectinomycin resistance gene and cytoplasmic male sterility encoded in the mitochondrial genome. Grafting triggers formation of new cell-to-cell contacts, creating an opportunity to detect cell-to-cell organelle movement between the genetically distinct plants.

We report movement of Nicotiana sylvestris mitochondria into Nicotiana tabacum cells through a graft junction. Co-transfer of mitochondria with chloroplasts was observed in plants regenerated from the graft zone. The flowers of N. tabacum are male sterile due to a sterility causing mitochondrial genome. Evidence of N. sylvestris mitochondrial transfer was recognized by restoration of pollen fertility in some flowers (Figure 1). Analyses of the mitochondrial genomes...
plants only one. Under low light conditions many isoforms of the D1 protein, whereas algae and higher phototrophs. Cyanobacteria encode at least two separation and water oxidation in all oxygenic the primary photochemistry of light-driven charge core polypeptides that make up the unit performing the D1 protein of photosystem II (PSII) is one of six core polypeptides that make up the unit performing the primary photochemistry of light-driven charge separation and water oxidation in all oxygenic phototrophs. Cyanobacteria encode at least two isoforms of the D1 protein, whereas algae and higher plants only one. Under low light conditions many cyanobacteria express a standard low-light inducible D1 isoform that has greater photochemical efficiency at low light intensity. However, when the cells are exposed to a stress such as even moderate light intensity, the expression of a more robust light-tolerant D1:2 isoform is upregulated and preferentially incorporated into PSII. Following the cyanobacterial design, we modified the tobacco native highlight D1 isoform into low light (LL) and high light (HL) variants. The photochemistry characteristics, including PSII charge separation rate, \([P680+QA-]\) recombination kinetics and WOC cycling efficiency, of the LL and HL tobacco D1 are consistent with those of cyanobacterial D1 isoforms. Light utilization of the plants with the engineered D1 isoforms are also evaluated by measuring biomass accumulation rate at different solar intensities. These experiments are carried out in collaboration with the Charles Dismukes laboratory.

Engineering Photosystem II to Improve Photochemical Efficiency

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Bioenergy, Shotgun DNA Sequencing, Genome Evolution, Genome Structure

Cultivated and non-cultivated plants are playing an ever-increasing role for sustaining life on earth. They reduce carbon with solar energy, thereby providing air to breathe, food and feed, medicine and vital supplements, a cleaner environment, building material, and renewable energy. The list is probably not complete, but it illustrates that there are many essential products that are derived from plants. Therefore, basic studies of different plant species will play an important economic role in the future. In our laboratory, we work with two species that hold great promise for future energy applications, sorghum and duckweeds.

In a consortium of laboratories, we have recently sequenced and annotated a sorghum cultivar Btx623, which is mainly used as a feed source in southern of the US. However, there is a cultivar that accumulates a high level of sugars in the stem compared to grain sorghum. This cultivar is known as Rio. Given these two phenotypes, we can take a genetic approach to identify genes that play a role in carbon reallocation during growth. A better understanding of carbon allocation would be critical to breeders in selecting new crop varieties that would be critical as a renewable energy source. In this respect, we were surprised to find that in this cultivar Rio carries an allelic copy of the miRNA169 gene family that is linked to higher sugar content. Previously, it was known that copies of this gene family contribute to the drought tolerance of sorghum. A patent application has been filed to permit practical applications.

A major limitation for crops is their limits in the amount of water for yield. Although it might not be intuitive, aquatic plants and algae require less water for growth than terrestrial plants. They require water as a surface to start with but the total amounts to less in one season than what is used in irrigation. An example of aquatic plants is duckweeds or species of the Lemnoideae that grow on still water or slow moving streams. They have a reduced plant body structure, called fronds, which are thin leaves. Because of a size lager than algae they are easily separated from water, which would be a substantial cost in using biomass. The Lemnoideae are also monocots like the grasses, but belong to a different order.

We previously analyzed the first reference genome in this order belonging to the duckweed Spirodela polyrhiza, which has a similar size as the dicot Arabidopsis with 158 Mb. Still, it has with 19,623 predicted genes 25% less than Arabidopsis and 50% less than rice. Our whole-genome shotgun sequencing assembly produced 32 supercontigs although there were only 20 chromosomes, clearly indicating that some of those were chimeric. Therefore, we selected several 100 BACs positioning their end-sequences to the supercontigs and reconstructing their full-length sequences. Then, we selected those that were low in repeat elements and spread throughout the set of

Molecular Genetics

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supercontigs. About 100 of the selected BACs served as hybridization probes for multicolor fluorescence in situ hybridization (mcFISH) to break the supercontigs into chromosome segments that could be fused to yield the full-set of 20 chromosomes, which will serve as a reference for the karyotyping of all the species in the Lemnoidae.

Protein Quality

The emergence of a nation-wide bio-ethanol production network has created several undesirable side effects. Among them is the use of maize starch for ethanol production rather than feed. Maize like sorghum, oats, barley, and wheat belong to the family of grasses, which provide the major crops for nutrition. The latter three belong to the tribe of the Triticeae in the subfamily of the Pooidae. They end up in many food products that are directly consumed, as for instance breads and noodles. Because their genomes are very large in size, another member of the same subfamily, Brachypodium, with a small size of 272 Mb has been sequenced and used as a reference genome for the others, in particular for wheat. The seed storage proteins in this subfamily have diverged from the proteins of species in other subfamily in such a way that only wheat can be used to make flour for bread and noodles. However, when proteins from wheat are digested, people with having alleles of HLA-DQ2 and HLA DQ8 can produce an autoimmune response, a medical condition called celiac disease. This affects about 1% of the population, requiring them to live a gluten-free diet. We are now investigating whether we can develop a novel cereal that produces flour with the sensory and baking characteristics of wheat but is non-toxic to individuals with celiac disease.

Despite the divergence of the proteins, which are also called prolamins because of the preponderance of glutamine and proline in their sequences, we were curious whether the divergence would also extend to the regulatory regions of the prolamin genes. We were able to take a unique approach because one can make wide crosses between oats and maize that can result in the addition of single maize chromosomes to the oat genome. Such oat-maize addition lines can therefore be used to investigate, whether maize prolamin genes can be regulated by oat-genes or the other way around. Based on previous phylogenetic studies of the prolamin genes in grasses, we could conclude that older genes were conserved in their regulation, but younger ones acquired new sets of transcriptional activators.

The divergence of regulation of gene expression in plants can be affected in different ways, one by the spreading of transposable elements in genomic regions of the genome, the other by epigenetic regulation of gene expression. We have explored this on two fronts. Within a cluster of maize prolamin genes, we discovered a transposable element that inserts into non-coding sequences of genic regions. This transposable element is also referred to as terminal-repeat transposon in miniature or TRIM, which mostly spread after allotetraploidization and has been found in many landraces and improved maize lines. Although, there are not yet specific examples how TRIMs create Mendelian phenotypes, they certainly provide useful molecular markers because each sequence-specific TRIM family has a relatively small copy number and each copy is linked to specific genes.

In respect to epigenetic regulation of gene expression, we investigated the acetylation pattern of lysine 27 of histone 3 in the maize genome. Because the maize genome has a rather large content of transposable elements, any specific epigenetic modification of genes would therefore be localized to a rather small proportion of the genome. Indeed, we could show that acetylation of lysine 27 of histone 3 is localized in gene-rich regions and largely absent in gene-poor regions. Moreover, inspecting known gene loci, acetylation marked actively transcribed genes. Given the preservation of these marks after the maize genome gained most of its DNA in the last four million years after allotetraploidization, one would expect that such an epigenetic mark for gene expression is conserved throughout the plant kingdom.
**CORE FACILITIES**

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

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

The Waksman Confocal Core Facility has approximately 35 trained users, primarily Waksman researchers, from thirteen 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.

**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.

**Waksman Genomics Core Facility**
Waksman Genomics Core Facility (WGCF) is a state-of-the-art laboratory facility, providing high-throughput next generation sequencing services to the Rutgers research community and to the broader scientific community. With three sequencers, NextSeq500, MiSeq and Ion Proton, WGCF covers a broad range of NGS requirements. NextSeq500, Illumina’s newest desktop sequencing instrument is most suitable for large-scale sequencing needs. It provides roughly 120 Gb data from its 300 cycle run in 2x50 bp configuration. NextSeq 500’s push-button operation provides a thirty-hour turnaround time for an array of popular sequencing applications such as single human genome, 20 transcriptomes or up to 16 exomes in a single run. Our second instrument, MiSeq with relatively long read-length and low throughput, is best suitable for small bacterial size genomes and targeted sequencing. Semiconductor-based sequencer Ion Proton, with 80 million reads per run adds another dimension to our capabilities. Ion Proton 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 times of four hours allowed us to reduce the turnaround time. The equipment and services provided by the genomics core are aimed for reducing the time required and designing experiments or sequencing strategies. In addition to the sequencing, WGCF also offers Real-Time PCR on Thermo Fisher’s StepOnePlus system, Digital PCR, DNA shearing services using Covaris, as well as DNA qualification services using Fluorometer Qubit, NanoDrop and Agilent Bioanalyzer.

Our mission is to keep the core facility as comprehensive and accessible as possible in order to increase research productivity. WGCF accepts raw samples as well as prepared libraries, giving researcher’s flexibility to try their protocols. Our projects involved seven Waksman Principal Investigators, across the full spectrum of organisms including bacteria, plants, as well as mammals, reflecting the diversity in research at the Waksman Institute. During 2014-15, the WGCF provides sequencing services for 12 projects. We were able to deliver more than 300 transcriptomes and over 30 whole genomes sequences. In addition to sequencing, the WGCF has delivered primary analysis of the resulting data as well as enhanced bioinformatic support for Waksman investigators and its collaborators. WGCF provided DNA mapping against reference genomes, de-novo assembly, ChIP-seq and RNA-seq analysis as part of the DNA sequencing services. WGCF collaborates 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.

In the year ahead, we will continue to offer an accurate and innovative sequencing and bioinformatic tools to facilitate research improving our support to Waksman investigators in providing deep sequencing and project data analysis.

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Cell and Cell Products Fermentation Facility
For more than sixty years, the Cell and Cell Products Fermentation Facility has provided affordable fermentation services to a highly diverse client base.

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/cosmetic industries. The facility also serves as a production unit and an incubator for virtual companies.

Designated BL2-LS for containment of recombinant organisms, and licensed by the EPA for the production of bioinsecticides and bioherbicides, the facility is; self-supportive through its fee-for-service structure, validated, certified to NIST standards and follows cGMP requirements for the production of 'Preclinical Biologics'. The facility’s bioreactors range from 30 to 1000 liters.

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Postdoctoral Research

Research Summary
Over the last year I have primarily worked on two projects that relate to different aspects of transcription by multisubunit RNA polymerases in prokaryotes:

Project 1: NAD and 3'dp-CoA Capping of RNA 5' Ends Occurs at Initiation During Transcription Initiation
Recent work has shown that certain RNAs carry 5' caps resembling nicotinamide adenine dinucleotide (NAD) or 3'-diphospho-coenzyme A (dpCoA). It has been proposed that NAD and dpCoA caps are added to RNAs post-transcriptionally, in a manner analogous to addition of γ-methylguanilate caps. I have performed experiments that show NAD and dpCoA are incorporated into RNA 5' ends during transcription initiation, functioning as non-canonical initiating nucleotides (NCINs). Both bacterial RNA polymerase and eukaryotic RNA polymerase II are capable of incorporating these NCINs during initiation. Additionally, Yu Zhang from the Ebright lab has solved crystal structures of bacterial transcription initiation complexes containing NAD and dpCoA-capped products. Also, promoter sequences affect the efficiency of NAD capping. Some promoters, such as chromosomally encoded gadY and the plasmid origin gene rnaI, are significantly better (10 to 100-fold) at initiating with NCINs than many promoters commonly used in transcription work like rrnBP1, N25 and T7A1. These results define the mechanism and structural basis of NAD and CoA capping and suggest that ab initio at initiating with NCINs than many promoters commonly used in transcription work like rrnBP1, N25 and T7A1. These results define the mechanism and structural basis of NAD and CoA capping and suggest that ab initio

Project 2: Sigma-70 Dependent Transcriptional Pauses are Modulated by a General Consensus Pause Element and Core Recognition Element (CRE) Recognition.
It has been known for some time that many prokaryotic and lambdoid phage late genes contain a promoter proximal transcriptional pause that is dependent on sigma-70 initiation factor recognizing and interacting with a near duplication of the -10 promoter element. These pauses occur approximately 10 to 12 nucleotides downstream of the -10-like element. In my graduate work I showed that the phage 82 late gene pR' sigma-70 dependent pause contain a second element that is responsible for the determination of nucleotide at which the pause occurs and that this element resembles the general pause consensus element published in Larson et al. (2014) and Vvedenskaya et al. (2014). Although the 82 pR' pause element is not a direct match to the consensus reported in those papers. For this project I performed transcriptional analysis of the Lambda phage pK' sigma-70 dependent pause. This pause contained a 3-6 of match to the consensus pause element [G-10(CY)G+] from our previous work (Vvedenskaya et al. 2014). Mutating these three positions weakened pausing predictably in both in vitro transcription and in vivo northern blotting experiments confirming that these three consensus bases are an additional requirement for factor dependent pausing to occur. Additionally, experiments with RNAP polymerase deficient for interaction with CRE motifs (RNAP Rd46A) show that RNAP CRE interaction is also modulating these pauses through that G+1 base, again, like the previously studied factor independent pauses.

Biological hydrogen production offers the cleanest energy carrier and when derived from photosynthetic biomass, offers the lowest net carbon emission among all biofuels. However, lower H2 yields and slow fermentation rate pose bottlenecks for commercialization. Here we demonstrate major improvement in H2 yield produced by metabolic engineering of the catabolic pathways that control the autofermentation of intracellular glycogen produced during prior photosynthetic growth. NiFe-hydrogenase functions as the terminal H2 producing/uptake enzyme under dark anoxic conditions. In earlier work we showed that eliminating competing sinks for NADH by genetic knockout of lactate dehydrogenase (LDH) significantly increased the H2 production rate (12% for ldhA knockout or lKo) and yield, but fermentative rates and yields were still low. Here we adopt two strategically different genetic approaches: 1) reroute autofermentative glycogen catabolism from low-to-high yielding NADPH pathways by knocking out glyceraldehyde phosphate dehydrogenase (GAPDH-1, gap1 (gKo)), and 2) accelerate both gluconeogenesis during the photosynthetic phase and glycogen catabolism during the autofermentative phase by overexpressing gap (gEx). Both strategies were tested in Synechococcus sp. PCC 7002 and were combined with the LDH knockout (lKo) to create double mutants. We confirmed that the gKolKo mutant forces flux through the oxidative pentose-phosphate (OPP) pathway, using Agilent's 6490 QQQ mass analyzer coupled to 1200 series HPLC. This highly sensitive QQQ machine was also employed to quantify intracellular redox status (NADPH and NADP+). An analysis of the steady-state rate of anaerobic catabolism and the static pool sizes of both OPP and glycolytic metabolites (determined by LC-tandem-MS) reveals that a kinetic bottleneck exists at GAPDH-1, and that the NAD(P)H/NAD(P) redox poise, not the glycogen content, limits the catabolic flux of all three engineered strains. H2 uptake strongly limits the H2 production rate and yield, but can be overcome by removal of H2 from the headspace or directly from solution, consistent with reversible hydrogenase equilibrium: H2+NADH → H2O+2NADH. Under H2 milking, the gKolKo mutant produced the largest amount of H2 (334 nmol H2/mg DCW, DCW), with a glucose to H2 energy conversion efficiency (ECE) of 53.3 % and an average rate of 28.4 nmol H2/mg DCW/hr, followed by gExKo producing 334 and 265 nmol H2/mg DCW and rates of 13.9 and 11.0 nmol H2/mg DCW/hr, respectively, under H2 milking conditions. This is the highest H2 yield and rate from any cyanobacterium under autofermentation, and results from shunting glycogen catabolism through the OPP pathway, blocking LDH, and H2 milking strategies.

50

51
The coordinated regulation of genes and hormones is required for the initiation and maintenance of groups of pluripotent stem-cells called meristems, whose activities in maize regulate the formation of its inflorescences, the uppermost male tassel and the lateral female ears. All meristems have two basic functions: i) to maintain an active population of stem cells, and ii) to form lateral organs, such as leaves and flowers. My project in Dr. Gallavotti’s lab is focused on cloning and characterizing a novel semi-dominant maize mutant, called Barren inflorescence3 (Bif3). Bif3 mutants show severe inflorescence phenotypes. Cloning and characterization of bif3 gene will increase our understanding of the molecular mechanisms regulating plant stem cell formation and maintenance in maize, an economically important crop.

The Bif3 semi-dominant mutant shows striking inflorescence meristem termination defects that result in severely malformed tassels and ears. The formation of tassels with shortened central spikes and ears that are stunted and partially bald. Detailed morphological analysis of Bif3 mutant inflorescences shows a significant decrease in the number of paired spikelets, which are instead often replaced by single spikelets or by barren regions completely devoid of spikelets. Furthermore, the inflorescence meristem often appears to collapse during development, thus resulting in smaller tassels and ears. These phenotypes suggest that bif3 is required for both the initiation and, in particular, the maintenance of meristem activity.

Using BSA (Bulk Segregation Analysis), the region containing the bif3 locus was mapped to chromosome 2. Further genetic mapping has narrowed the causative locus to a 50kb region that encompasses five expressed genes. Among the candidate genes found in this small window, there is a co-ortholog of the Arabidopsis WUSCHEL (WUS) gene, known to regulate meristem maintenance.

Preliminary data by qRT-PCR with the four genes showed a four-fold increase in the expression of ZmWUSs in Bif3 homozygous mutant ears relative to wild type, but no significant changes in gene expression for the other four genes. The significantly unregulated gene in Bif3 mutant inflorescences suggests that its misexpression may underlie the observed phenotype.

Sequenceing of the region surrounding the ZmWUS gene identified a 380bp hAT transposon Ins2 insertion around 7kb upstream of ZmWUSs start codon found in Bif3 mutants. Subsequent sequencing of this same region in 27 maize inbred lines showed that this transposon insertion is unique to Bif3 mutants. Taken together, my data strongly suggest that ZmWUSs corresponds to the Bif3 locus.

Last but not least, I established a transgenic platform for the Gallavotti lab and I’ve made a significant achievement since it allowed us to obtain maize transgenic lines at a fraction of the cost normally charged by the Iowa State Transformation Facility, as well as providing the flexibility for generating new transgenic lines when needed, since it allowed us to obtain maize transgenic lines at a fraction of the cost normally charged by the Iowa State.

Two molecular systems are involved in establishment and maintenance of PCP: Frizzled (Fz) PCP pathway and Dachsous (Ds)-Fat PCP pathway. The Ds-Fat PCP pathway includes the cadherin family proteins Ds and Fat, a Golgi localized kinase Four-jointed (Fj) and an unconventional myosin Dachs. Ds and Fat interact between neighboring cells and Fj modulates the binding between them. Ds and Fj are expressed in opposing gradients in Drosophila tissues. Earlier I have shown that Ds and Fat are asymmetrically localized in a cell. Fat accumulates on the side where it contacts cells with higher Ds and lower Fj, while Ds localizes in a complementary orientation. Fat influences the localization of Dachs, which accumulates on the same side as Ds. The Fz pathway includes transmembrane proteins Fz and Van Gogh (Vang), cadherin family protein Starry night (Stan) and cytoplasmic proteins Dishevelled (Dsh), Diego (Dgo) and Prickle-Spiny legs (Pk-Sple). Stan-Vang interacts with Stan-Fz heterodimers in neighboring cells. Dgo and Dsh associates with Stan-Fz while Pk-Sple associates with Stan-Vang.

It was not clear how the Ds-Fat pathway interacts with Fz pathway to coordinate polarity along the axis of a tissue. I have now shown that Dachs and Ds can each physically interact with Spiny-legs through the N-terminal region of Sple. Dachs and Ds influence Sple localization in different regions of wing imaginal disc and contribute to hair polarity in adult wing. Also, Ds can polarize Sple independently of Dachs in eye imaginal disc and regulate ommatidial polarity. Similar influence of Dachs and Ds is observed on Sple localization and hair polarity in abdomen, along with differences in few regions. These observations identified the molecular mechanism by which the Ds-Fat pathway interacts with the Fz pathway to influence PCP. Overall, these studies help in understanding how the Fat-Hippo signaling network regulates PCP, growth and patterning to yield organs of appropriate size and shape.

Genotyping Reveals Uniformity of Plastids in cms-T Maize

Cytoplasmic male sterility (CMS) is the failure of plants to produce functional pollen as a result of the expression of a toxic mitochondrial gene. Nuclear restorer genes suppress this mitochondrial effect, restoring pollen fertility. CMS lines in maize have been classified by their response to specific restorer genes into three categories: cms-C, cms-S, and cms-T. To identify markers for plastid genotyping, we sequenced the plastid genomes (ptDNA) of three fertile maize lines (B73, A88) and the B73 cms-C, cms-S, and cms-T lines. To gain insight into the origin and transmission of the cms-T trait, we identified three SNP's 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 cytoplasms. We found that each of the 27 cms-T group accessions have the same plastids indicating a single origin and maternal transmission of the cms-T mitochondria and plastids to the seed progeny. Our data exclude exceptional pollen transmission of mitochondria or multiple horizontal gene transfer events as the source of the urf3-T gene in the cms-T cytoplasms. Plastid genotyping enables a reassessment of evolutionary relationships of cytoplasms in cultivated maize.
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.

Microtubule Associated Proteins Regulate Stomatal Development and Patterning in Arabidopsis

Asymmetric cell division (ACD) is a fundamental process in the development of almost all multicellular organisms. During ACD, precursor cells divide to create daughter cells that differ in size, location, and cellular components. Such division functions to maintain stem cell population, specify various cell fates, and provide patterning.

Our recent work established a positive feedback loop between the polarity protein BASL and the YODA (YDA) MAPK pathway, which directs protein polarization and connects cell polarity to fate differentiation during stem cell ACD in plants (Zhang et al., 2015). The flowing key question is how BASL-YDA polarity governs the asymmetric placement of the division plane during ACD. A specialized microtubule organization at the cell cortex, called the preprophase band (PPB), has been found to predict where the nascent cell wall to be placed. The PPB is assembled at the preprophase and disassembled at the metaphase, leaving a cortical division site (CDS) to orientate the division place.

Recently we identified a novel protein family, YIPs (YDA Interacting Proteins), that physically associate with the MAPKKK YDA to regulate the PPB placement and stomatal asymmetric cell divisions. The Arabidopsis VIP family is comprised of 5 members, all of which were found actively transcribed in the early staged stomatal lineage cells. When tagged with GFP and expressed in the Arabidopsis stomatal lineage cells, all VIP members showed microtubule-like filamentous localization. Interestingly, overexpression of VIPs triggers stomatal clustering, a phenotype often resulted from the defects in stomatal ACD. I also found that the PPB organization and alignment was disrupted in these plants; the PPB expanded wider and some times two PPBs were generated in one cell. VIP-YIP was found along the cell cortex, in the nucleus, and at the PPB. We hypothesized that VIPs located at the plasma membrane and the microtubule may sense the positional cue at the cell cortex to regulate the PPB organization and positioning during asymmetric division. The physical association of VIPs with both YDA and BASL is under analysis and how the polarity cue is connected to VIPs and the PPB positioning will be pursued in my future study.

A. Primary Project:

1. RNA polymerase "core recognition element" (CRE): sequence determinants, recognition mechanism and functional roles:
   In recent published work, our laboratory identified a sequence-specific protein-DNA interaction between the RNA polymerase (RNAP) core enzyme and a sequence element—the "core recognition element" (CRE)–located in the transcription-bubble nontemplate strand, and showed that this interaction plays functional roles in promoter recognition during transcription initiation. Analysis of RNAP-CRE interactions constitutes my primary project in the lab in which I am seeking to answer three main questions: What is the recognition mechanism of CRE? What are the functional roles for RNAP-CRE interactions? I have defined the consensus sequence for RNAP-CRE interaction, identified the residues of bacterial RNAP that mediate RNAP-CRE interaction, and have demonstrated that RNAP-CRE interaction plays functional roles not only in transcription initiation but also in transcription elongation. My results show:
   (1) The CRE consensus sequence is 5’-t/g n n T-G-3’.
   (2) RNAP β subunit residues 151, 446 and 451 determine specificity at the CRE position 6 ("CRE-G").
   (3) RNAP β subunit residue 183 determines specificity at CRE position 5 ("CRE-T") and RNAP β subunit residue 371 determines specificity at CRE position 1 ("CRE-tg").
   (4) In transcription elongation, RNAP-CRE interactions stabilize the transcription open complex. Additionally, they favor start-site selection (TSS) at positions upstream of consensus CRE sequences (shown partly in collaboration with Nickels lab). Moreover, RNAP-CRE interactions affect abortive transcription in initial transcription.
   (5) In transcription elongation, RNAP-CRE interactions affect translocation bias by favoring post-translocated states at positions upstream of consensus CRE sequences. Additionally, these interactions counteract sequence-dependent pausing by facilitating forward translocation at pausing-prone 5’-YG-3’ sequences (partly in collaboration with Nickels lab).

   This work has resulted in one published paper (Vvedenskaya et al, Science 2014) and additional papers which are currently in preparation.

B. Secondary Projects:

In addition to my primary project, I have been involved in several secondary projects, the most important of which are listed below:

2. Transcription antitermination factor λQ: I have studied the DNA binding properties, including affinity and sequence specificity, of a crystallographically characterized protein fragment of λQ (Vorobiev et al, Structure, 22:488-495, 2013).

3. Small-molecule inhibitors of RNAP, targets and binding parameters: I have used stop-flow-fluorescence techniques to study binding kinetics of small-molecule inhibitors of RNAP (Zhang et al, nelife, 2014). In addition, I have used radiochemical equilibrium-dialysis techniques to determine binding constants for inhibitors and to demonstrate binding competition between inhibitors having overlapping binding sites.

4. Small-molecule inhibitors of RNAP, mechanisms: I have used pyrophosphorylation assays to assess effects of inhibitors on transcription complex transcriptional states and catalytic activity (Degen et al, elife, 2014).

5. Small-molecule inhibitors of RNAP, pharmacological parameters: I have used fluorescence-detected competition assays to quantify binding of inhibitors to serum albumin (a critical parameter for predicting activity in vivo and in mammals).
Cilia are morphologically and functionally diverse cellular organelles. Despite their diversity, all cilia contain a tubulin-based axoneme surrounded by plasma membrane and a conserved microtubule-based intraflagellar transport system that builds them. Molecular mechanisms that contribute to ciliary morphological and functional specializations are not well understood.

We use Caenorhabditis elegans as a model to understand ciliary specialization. We used Serial Electron Tomography and serial Transmission Electron Microscopy approaches to characterize the ultrastructure of the cephalic male neuronal (CEM) cilia in C.elegans. We found that in CEM cilia, nine microtubule doublets of the middle segment give rise to 18 singlets in the distal segment. This occurs by doublet splitting and extension of both A- and B-tubules as singlets. Some distal microtubule singlets become fused together at the distal tip of the CEM cilium. Microtubule doublet splitting has been observed in human spermatozoa although the functional consequences are not known.

To understand the molecular mechanism of how A-B doublet splits into A- and B-singlets, we took a candidate gene approach. tba-6 is expressed specifically in CEM cilia, whereas other tubulins such as tba-5 and tbb-4 are expressed in all ciliated neurons in hermaphrodites and males. We determined that TBA-6 is required for proper CEM cilia length, shape and function. In tba-6 mutant CEM cilia lack B-tubule singlets. In tba-6 mutant CEM cilia, only A-tubule singlets extend to the distal segment. B-tubules terminate at the end of the middle segment. This suggests that B-tubule singlets are of a different composition than the A-tubules and require TBA-6 for singlet extension.

Loss of tba-6 also alters the localization of ciliary proteins in CEM cilia. Using serial electron microscopy, we found that in tba-6 mutants CEM cilia have an elongated transition zone and ectopic Y-links in distal CEM cilia. However, tba-6 CEM cilia elongation is not accompanied by extended distribution of transition zone reporter NPHP-4::GFP. CEM cilia shed neuron-derived extracellular vesicles (EVs). We found that tba-6 mutants have EV cargo sorting defects, and are determining the role TBA-6 plays in EV-cargo selection. We are in the process of analyzing the localization patterns of GFP-tagged proteins that label CEM ciliary sub-structures in the tba-6 mutant background.

We conclude that axonemal B-tubule ultrastructure drives morphological and functional specialization of CEM cilia.


How To Specialize A Cilium: Alpha-Tubulin 6 Regulates Axonemal Ultrastructure

Investigating Tet Function in Drosophila

DNA methylation is a key epigenetic regulator of embryogenesis and stem cell differentiation in mammals. Tet(TET1/2/3) proteins have been recently identified as enzymes regulating active removal of the DNA methyl mark. They function as oxoglutarate- and iron-dependent dioxygenases which oxidize 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) [1]. In Drosophila, DNA methylation has been controversial and a recent whole genome sequencing study demonstrated only very low levels of DNA methylation in Drosophila embryos. Also, DNMT2, the only known candidate DNA methyltransferase in Drosophila does not recognize DNA as a substrate [2]. However, Tet is an essential gene in Drosophila. I have induced a null allele of Tet and found that the animals died at the pupal stage with strong ovary and brain abnormalities. In collaboration with Dr. Amjama Rao’s lab in San Diego working on vertebrate Tet, we determined that DNA 5-hmC, like 5-mC, is at most present at background levels in Drosophila. At about the same time that I obtained this result a study was published showing that Tet enzymes can also catalyze the formation of 5-hydroxymethylcytidine (5-hmC) in RNA in vitro and in tissue culture cells [3]. The biological functions of RNA methylation/hydroxymethylation are unknown. Dot blot experiment suggested that 5hmC did exist in Drosophila RNA and that it is highly enriched in mRNA. Because of the lack of DNA methylation/hydroxymethylation, Drosophila is an excellent model to study 5hmC in RNA

My hypothesis is that Drosophila TET catalyzes the conversion of 5-methylcytidine (5-mC) into 5-hmC and that this mark controls the processing, export or possibly translation of mRNAs. I have found that consistent with the Tetnull phenotype Tet is expressed in neural tissues in embryos, larvae and adults. I expect that the 5hmC mark has an important biological function during neuronal development and may be required for normal function of the nervous system. I will, in conjunction with our collaborators, Dr. Fuk’s laboratory at the Free University of Brussels, identify 5hmC-enriched genes in Drosophila embryos. The preliminary data suggest that Tetnull mutants display behavioral and neuron development defects. In my future experiments I will investigate if axon guidance and neuromuscular junction formation is affected in Tet mutants. My ultimate aim is to elucidate the biological and biochemical function of the 5hmC mark in mRNA. My work should result in the discovery and characterization of an entirely new epigenetic mechanism controlling gene expression in stem cells and developmental biology.

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 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 2014-2015

- Advanced Inorganic Chemistry
- Advanced Plant Genetics
- Byrne Seminar: Control of Gene Expression
- 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
- Honors Introduction to Molecular Biology and Biochemistry Research
- Honors Thesis Seminar
- Introduction to Molecular Biology and Biochemistry Research
- Microbial Biochemistry
- Microbiology
- Molecular Biology and Biochemistry
- Molecular Biology of Gene Regulation & Development
- Molecular Biosciences
- Mutant Isolation and Analysis
- Research in Biochemistry
- Research in Chemistry
- Seminar in Molecular Biology and Biochemistry
- Seminar of Molecular Biosciences
- Structural Biology, Structural Biophysics and Chemical Biology of Transcription
For over 20 years, faculty and scientists at the Waksman Institute (in conjunction with GE Healthcare Life-Sciences) 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 is funded by the National Science Foundation is titled: Conducting Authentic research, in an effort to bridge the gap between how scientific research is conducted versus how science is taught. 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 is funded by the National Science Foundation is titled: Conducting Authentic

The 2014 WSSP consisted of two interrelated parts: a Summer Institute (SI) and an Academic Year Program (AYP). In July 2014, 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 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

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 school, and conducted the project as an after school club. These courses and clubs provided additional students beyond those who attended the SI with opportunities to conduct 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 the 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 each participating school and teacher.

The Research Question
The 2014 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 bio remediation 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 2014-2015 SI and AYP, over 2121 plasmid clones were purified and 2534 were sequenced. To date, 1537 DNA sequences have been analyzed by the students and 1206 have 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 by Andrew Vershon at the Lawrence Livermore National Laboratory, Livermore, CA. As a result of these extended outreach activities and the growth in the number of schools participating locally in New Jersey, a total of 1246 students participated in the program this last year.

Upward Bound Program
In a separate outreach activity, the WSSP partnered with the Upward Bound Math Science Program at Rutgers University to hold a three-day DNA Workshop for 21 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.
**Waksman Annual Retreat**

**Presentations & Meeting Abstracts**

**Patents & Publications**

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**Waksman Annual Retreat**

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**Rutgers University Inn & Conference Center**

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September 15, 2014

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POSTER SESSION

Barr Lab
1. Alina Rashid, Branching Out: Determining the function of IL2 neurons in C. elegans dauer and non-dauer animals
2. Malan Silva, Alpha tubulin-6 and MT glutamylation regulate axoneme specialization in male specific CEM cilia in C. elegans

Dizmukes Lab
4. Yuan Zhang, Engineering of Photosystem II Dps Protein Subunit in Nicotiana tabacum by plastid transformation
5. Xiao Qian, Beyond flux balance analysis of photoautotrophic metabolism: Light intensity dependent partitioning of carbon into different biopolymers
6. Gennady Ananyev, Regulation of S-states cycling of Water-Oxidizing Complex in vivo in algae and cyanobacteria
7. Colin Gates, Physiological Adaptation to Sr-substitution in the Water-Oxidizing Complex
8. Xiao Qian, Beyond flux balance analysis of photoautotrophic metabolism: Light intensity dependent partitioning of carbon into different biopolymers

Dong Lab
9. Chao Bian, An Arabidopsis Mutant echo with Overproduced Stomata and Defective Patterning
10. Ying Zhang, BASL and MPK6 in stomatal development in Arabidopsis

Dooner Lab
11. Qinghua Wang, The unusual lifestyle of the maize dRemp retrotransposon
12. Yubin Li, A Sequence-Indexed Single Gene Knockout Resource for Maize
13. Wenwei Xiong, HelitronScanner uncovers a large overlooked cache of Helitron transposons in many genomes

Ebright Lab
14. Yu Zhang, Structural basis of de novo transcription initiation and structural basis of initial transcription pausing (also in Ebright Lab)
15. Hanif Vahedian Movahed, Sequence-specific RNAP-DNA interactions in transcription initiation and elongation: core recognition element (CRE)

Galvaggi Lab
16. Wei Li, Barren inflorescence3, a novel semi-dominant maize mutant defective in meristem initiation and maintenance
17. Qiuju Liu, Auxin signaling in maize inflorescence development
18. Iris Camehl, Dissecting the role of REL2, a key player in maize development
19. Mary Galli, Auxin and the Architecture of Maize Inflorescences

Genomics Core Facility
Waksman Institute Hosted Seminars
Dr. Jian-Kang Zhu, Purdue University. “Genome editing in plants using CRISPR/Cas.” September 12, 2014.
Dr. V. Siva Reddy, International Centre for Genetic Engineering & Biotechnology, New Delhi, India. “Chloroplast genetic engineering for high level expression of cellulosytic enzymes and antibiotics.” October 6, 2014.
Dr. Natalia Morsci, National Institute of Neurological Disorders and Stroke, NIH. “Age-related changes in neuronal mitochondrial load and dynamics in Caenorhabditis elegans.” April 17, 2015.
Waksman Student Scholars Programs
Waksman Student Scholars Summer Institute, Lawrence Livermore National Laboratory. July 28-August 1, 2015.
Barr Lab
• Barr, M. Exploring the Biology of GPCRs from in vitro to in vivo. Lorentz Center, Netherlands, August 2014.
• Barr, M. “Cilia in Development” session at the AAA meeting at Experimental Biology, Boston, March 2015.
• Barr, M. Kansas University Medical Center, Kidney Institute, April 2015.
Dissmues Lab
• Dissmues, G.C. KRC Photophysics, Bentley Univ. Waltham, MA, July, 2015.
• Dissmues, G.C. Eastern Regional Photosynthesis Conference. Wood Hole, MA, April, 2015.
• Dissmues, G.C. Invited speaker, NERM Northeast Regional Meeting American Chemical Society, Cornell Univ., June 11-12, 2015.
• Dissmues, G.C. Invited speaker, BASF Chemical Company, Ludwigshafen, Germany, July 22, 2014.
Dong Lab
• Dong, J. “Phospho-status of BASL regulates protein polar trafficking and coordinates cell fate in stomatal ACD.” Gordon Research Conference on Posttranslational Modification Networks at the Hong Kong University of Science and Technology, Hong Kong, China, 2015.
• Dong, J. “Re-organized MAPK signaling in asymmetric cell division.” Seminar in the 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. “Polarity-centered feed forward loop in stomatal asymmetric cell division.” 2013 American Society of Plant Biologists Mid-Atlantic Sectional Spring Meeting at Swarthmore College, Swarthmore, PA, 2015.
• Dong, J. “Re-organized MAPK Signaling in Asymmetric Cell Division.” Seminar in Tsing Hua University, Beijing, China, 2014.
• Dong, J. “Re-organized MAPK Signaling in Asymmetric Cell Division.” Seminar in Beijing Center for Plant Stress Biology, Shanghai, China, 2014.
• Dong, J. “Re-organized MAPK Signaling in Asymmetric Cell Division.” Seminar in Fujian Agriculture and Forestry University, Fuzhou, China, 2014.
Dooner Lab
• Dooner, H. “Gene Tagging with Green Ds,” Maize Genetics Conference, Pheasant Run, IL, March 12, 2015.
Ebright Lab
• Ebright, R. “Antibacterial agents: small-molecule inhibitors of bacterial RNA polymerase.” Department of Antibacterial Discovery, Novartis, Emeryville, California, 2015.
• Ebright, R. “Structural studies of transcription initiation and activation, and anti-tuberculosis drug discovery targeting transcription.” Department of Microbiology and Immunology, University of California, San Francisco, California, 2015.
• Ebright, R. “Structural studies of transcription initiation and activation, and anti-tuberculosis drug discovery targeting transcription.” National Institutes for Health, Department of Microbiology and Immunology, Harvard Medical School, Boston, Massachusetts, 2015.
• Ebright, R. “Structural basis of transcription initiation.” Biochemical Society/Wellcome Hardem Meeting, Transcription, Cambridge, United Kingdom, 2014.


Gallavotti, L.

Gallavotti, A. Molecular mechanisms of maize architecture. Purdue University, West Lafayette, IN, February 4, 2015.

• Cahill, M. and Gallavotti, A. Characterization of the maize boron efflux transporter family. Maize Genetics Conference Abstract 57:Ps56.

Gallavotti, A.

Gallavotti, A. Mechanisms of protein aggregation. Purdue University, West Lafayette, IN, February 4, 2015.

• Yuan, Z. and Gallavotti, A. Characterization of the maize boron efflux transporter family. Maize Genetics Conference Abstract 57:Ps56.
Ebright Lab


Dong Lab


Dissmukes Lab


Dooner Lab

press).


**Nickels Lab**


**Severinov Lab**


**Steward Lab**
