Waksman Institute of Microbiology

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Report of the Director

Mission Statement

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

Background

The principal mission of the Waksman Institute is research. While the initial emphasis of the institute at its founding was microbiology, its focus soon turned toward molecular genetics, and was later broadened to include organisms other than viruses, bacteria, and fungi. As a reflection of this new, broadened, vision of research at the institute, under previous directors fruit flies and plants were also studied. Since my assumption of the institute's directorship, I have strived to expand its investigative horizons to include computational and structural biology, and a further emphasis on the molecular genetics of regulation of gene expression and biomolecular interactions.

This new expansion of the Waksman Institute's investigative goals has stimulated the introduction of interdisciplinary programs with chemistry, computer science, and plant science. Indeed, the institute's research mission has evolved from a diversity of disciplines centered on antibiotics to a unified discipline of molecular genetics with a more diverse set of biological problems. The institute today employs faculty teams that concentrate on certain classes of organisms amenable to genetic analysis such as bacteria and fungi (*Escherichia coli and yeast*), animal systems (e.g., *Drosophila* and *C. elegans*), and plants (*Arabidopsis*, tobacco, and maize). Although the institute focuses on basic academic questions in microbial, animal, and plant research, it continues to seek practical and commercially viable applications of its discoveries. Historically, in fact, the institute owes its existence to the symbiotic relationship that exists between academic research institutions and the private sector. In 1939 Dr. Selman Waksman, the institute's founder and namesake, entered into an agreement with Merck & Company of Rahway, New Jersey, to study the production of antimicrobial agents by soil bacteria. Within four years, streptomycin, the first effective antibiotic against tuberculosis, was discovered, patented, and licensed to the pharmaceutical industry by Rutgers University.

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

Organization

The Waksman Institute is a research unit of the New Brunswick campus of Rutgers University, The State University of New Jersey. It receives a budget from the state to support the recruitment and appointment of faculty on separate budgeted research (SBR) lines that are split with instructional (IDR) lines from the decanal units of the campus. 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. The faculty of the institute also participate in the various graduate programs, thereby becoming fully integrated into the state university system.

Facilities at the Waksman Institute include:
A reading room with over 20,000 volumes on microbiology, biochemistry, and genetics; the Molecular Biology Computing Laboratory serving 203 users and 157 workstations, maintaining 20 servers (Apple Xserve, IBM Linux, SGI Origin, Sun v880) with a combined total of 15.9 TB (15,900 GB), holding site licenses for DNA sequence analysis packages that include FGENESH, GCG/SeqWeb, Jellyfish, Lasergene, and Vector NTI; a confocal microscopy suite for molecular and cellular biology; a modern computerized cell and cell products fermentation facility that has completed equipment validation and prepared standard operating procedures for such equipment. This validation allows us to meet requests for the manufacture of preclinical products under current Good Laboratory Practices (cGLP) and current Good Manufacturing Practices (cGMP) compliance.

Personnel and Budget

The institute currently consists of thirteen resident faculty members, four with non-resident membership, one with adjunct membership, and four with emeritus status. The institute accommodates one research assistant professor, ten visiting researchers, fourteen research associates, nine research assistants, thirteen postdoctoral researchers, twelve technical assistants, twenty-one graduate students, and seven visiting students. The Waksman Institute's total resident population is currently 121, which does not include the 30 undergraduate students that did independent research during the last year.
There was a significant decrease of Ph.D. level researchers in the institute last year to about the level we had three years ago, while the number of graduate students has stayed steady now for the last two years. The major factor for this change is the current drop in federally funded basic research. A problem that affected graduate student levels was the increased rejection of granting visas for foreign students by the State Department, which has fortunately leveled off.

If we track the institute's external funding from both government and

private grants over the last 10 years, income per faculty member has risen sharply, setting records for the Institute. In this period, external funding has risen from $6,308,295 to $13,026,581 this year or has more than doubled in 10 years. During the same period state funding has increased from $2,900,679 to $3,480,123 or 20%. State funding has therefore actually declined after inflation adjustments are made. Outside funding has also declined by 14% in part due to cuts in the federal funding of basic research and the loss of a faculty member. To maintain a strong external funding level at times where competitive renewals of basic research grants in the life sciences have taken a nosedive reflects the strength of our faculty to receive funding based on merit.

Recruitment, Promotions, Awards
In 2005, the institute conducted a search for a full-time faculty member for the replacement of Dr. Xuemei Chen's position in plant genetics jointly with the Department of Genetics and the Department of Plant Biology and Pathology. We had some excellent candidates, but, unfortunately, the finalist decided to take a different position at the University of Pennsylvania. However, we made one non-resident appointment with Dr. Robert Goodman, who is a member of the Department of Ecology, Evolution, and Natural Resources. He is a plant microbiologist and came from the University of Wisconsin. Here, he is now the Executive Dean of Cook College and the Director of the Experiment Station. We also had three promotions. Dr. Kenneth Irvine moved up to Full Professor. Drs. Chris Rongo and Andy Singson moved to Associate Professor and received tenure. This year Dr. Pal Maliga received the honorary degree of "Doctor Honoris Causa" from the University of Debrecen in Hungary for his work on plastid transformation and its application to plant biotechnology. Congratulations to their promotions, Dr. Maliga's recognition, and welcome to our new colleague at the institute.

Steve Lawrence, who joined the institute in 1988, announced his retirement last year. Steve was the Executive Officer of the institute and also served as the Executive Director of the Rutgers Research and Education Foundation, which administers our endowment. Steve was well liked by faculty and staff for his communication skills and his management style. He managed the daily operations of the institute, interacted with sponsors, and served on university-wide committees. During this tenure, the institute experienced major renovations and expansions. Even under budget constraints the institute has been regarded as one of the best maintained buildings at the university. Faced with the departure of Steve, it became a major challenge for us to search for a replacement. A search with faculty and staff input was conducted with the help of Steve and we attracted Robert Rossi to join us with a brief overlap of Steve. Bob has a Bachelor degree in Science, Business Administration from Susquehanna University in Pennsylvania and has served in administrative capacity at the Institute of Advanced Studies in Princeton, the joint managers of the Advanced Technology Centers of Rutgers and UMDNJ, and at the Institute of Marine

and Coastal Sciences. We also welcome him to our institute.

There are currently five institute faculty members in the Department of Molecular Biology and Biochemistry, three in the Department of Genetics, three in the Department of Plant Biology and Pathology, two in the Department of Chemistry, one in the Department of Ecology, Evolution, and Natural Resources, and one in the Department of Computer Science. Of the thirteen resident, four non-resident, and one adjunct members of the faculty, one member is assistant professor, three are associate professors, six are professors, four are professors II, two are Board of Governors professors, and two are university professors. The Institute currently has four professor emeriti, who are all well, and occasionally join us here for events. Two professors are members of the National Academy of Sciences, one is a member of the National Institute of Medicine, and five are Fellows of AAAS.

Lectures

This year's Harry Yale Lecturer was Dr. Hermann Steller from Rockefeller University. Dr. Steller talked about mechanism and regulation of apoptosis. Apoptosis is a programmed cell death, which is associated with a variety of diseases, including cancer, autoimmune diseases, stroke and neurodegenerative disorders and can occur in plants as well. His studies take a genetic approach by using the fruit fly as model organism. A similar concept, using a simple animal system that is genetically tractable to study vital processes in mammalian systems, is taken by a number of our faculty members that work with the fruit fly *Drosophila* and the worm *C. elegans*.

The institute also supported a number of outside speakers that complement the various seminar and lecture programs on the Busch and the Cook campus. For instance, our faculty that are members of the Plant Biology and Pathology Department have organized this year a graduate student course, where original research is presented by speakers directly involved in the subject area. Students had to read key papers of those speakers before they came, making this a very attractive program. Speakers in this program that were supported by the institute were Dr. Robert Fischer from the University of California at Berkeley, who spoke about genomic imprinting in plants, Dr. Ian Small from the Institut National de la Recherche Agronomique in Paris, who spoke about high-throughput RNAi approaches and the pentatricopeptide repeat (PPR) gene family in plants, and Maureen Hanson from Cornell University, who spoke about the role of mitochondrial gene expression in plant development.

Other outside speakers include Shai Shaham of Rockefeller University on "Essential Roles for Glia in the Nervous System, Form and Function," James S. Clegg of the University of California at Davis on "Molecular and Chemical Chaperones in the Animal Extremophile, Artemia," Cedric Wesley of the University of Vermont on "Notch Autoregulation and Neurogenesis in Drosophila," and Ralph Bock of the Max Planck Institute for Molecular Plant Physiology on "Gene Transfer into and out of Plastids." Dr. Bock was a postdoctoral researcher in the Maliga laboratory at the institute and became one of the youngest directors of a Max Planck Institute.

Structural Biology and Bioinformatics

Dr. Helen M. Berman
Board of Governors Professor of Chemistry and Chemical Biology

Dr. Catherine Lawson
Associate Research Professor

Dr. John Westbrook
Associate Research Professor

Dr. Zukang Feng
Assistant Research Professor

Kyle Burkhardt
Research Associate

Dr. Shuchismita Dutta
Research Associate

Judith L. Flippen-Anderson
Research Associate

Dr. Shri Jain
Research Associate

Dr. Brian Hudson
Research Associate

Dr. Andrei Kouranov
Research Associate

Dr. Jeramia Ory
Research Associate

Introduction

Our goals are to understand the structural properties of biological molecules and to relate these structures to their biological functions. A key focus of our work has been to establish methods to collect and archive structural data for analysis. Since 1991, we have developed and managed the Nucleic Acid Database. In October 1998, the management of the Protein Data Bank was moved to the Research Collaboratory for Structural Bioinformatics, of which Rutgers is the lead site.

In addition to these bioinformatics activities, we have continued structural studies on nucleic acid-protein complexes. Our current focus is on structural analysis of simple molecular complexes formed in transcription activation in E. coli. Our goals here are to help to explain the principles that underlie interactions between nucleic acids and proteins, as well as to increase our understanding of the role of water in mediating intermolecular interactions.

Structural Bioinformatics

RCSB Protein Data Bank

The RCSB Protein Data Bank (PDB) is managed by Rutgers, The State University of New Jersey and the San Diego Supercomputer Center (SDSC) at the University of California San Diego (UCSD)–two members of the Research Collaboratory for Structural Bioinformatics (RCSB). The RCSB PDB is a member of an international collaboration that manages the PDB archive called the wwPDB (http://www.wwpdb.org/). As of April 4, 2006, the PDB contained 35917 structures.

On December 30, 2005, the RCSB PDB upgraded http://www.pdb.org to the improved database and website that had been in beta testing since July 2004.

In addition to enhanced navigation and more accurate searching and reporting, the new site has also brought significant performance enhancements, such as faster searching and page display.

The RCSB PDB website is accessed by about 100,000 unique visitors per month from nearly 140 different countries. More than 600 GigaBytes of data are transferred each month. On a typical weekday, two pages from the site are viewed every second.

The new features of the site, which include enhanced searching, browsing, navigating, and reporting, have received significant usage. For example, the option to explore structures using browers that navigate the PDB archives using classifications from Gene Ontology, EC nomenclature,

source organism, disease, genome, SCOP, and CATH were used more than 6,000 times in January alone. The narrated flash tutorial, which provides an introduction to using the new site, was viewed more than 11,000 times.

Nucleic Acid Database (NDB)
The NDB currently contains 3022 structures. Structures are deposited and processed at the NDB using the same tools as the PDB. The NDB website (http://ndbserver.rutgers.edu/) offers a variety of methods for searching and reporting. A special NDB feature is the Atlas -- a collection of summary pages with data, images, and coordinate files for each entry in the NDB. The website also hosts galleries of images with links to Atlas entries that organized by structure type, CATH and SCOP classifications, and Gene Ontology identifiers.

RNA Conformational Classification
In collaboration with the Richardson lab at Duke University, we have executed an across-the-database study of RNA structures. With this study, we combined our independently developed approaches toward RNA conformation analysis. The work utilized RNA structural data from the NDB in order to relate torsion angles of dinucleotides (di-nt) to conformation classification. First, the RNA structures were filtered by crystallographic criteria and stereochemical quality. The resulting data set contained roughly 4000 di-nt. Torsion distributions of these fragments were then analyzed via seventeen 3D scattergrams ("maps") by a Fourier averaging technique developed previously by the Berman lab in order to pinpoint areas of high torsion frequency. Maps with the highest information content were then used to cluster the data points. Conformational clusters were independently determined using protocols by both groups, and to further the study, a novel computational clustering technique based on multiple alignment algorithms is under development at Rutgers. Once the geometries of these two sets of conformational clusters are compared and validated, a set of consensus RNA conformational families will be determined.

Cryo-EM Data Dictionary
Recent advances in preparation, imaging and 3D reconstruction of large biological complexes by cryo-electron microscopy (cryo-EM) have led to a significant increase in cryo-EM depositions to the PDB. Over 40 entries have been deposited in the past two years. In collaboration with the Computational Center for Biomolecular Complexes (http://ncmi.bcm.tmc.edu/ccbc), the RCSB PDB is working to develop a comprehensive dictionary of data items for collection and archiving of cryo-EM experimental data. Our current dictionary proposal is available at the project website (http://rcsb-cryo-em-development.rutgers.edu).

Structural Biology
Binary, Ternary and Quaternary Complexes with Catabolite Activating Protein (CAP)
We are collaborating with the Ebright group on structural studies of the

Dr. Helen M. Berman

Dr. Irina Persikova
Research Associate

Dr. Massy Rajabzadeh
Research Associate

Dr. Bohdan Schneider
Research Associate

Dr. Monica Sundd
Research Associate

Dr. Huanwang Yang
Research Associate

Dr. Jasmine Young
Research Associate

Christine Zardecki
Research Associate

Dr. Jens Birktoft
Research Specialist

Li Chen
Research Assistant

interactions of Catabolite Activator Protein (CAP) with DNA and RNA polymerase. CAP activates transcription at Plac and other promoters through interactions with the RNA polymerase α subunit C-terminal domain (αCTD). The αCTD, in turn, interacts with region 4 the RNA polymerase σ subunit (SR4). Our crystallographic structure determination of the CAP-αCTD-DNA complex at 3.1 Å resolution established that CAP makes direct protein-protein interactions with α, and that αCTD makes direct protein-DNA interactions with the DNA segment adjacent to the DNA site for CAP. This structure is the first of a complex between a transcriptional activator and a functional target within the general transcription machinery. We are working to improve diffraction of crystals containing CAP, αCTD, SR4, and a 68 bpDNA duplex. In collaboration with the Arnold group, we are also carrying out analyses of intact CAP-RNA polymerase-DNA complexes. Single-particle cryoelectron microscopy studies are in progress to define their overall shape (collaboration with Bridget Carragher at the Scripps Research Institute, La Jolla). These studies will improve our fundamental understanding of factor-mediated transcription activation.

Joanna De La Cruz
Research Assistant

Vladamir Guranovic
Research Assistant

Suzanne Richman
Research Assistant

David Micallef
Graduate Assistant

Allison Clarke
Operations Coordinator

William Abbott
Systems Administrator

James Croker
Systems Administrator

Lew-Christiane Fernandez
Systems Administrator

Rose Fredua
Administrative Assistant

Plant Genetics

Dr. Hugo Dooner
Professor

Dr. Gregorio Segal
Research Associate

Dr. Yubin Li
Research Assistant

Dr. Changfa Lin
Research Assistant

Dr. Qinghua Wang
Research Assistant

Limei He
Laboratory Researcher

Steve Maurais
Laboratory Technician

Krystyna Dooner
Laboratory Assistant

Jun Huang
Graduate Fellow

Michael Peterczak
Supervisor Waksman Greenhouse

Our lab performs studies on genome structure, homologous meiotic recombination, and functional genomics in maize.

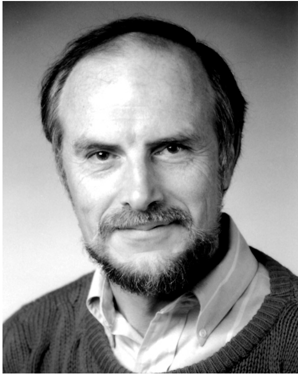
The many genomes of maize: unprecedented haplotype variability at the bz locus

Maize may be the most diverse of all plant species. Unexpectedly large differences among haplotypes were first revealed in a comparison of the bz genomic regions of two different US Corn Belt inbred lines. Retrotransposon clusters, which comprise most of the repetitive DNA in maize, varied markedly in make-up and location relative to the genes in the region and certain genic sequences, later shown to be carried by two Helitron transposons, were present in only one of the two lines. Thus, the allelic bz regions of these two inbreds shared only a minority of the total sequence.

This finding prompted us to investigate the organization of the bz genomic region in inbred lines and land races of widely variable geographic origin. We have found that no two lines are alike. The vertical comparison of eight different haplotypes revealed the existence of several new helitrons, new retrotransposons, members of every superfamily of DNA transposons, numerous MITEs (Miniature Inverted-repeat Transposable Elements), and novel insertions flanked at either end by TA repeats, which we call TAFTs for TA-flanked transposons (Figure). The extent of variation in the region is remarkable. In pairwise comparisons of eight bz haplotypes, the percentage of shared sequences ranges from 25% to 84%. Chimeric haplotypes exist that combine retrotransposon clusters found in different haplotypes. Recombination in the common gene space has shuffled intergenic retrotransposon clusters, greatly amplifying the variability produced by the retrotransposition explosion in the maize ancestry and giving rise to the highly heterogeneous genome organization of modern maize.

Homologous meiotic recombination

Meiotic recombination is a fundamental mechanism in the creation of novel genotypes in sexually reproducing organisms. We are using the bz locus of maize, a uniquely advantageous system, to attempt to obtain answers to basic questions regarding the process of homologous meiotic recombination in plants. The bz gene affects seed pigmentation and is at least 100 times more recombinogenic than the average DNA segment in maize, so recombinants can be generated and identified with relative ease.



Dr. Hugo Dooner

As described above, the organization of the bz genomic region varies from line to line. In some lines, a 26-kb retrotransposon block separates bz from stc1, the next distal gene, whereas in others, that block is absent (Figure). We are presently investigating the effect of this large block on recombination. We find that the size of the same genetic interval is twice as large in the absence of the retrotransposon cluster. The suppressing effect is most pronounced in the adjacent regions: four-fold in bz and two-fold in the closest stc1 segment. Therefore, as might have been expected, the presence of the retrotransposon cluster reduces the frequency and changes the distribution of recombination events between markers. Our finding implies that haplotype structure will profoundly affect the correlation between genetic and physical distance for the same interval in maize and indicates that the distribution of recombination along a chromosome may be yet another polymorphic trait in maize.

Maize transposable elements in functional genomics

Transposons are invaluable tools in genetic analysis. They enable us to clone a gene on the basis of its mutant phenotype, rather than prior knowledge of its function, and to search for genes in organisms with a high content of repetitive DNA, such as maize. Functional analysis of the human-size maize genome appears daunting on the surface. However, genes are concentrated in regions of undermethylated DNA, which is also where the transposon Activator (Ac) tends to insert. Thus, genes can be identified as Ac receptor sites and isolated as the DNA adjacent to the transposed Acs. This approach generates a sequence that can be compared to existing databases and an insertion library that can be screened intelligently for subtle mutant phenotypes.

Our lab is funded by the NSF Plant Genome Program to optimize Ac as a gene searching engine in maize . We developed simple and efficient Ac transposition assays and generated a collection of over 1300 independent Ac transposants. Because Ac has a strong tendency to transpose to nearby sites, about one-half of our transposed Acs are linked to the donor loci. Clearly, it would be desirable to mobilize Ac from different launching platforms in the genome. Towards that end, we have developed a transformable genetic line and have begun to produce transgenic maize plants carrying a transposon that is modified to facilitate the isolation of adjacent DNA. Our construct should integrate at random sites in the genome, providing starting platforms for future transposon mobilization from many locations in the genome.

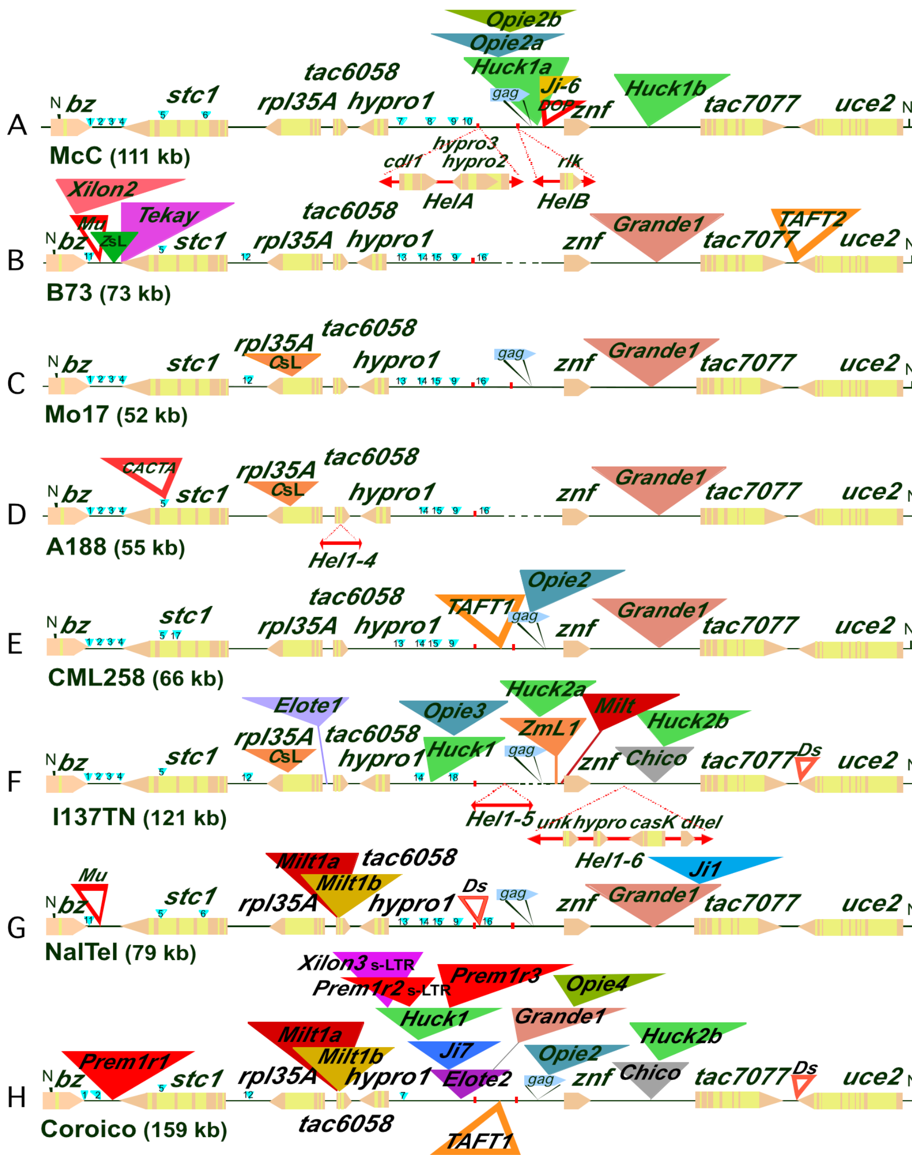


Figure. Organization of eight bz haplotypes. Each haplotype is identified by the name of the genetic line, followed by the size of the cloned NotI (N) fragment, in parentheses. Genes are shown as pentagons pointing in the direction of transcription; exons are in bronze and introns in yellow. There are eight genes in the region: bz, stc1, rpl35A, tac6058, hypro1, znf, tac7077, and uce2. Helitrons (Hels) carrying gene fragments are represented as bidirectional arrows below the line for each haplotype. Dashed lines represent deletions. Retrotransposons are indicated by solid triangles of different colors. DNA transposons and TAFTs, which are probably also DNA transposons, are indicated by open triangles of red and orange color, respectively. MITEs are indicated in light blue color. Only the genes have been drawn to scale.

Protein Biochemistry

Dr. Richard Ebright
Professor

Dr. Yon Ebright
Research Associate

Dr. Vladimir Mekler
Research Associate

Dr. Aashish Srivastava
Research Associate

Dr. Anatoliy Kravets
Research Associate

Dr. Jayanta Mukhopadhyay
Research Specialist

Dr. Sergei Druzhinin
Research Specialist

Dr. Rui Rong
Postdoctoral Fellow

Ekaterine Korkthonjia
Graduate Assistant

Dongye Wang
Graduate Assistant

Transcription is the first step in gene expression and is the step at which most regulation of gene expression occurs. Our laboratory seeks to understand the structure, function, and regulation of transcription complexes, and to identify and characterize inhibitors of bacterial transcription for use as potential antibacterial agents.

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

Transcription initiation at a eukaryotic protein encoding gene involves RNA polymerase II, mediator, and up to six general transcription factors: IIA, IIB, IID, IIE, IIF, and IIH. The fully assembled eukaryotic transcription initiation complex contains more than 50 polypeptides (12 in RNA polymerase II, at least 15 in mediator, and at least 26 in general transcription factors) and promoter DNA, and has a molecular mass in excess of 3 MDa.

Understanding transcription initiation in bacteria and eukaryotes will require understanding the structures of the polypeptides in the respective transcription initiation complexes and the arrangement of these polypeptides relative to each other and relative to promoter DNA.

Crystallographic structures have been determined for several components of the bacterial and eukaryotic transcription initiation complexes. However, intact transcription initiation complexes have proved refractory to crystallographic structure determination. Therefore, efforts to understand the arrangement of polypeptides within intact transcription initiation complexes rely heavily on biophysical data defining distances within complexes and on biochemical and genetic data defining contacts within complexes.

We are carrying out systematic analyses of distances, protein-protein contacts, and protein-DNA contacts within the bacterial and eukaryotic transcription initiation complexes. We are using fluorescence resonance energy transfer (FRET) to define distances between pairs of site-specifically incorporated fluorescent probes, photocrosslinking to define polypeptides near to site-specifically incorporated photocrosslinking probes, and protein footprinting and residue scanning to define residues involved in contacts. In addition, we are using binding-site selection to define new promoter DNA-sequence elements recognized by polypeptides and

polypeptide fragments. Finally, we are developing and using automated constrained docking algorithms to integrate structural, biophysical, biochemical, and genetic data in order to construct models for the structures of complexes.

Function of Transcription Complexes

The bacterial and eukaryotic transcription initiation complexes are molecular machines that carry out complex, multi-step reactions. The transcription initiation pathway involves: (i) binding of RNA polymerase and initiation factor(s) to promoter DNA to form a "closed complex" with duplex DNA; (ii) isomerization through several intermediates to form an "open complex" with an "~14-nucleotide region of melted, single-stranded DNA surrounding the transcription start; (iii) abortive cycles of synthesis and release of 2- to 8-nucleotide RNA oligomers as an "initial transcribing complex"; and (iv) upon synthesis of a 9-nucleotide RNA oligomer, isomerization to break protein-DNA interactions between RNA polymerase and the promoter and to break, or weaken, protein-protein interactions between RNA polymerase and initiation factor(s), resulting in an "elongation complex" that processively translocates along DNA and extends the RNA product.

Each of the steps in this pathway appears to involve conformational changes in both RNA polymerase and promoter DNA. Understanding transcription initiation will require defining the structure of the complex at each step, defining the conformational transitions, and defining the kinetics of the transitions.

We are addressing these issues in studies of the smaller, and thus more experimentally tractable, bacterial transcription complex. We are using the fluorescence resonance energy transfer and photocrosslinking methods of the preceding section to define distances and contacts within trapped intermediates (e.g., closed complexes trapped at 4°C, intermediate complexes trapped at 15°C, open complexes trapped at 37°C in the absence of NTPs, initial transcribing complexes trapped at 37°C in the presence of specific subsets of NTPs). In addition, we are using fluorescence resonance energy transfer with stopped-flow rapid mixing, and photocrosslinking with quenched-flow rapid mixing and laser flash photolysis, to monitor kinetics of transitions. Finally, we are using single-molecule optical microscopy, single-molecule DNA nanomanipulation, and combined single-molecule optical microscopy and single-molecule DNA nanomanipulation, for single-molecule, millisecond to second scale, analysis of transitions within transcription complexes.

Regulation of Transcription Complexes

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

Dr. Richard Ebright

Chenyu Liu
Graduate Assistant

Mohan Tripathi
Undergraduate Assistant

Trevor Michalchuk
Research Assistant, Laboratory Manager

Pris Zhou
Laboratory Manager

With the objective of providing the first complete structural and mechanistic descriptions of activation, we study two of the simplest examples of activation in bacteria: (i) activation of the lac promoter by catabolite activator protein (CAP), and (ii) activation of the gal promoter by CAP. These model systems each involve only a single activator molecule and a single activator DNA site, and, as such, are more experimentally tractable than typical examples of activation in bacteria and substantially more experimentally tractable than typical examples of activation in eukaryotes (which can involve tens of activator molecules and activator DNA sites).

In work to date, we have established that activation at lac involves an interaction between CAP and the RNA polymerase α subunit C terminal domain that facilitates closed-complex formation. Activation at gal involves this same interaction and also an interaction between CAP and the RNA polymerase αsubunit N-terminal domain, and between CAP and σ, that facilitate isomerization of closed complex to open complex.

In current work, we are using x-ray crystallography and cry-electron microscopy to determine the structures of the interfaces between CAP and its targets on RNA polymerase, and we are using photocrosslinking, FRET, single-molecule-optical-microscopy and single-molecule-DNA-nanomanipulation methods to define when each CAP-RNA polymerase interaction is made as RNA polymerase enters the promoter and when each interaction is broken as RNA polymerase leaves the promoter.

Inhibitors of Bacterial Transcription

Bacterial transcription is a proven target for antibacterial therapy. The rifamycin antibacterial agents--notably rifampicin, rifabutin, and rifapentine--function by binding to, and inhibiting, bacterial transcription complexes. Due to the public-health threat posed by drug-resistant and multidrug-resistant bacterial infection, there is an urgent need for novel classes of antibacterial agents that target bacterial transcription complexes (and thus have the same biochemical effects as rifamycin antibacterial agents) but that target different, non overlapping structural elements within bacterial transcription complexes (and thus do not show cross-resistance with rifamycin antibacterial agents).

We systematically are identifying and characterizing low-molecular-weight compounds that target specific structural elements within bacterial transcription complexes, and/or that inhibit specific reaction steps of bacterial transcription complexes. We are using genetic, biochemical, spectroscopic, and crystallographic approaches to define the mechanism of action of each known inhibitor of bacterial transcription; peptidomimetic-chemistry approaches to develop improved inhibitors of bacterial transcription; and combinatorial-chemistry and high-throughput screening approaches to identify novel inhibitors of transcription. In addition, in support of this work, we are developing and testing methods for optically encoded combinatorial chemistry.

Developmental Biology

Dr. Ken Irvine
Professor

Dr. Aiguo Xu
Research Specialist

Yaopan Mao
Postdoctoral Associate

Marie-Agnes Michellod
Postdoctoral Associate

Venugopala Reddy
Postdoctoral Associate

Hyangyee Oh
Postdoctoral Associate

Yongqiang Feng
Graduate Assistant

Yuh-ru Lin
Graduate Assistant

Robert Major
Graduate Assistant

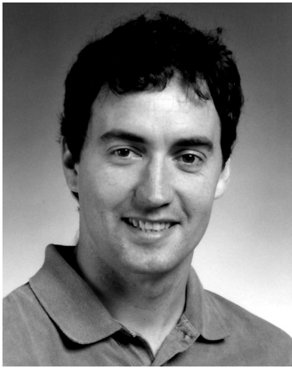
Dragana Rogulja
Graduate Assistant

Research in my laboratory is directed toward understanding the regulation and coordination of tissue patterning, growth and morphogenesis during animal development. 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.

Glycosylation and Notch Signaling
One focus of our research has been the regulation of the Notch signaling pathway. Notch is a receptor protein that mediates a wide range of cell fate decisions during animal development. In humans, aberrant Notch signaling has been linked to leukemia (TAN-1), and congenital syndromes associated with stroke and dementia (CADASIL), and liver, cardiovascular, and skeletal defects (Alagille, spondylocostal dysostosis).

The Notch receptor and its ligands are modified by an unusual form of glycosylation, which is initiated by the attachment of fucose to Serines or Threonines within epidermal growth factor-like (EGF) repeats. We have studied the influence of this post-translational modification using a combination of Drosophila genetics, cell culture, and biochemistry. Decreasing the expression of Protein O-fucosyltransferase 1 (OFUT1), the enzyme that initiates the synthesis of O-linked fucose, demonstrated that OFUT1 is positively required for Notch signaling. We recently discovered, however, that OFUT1 actually plays two distinct roles in Notch signaling. It acts both as a fucosyltransferase to modify the Notch receptor, and as a chaperone to promote Notch receptor folding. These two roles are genetically separable, because the chaperone activity of OFUT1 does not require its fucosyltransferase activity. The chaperone activity is required for all Notch functions, but the fucosyltransferase activity is principally required to allow Notch to be further glycosylated.

Fringe is a glycosyltransferase that modifies the O-linked fucose on Notch by addition of ß1,3 linked N-acetylglucosamine. This further glycosylation of Notch both inhibits the activation of Notch by one ligand, Serrate, and potentiates the activation of Notch by another ligand, Delta. The influence of this glycosylation on Notch activation can be accounted for an effect on Notch-ligand binding. By reproducing the influence of glycosylation on ligand binding in vitro with purified components, we have been able to demonstrate that the simple addition of N-acetylglucosamine to Notch is sufficient to alter the interaction of Notch with its ligands, and



Dr. Ken Irvine

Binnaz Kucuk
Graduate Assistant

Angelina Lalova
Undergraduate Assistant

Michael Yang
Undergraduate Assistant

Shemeeakah Powell
Research Technician

that this influence of glycosylation does not require the participation of any accessory proteins.

In another line of experiments, we have used site-specific mutagenesis to assess the requirements for glycosylation of different EGF repeats of Notch. Characterization of mutant forms of Notch that can not be fucosylated on specific EGF repeats, or that lack specific EGF repeats, has indicated that Fringe acts through multiple, independent sites to modulate ligand binding, and have led us to propose a model for how Notch interacts with its ligands.

Developmental Functions of Notch Signaling
Notch signaling is required for an enormous number cell fate decisions in metazoans, but Fringe is only required for a subset of Notch signaling events. We have taken advantage of this over the years to identify and characterize requirements for Notch signaling in different tissues. Currently, we are focusing on a critical yet poorly understood role for Notch in the subdivision of the developing Drosophila wing into distinct groups of cells that do not intermix, called compartments. Because Notch has been implicated in separating cells in a wide variety of contexts, from somitogenesis and brain compartmentalization in vertebrates to leg and body segmentation in insects, we think that the results of these studies will be broadly relevant. Notch is required for the maintenance of a boundary between dorsal and ventral cells in the wing, and our characterization of the role of Notch has led us to propose that it effects compartmentalization in a novel way: rather than establishing distinct dorsal- or ventral-type cell affinities, it induces a property or behavior of cells at the border that prevents them from intermixing. In trying to define the cellular and molecular basis for this Notch-dependent cell separation, we discovered a distinct, Notch-dependent organization of the actin cytoskeleton, and identified a requirement for a regulator of actin polymerization, Capulet, in dorsal-ventral compartmentalization. Our results also suggest that the influence of Notch on actin and dorsal-ventral compartmentalization involves a non-transcriptional pathway. Our continuing studies of dorsal-ventral compartmentalization are directed towards understanding the cellular mechanism downstream of Notch that effects cell separation, and how Notch influences this process.

Developmental Glycobiology
There are an increasing number of examples in which post-translational modification of proteins by glycosylation plays important roles in regulating their activity, but the requirements for some forms of glycoylation remain poorly understood. In order to identify and characterize additional requirements for glycosylation, we have conducted genetic and biochemical studies in Drosophila on several genes predicted to encode glycosyltransferases, including sialyltransferase, β,4-galactosyltransferases, and fucosyltransferases. Currently we are focusing on β,3 galactosyltransferases that we have found are essential for viability in Drosophila. One gene of interest is closely related to mammalian core 1 β,3 galactosyltransferase,

which transfers Galactose onto O-linked GalNAc. This is of particular interest because alterations in O-GalNAc glycans have been correlated with tumor metastasis. We have identified a Drosophila core 1 β,3 galactosyltransferase that is essential for morphogenesis, and we are currently characterizing its requirements in detail.

Patterning and growth during development

Understanding how growth is controlled is a major goal of developmental biology. Decades ago, regeneration experiments revealed an intimate relationship between tissue patterning and tissue growth, but the molecular basis for this relationship has remained elusive. We are currently engaged in projects whose long term goal is to define the relationship between patterning and growth in developing tissues.

One approach we have taken is to reexamine the influence of long range morphogens on Drosophila wing growth. For example, While Decapentaplegic, a member of the TGFß family, has long been known to be important for wing growth, how it actually influences growth has remained unclear. We have been reexamining this, using a new approach for regulating gene expression in Drosophila, which has enabled us to exercise quantitative and temporal control over expression of transgenes in clones of cells.

In a second approach, we have been investigating a set of genes that we think constitute a new signaling pathway which affects growth and patterning during development. Studies in Drosophila have been instrumental in the identification and functional characterization of many key components of intercellular signaling pathways. This new pathway, the Fat pathway, has been studied mostly for its role in the regulation of cell polarity. Fat also influences growth and gene expression in developing tissues, but the mechanism by which it does so has not yet been described. We are actively engaged in genetic and biochemical studies to elucidate this intracellular Fat signal transduction pathway. In these experiments, we are taking advantage of insights our research has provided into developmental roles of Fat signaling to identify and characterize new components of the Fat pathway. We are also beginning to extend our studies to investigations of Fat signaling mammals.

Molecular Genetics of Leaf Development

Dr. Randall A. Kerstetter
Assistant Professor

Dr. Irina Vvedenskaya
Postdoctoral Associate

Tengbo Huang
Graduate Fellow

Rajani Pemmaraju
Laboratory Assistant

Thomas Morley
Undergraduate Researcher

Ken Chen
Undergraduate Researcher

Robert Pepitone
Undergraduate Researcher

Naznin Daginawala
Undergraduate Researcher

The leaves of flowering plants display noticeable anatomical differences between their adaxial (top or dorsal) side and abaxial (bottom or ventral) side. These differences reflect functional specialization. For example, the adaxial side of the leaf typically appears darker green due to the tight clustering of photosynthetic cells responsible for capturing light. In contrast, the abaxial side of the leaf often appears pale green due to the presence of air spaces between cells that facilitate the exchange of atmospheric gases. Normal leaf morphogenesis requires the proper specification of adaxial-abaxial polarity both during formation of the leaf primordium in the shoot apical meristem and during its subsequent outgrowth.

Members of the KANADI (KAN) family of myb-related transcriptional regulators have been demonstrated to play essential roles in the specification of abaxial fate in leaf development. Combining loss of function mutations in members of the KAN family leads to progressive loss of abaxial identity. Single kan1 mutants form trichomes (an adaxial trait) on the abaxial leaf surface. Double kan1 kan2 mutants have dramatically reduced leaf expansion and form ectopic leaf blade outgrowths on the abaxial leaf surface. Triple kan1 kan2 kan3 mutants further reduce blade expansion, do not produce ectopic leaf blade outgrowths, and form nearly unifacial, adaxialized leaf blades. In order to clarify the molecular mechanisms that regulate adaxial-abaxial polarity, we are pursuing three independent molecular genetic approaches to identify critical factors that contribute to the specification of leaf polarity during development.

Downstream Targets of KANADI

Microarray analysis comparing wild-type seedlings with kan1 single and kan1 kan2 double mutants indicates that KAN1 may function primarily as a negative regulator. Far more genes were found to be significantly up regulated in the mutants than are down regulated. Transgenic seedlings over-expressing a post-translationally activated form of KAN1 have been used to identify putative direct downstream targets. Almost three times as many genes are significantly down regulated as are up regulated in response to ectopic KAN1 activation.

Among the many candidate targets genes, two groups are of particular interest. Twenty-four genes are up regulated even in the presence of a protein synthesis inhibitor indicating they are direct downstream targets of KAN1. Members of this group of genes are transcription factors and proteins implicated in signal transduction. The second group of genes display reciprocal changes of expression in the loss-of-function mutants and

post-translationally activated KAN1. Among these, IAA2, a regulator of auxin responses, is strongly up regulated in kan1 kan2 and strongly down regulated upon KAN1 over-expression. Quantitative reverse-transcription PCR (qPCR) experiments have confirmed that KAN1 represses IAA2 gene expression and chromatin immunoprecipitation experiments confirm that the KAN1 protein associates with the IAA2 promoter. Auxin is known to play diverse roles in growth and morphogenesis throughout plant development. Our efforts to identify direct downstream target genes of KAN1 have yielded several other genes implicated in auxin responses. This result uncovers a direct connection between KAN1 and genes involved in differential growth processes that may determine some of the morphological differences that distinguish adaxial from abaxial tissues.

We have determined the DNA sequence to which the KAN1 protein binds in vitro and shown that it is distinct from sequences bound by other myb-related transcription factors. We have analyzed the promoter sequences of the candidate KAN1 target genes and determined that the binding site is significantly over-represented in their promoters. This information provides further evidence that the genes we have identified are directly regulated by KAN1.

Dr. Randall A. Kerstetter

ARROW1, a Novel Enhancer of KANADI

We identified arrow1 (aro1) in a screen for mutants that shift leaf development in the kan1 kan2 double mutant further toward a fully adaxialized fate resembling the kan1 kan2 kan3 triple mutant. Plants triply mutant for kan1 kan2 aro1 form radially symmetric adaxialized leaves. In addition, genetic analysis indicates that aro1 enhances the phenotypes of mutations in genes that specify adaxial leaf fate including asymmetric leaves 1 & 2 and revoluta. As a single mutant, aro1 has pleiotropic developmental defects including narrow, serrated leaves, delays in root growth, flowering time, and vascular differentiation. ARROW1 encodes a Pumilio-related RNA-binding protein. Related proteins in yeast, Drosophila and C. elegans function as sequence-specific RNA-binding proteins that repress translation of their target mRNAs. This mechanism of post-transcriptional regulation of expression is critical for anterior-posterior patterning of the Drosophila embryo and in stem cell maintenance during neurogenesis and germ cell formation in both Drosophila and C. elegans. We are currently working to determine if ARO1 functions in similar manner in plants.

FLAVODENTATA Links Polarity and Chloroplast Function

The flavodentata (flv) mutant has serrated, pale green leaves and occasionally forms radialized or needle-shaped leaves. The pale green leaves observed in flv indicate defects either in chloroplast development or photosynthesis and needle-shaped leaves suggest defects in the proper specification of adaxial leaf fate. We have crossed flv to a number of mutants that display defects in either adaxial or abaxial leaf development. Strong synergistic phenotypes in double mutants with kanadi, filamentous flower, asymmetric leaves 1, revoluta, and serrate indicate that FLV functions in an independent genetic pathway to specify normal leaf polarity.

FLV encodes a pentatricopeptide repeat (PPR) protein with a chloroplast signal sequence. PPR proteins are a large family of RNA-binding proteins in plants. Members of this family have been shown to catalyze RNA processing events in plastids and mitochondria. Growing flv plants under low light conditions suppresses both the greening defects and the defects in leaf morphogenesis. This result suggests that defects in leaf polarity may be a consequence of defects in chloroplast development or function. The flv mutation therefore uncovers a novel link between chloroplast function and the proper specification of adaxial-abaxial leaf polarity in Arabidopsis. Asymmetry and polarity establishment are fundamental processes in all multi-cellular organisms. Identifying the molecules and mechanisms employed by plants to form leaves will enrich our knowledge of cell fate specification and provide a meaningful comparison for developmental processes that are increasingly well understood in animal systems. In addition, because the bulk of photosynthesis that contributes to plant-derived commodities, such as food, fiber, wood, and fuel, depends on leaves, understanding leaf morphogenesis has the potential to provide powerful tools for modifying future agricultural products.

Computational Bioinformatics Laboratory

Casimir A. Kulikowski
Professor

Ilya Muchnik
Senior Investigator

Andrei Anghelescu
Graduate Assistant

Thang Le
Graduate Assistant

Akshay Vashist
Graduate Assistant

Jiankuan Ye
Graduate Assistant

Hwaseob Yun
Research Assistant

Akshay Vashist
Graduate Assistant

Jiankuan Ye
Graduate Assistant

HwaSeob Joseph Yun
Graduate Assistant

The research group on biomedical informatics headed by Professor Kulikowski is currently working on problems involving the discovery of orthologs and paralogs by combinatorial clustering techniques, prediction of protein-protein interactions, text mining methods for the annotation of biomolecular data, and graphics and imaging techniques for characterizing biomotion.

Plastid Molecular Genetics

Dr. Pal Maliga
Professor

Dr. Zora Svab
Research Associate

Arun Azhagiri
Graduate Fellow

Massimo Bosacchi
Graduate Fellow

Chokchai Kittiwongwattana
Graduate Fellow

Paolo Lenzi
Graduate Fellow

Kerry Lutz
Graduate Fellow

Tarinee Tungsuchat
Graduate Fellow

Mark Clark
Undergraduate Research Fellow

Plastids of higher plants are genetically semi-autonomous: ~100 genes are encoded in the plastid genome (ptDNA) while most functions are encoded in nuclear genes targeting proteins to plastids. Our current focus is development of a system for plastid transformation in *Arabidopsis thaliana*, a model plant species that would enable mutant screens to study nuclear control of plastid gene expression. Additional research areas in the laboratory are related to transgene biosafety: study of rare paternal pollen transmission and excision of marker genes by site-specific recombinases. We are also interested in engineering metabolic pathways by exploiting plastid localization of transgenes and the plastid's unique transcription and translation machinery.

Plastid Transformation in Arabidopsis thaliana

Plastid transformation is tissue culture based, as gradual replacement of the thousands of wild-type plastid genome copies with transgenic ptDNA can be best accomplished in the tissue culture environment. One bottleneck of plastid transformation in *Arabidopsis thaliana* is the difficulty to maintain the normal diploid state in cell culture. Although most leaf cells become polyploid, the diploid state is maintained in plants in the germline cells of embryos and the shoot apex. During the past year we developed a novel tissue culture system in which the embryogenic state of Arabidopsis cells is maintained by expression of a plant transcription factor, BABY BOOM (BBM), under inducible control. BBM, when constitutively expressed, is imported into the plant nucleus. Translational fusion of BBM with a glucocorticoid-receptor (GR) makes import of BBM-GR dependent on dexamethasone. We have shown that transgenic Arabidopsis plants develop normally in the absence of the inducer but form prolific embryogenic cultures when the inducer is incorporated in the culture medium. The utility of the system is currently tested by selection of spectinomycin resistant mutants, a process that is also suitable for the recovery of transplastomic clones.

A second problem of plastid transformation in *Arabidopsis thaliana* is inefficient incorporation of the transforming DNA. INT, the phiC31 phage integrase mediates recombination between attB and attP sequences. We have shown that INT efficiently incorporates the transforming DNA in plastids by recombination between the vector attP site and the attB site incorporated in the tobacco plastid genome. During the past year we constructed Arabidopsis-specific INT vectors that are currently utilized for plastid transformation in Arabidopsis using the steroid-regulated plant

regeneration system. When a suitable INT recipient line is obtained, we will test whether or not low transformation efficiency in Arabidopsis can be overcome by INT-mediated transformation.

Biosafety Evaluation of Plastid Transgenes

Plastid localization is an attractive idea to control transgene flow via pollen in species with strict maternal plastid inheritance. However, low frequency of paternal pollen transmission of plastids was shown in crosses involving plants with an alien cytoplasm. Our objective is to evaluate the significance of exceptional paternal ptDNA inheritance for transgene containment in tobacco (*Nicotiana tabacum*). We track paternal ptDNA transmission to the progeny by expression of antibiotic (spectinomycin) resistance genes encoded in the ptDNA. The seedlings derive from crosses with maternal parents carrying either alien or normal cytoplasms. We have now confirmed low frequency paternal ptDNA transmission (~1 in 5,000) to alloplasmic tobacco seedlings. We have also shown that the entire ptDNA is transmitted by pollen, rather than only small ptDNA fragments involving a transformation-like process. We are currently testing, if paternal pollen transmission is unique to alloplasmic substitution lines, or it also occurs in plants with a normal cytoplasm.

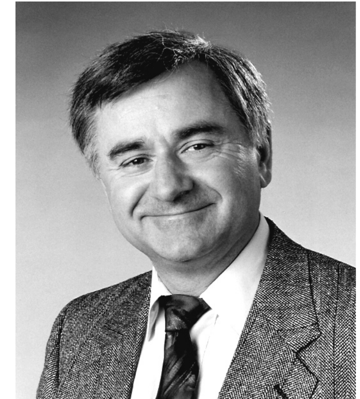
Nicotiana tabacum is a polyploid species that is not suitable for the isolation of recessive mutants. Therefore, we are testing paternal pollen transmission in diploid species, such as *Arabidopsis thaliana* and *Nicotiana sylvestris*, with an interest to develop mutant screens for the identification of nuclear genes controlling paternal plastid transmission.

Diversity of Plastid Genomes in Arabidopsis thaliana

To enable tracking of ptDNA in Arabidopsis crosses, we tested the diversity of plastid genomes in 26 different ecotypes. We identified 3 polymorphic sites in the *rpl2-psbA* region and five polymorphic sites in the *rbcL-accD* region by sequencing the 481 bp (*rpl2-psbA*) and 677 bp (*rbcL-accD*) intergenic regions. Five sites involved changes in the length of poly-A or poly-T mononucleotide repeats. Three of the polymorphic sites are base substitutions, one of which leads to the creation of an MseI restriction site. Phylogeographic analyses showed that ecotypes with specific markers could be grouped based on their geographic location. The polymorphisms identified in Arabidopsis thaliana will be useful as genetic markers to track plastid inheritance and give new insights into the intraspecific diversity and microevolution within this important model plant species.

Plastid Marker Gene Excision with a Transiently Expressed CRE Site-Specific Recombinase

Plastid transformation requires uniform alteration of the 1,000-to 10,000-plastid genome copies present in a cell by selection for antibiotic resistance encoded in a marker gene. Once all plastid DNA copies are transformed the marker gene is no longer needed to maintain the transformed state. We have shown earlier that *loxP*-flanked plastid marker genes can be



Dr. Pal. Maliga

efficiently excised with nuclear-encoded, plastid targeted CRE site-specific recombinase. We now developed a novel protocol for plastid marker gene excision with a transiently expressed CRE that enables rapid removal of marker genes from the ~10,000 plastid genome copies without transformation of the plant nucleus. Marker excision by a transiently expressed CRE involves introduction of CRE in transplastomic leaves by agroinfiltration, followed by plant regeneration yielding plastid and nuclear marker free plants at ~10% frequency.

Engineering of Methionine and Folate Production

We are currently testing if increased levels of free methionine and folate can be obtained by manipulating the network of tetrahydrofolate-bound one-carbon units in tobacco plastids. The targets for engineering are methionine synthase (MS) and/or 5,10-methylenetetrahydrofolate reductase (MTHFR). The enzymes are expressed from both plastid and nuclear genes. These experiments are carried out in collaboration with Dr. S. Roje, Washington State University, Pullman, WA. Engineered plants with increased folate content will represent a dietary source of natural folates. This could potentially solve the problem of poor folate uptake associated health disorders linked to anemia, birth defects and vascular disease.

Molecular Genetics of Meiotic Recombinations and Chromosome Segregaton

Dr. Kim S. McKim
Associate Professor

Janet Jang
Laboratory Researcher

Erica Kolibas
Laboratory Technician

Jeffry Cesario
Graduate Student

Eric Joyce
Graduate Student

Sonam Mehrotra
Graduate Fellow

Sejal Ghandi
Undergraduate Student

Matthew Hanlon
Undergraduate Student

Sona Jasani
Undergraduate Student

Apple Long
Undergraduate Student

Shree Tanneti
Undergraduate Student

Meiotic crossing over provides a linkage between homologous chromosomes that directs their segregation at the first meiotic or reductional division. The homologous chromosomes are aligned and held together along their length by the synaptonemal complex (= SC) and recombination is initiated with a double strand break (DSB). Repair of the DSBs results in either gene-conversion or crossing over. In the absence of recombination, homologous chromosomes do not segregate properly, resulting in aneuploidy and usually death of the embryo. In certain cases, these aneuploids survive; in humans, this results in syndromes such as Down's, Turner's, and Klinefelter's.

Research in the laboratory is directed at understanding meiosis in Drosophila melanogaster females. Our studies have focused on two important aspects of the meiotic pathway. The first is to characterize the genes which are involved in the initiation and repair of meiotic DSBs. The second is to characterize the mechanisms controlling the assembly of the metaphase I spindle in the Drosophila oocyte. Many of the important genes in this process are also involved in DNA repair or the fidelity of chromosome division in other cell types. Therefore, these studies will likely provide insights into the factors affecting genome stability in mitotic cells.

The Initiation and Repair of Meiotic Double Strand Breaks

Most organisms initiate meiotic recombination with the formation of double stand breaks (DSB). Drosophila females are among the group of organisms that can form synaptonemal complex in the absence of DSBs. Using an antibody against the phosphorylated form of His2Av (γ-His2Av), we have investigated whether DSB formation depends on the synaptonemal complex and what factors regulate the repair of DSBs. Since we have found situations where the SC is not required for DSB formation, we suggest that the SC has a regulatory role. One possibility consistent with our data is that the SC functions to counteract the effects of a negative regulator of DSB formation. The SC is clearly not sufficient for DSB formation, however, since DSBs were found to be absent from the heterochromatin even though SC formation occurs in these regions. Insights into how DSB repair is regulated have come from experiments using X-ray irradiation and comparing the frequencies of γ-His2Av foci and crossing over in a variety of recombination defective mutants. These results suggest that the response to meiotic DSBs is quicker than the response to X-ray induced DSBs. One interpretation of these results is that the response of early

pachytene cells to meiotic DSBs depends on pre-assembling repair complexes before the break is made (Figure 1). Crossing over may also be dependent on events or conditions established independent of DSB formation. Since we found a linear relationship between the numbers of γ-His2Av foci and crossovers, it appears that Drosophila females lack a compensatory mechanism that ensures a crossover in the presence of a low number of DSBs. The probability that a DSB becomes a crossover depends largely on the location of the break.

Dr. Kim S. McKim

A Link between the Meiotic Crossover Pathway and Oocyte Differentiation
DSBs are repaired using the homolog as a template and results in either a simple gene-conversion or crossing over. hold'em (hdm) is a new meiotic gene on the X-chromosome. All three mutant alleles cause reductions in crossing over to approximately 30% of wild-type and increase nondisjunction. Phenotypic analysis of these mutants suggests that hdm is a member of the exchange class (e.g. mei-9) that is proposed to be directly involved in resolving recombination intermediates as crossovers. Consistent with this conclusion, staining with an antibody to γ-HIS2AV showed that hdm mutants were competent to induce and repair DSBs. Interestingly, there appeared to be a delay in the appearance of the γ-HIS2AV foci as well as persistence of two oocytes in the germarium, suggesting a delay in multiple aspects of oocyte differentiation. The two oocyte phenotype was also observed with two other exchange class mutants (mei-9 and mus312) and some mutants with general defects in DSB repair such as mei-41, okr and spn-A. The delay in γ-HIS2AV staining and the two oocytes phenotypes were suppressed by mei-218 mutants, suggesting both phenotypes result from defects in the meiotic recombination pathway. Therefore, it was unexpected that mei-P22 and mei-W68 mutants, which fail to generate DSBs, failed to suppress the two oocyte phenotype. These results along with other epistasis experiments lead to the hypothesis that hdm, mei-9, and mus312 have a function which is part of the recombination pathway but is independent of DSB formation (Figure 1).

Meiotic Spindle Formation and Chromosome Segregation
In the oocytes of many species, bipolar spindles form in the absence of centrosomes. Drosophila melanogaster oocyte chromosomes have a major role in nucleating microtubules, which precedes the bundling and assembly of these microtubules into a bipolar spindle. We have found that a region similar to the anaphase central spindle functions to organize acentrosomal spindles. Subito mutants are characterized by the formation of tripolar or monopolar spindles and nondisjunction of homologous chromosomes at meiosis I. Subito encodes a kinesin-like protein and associates with the meiotic central spindle, consistent with its classification in the Kinesin 6 family. This class of proteins is known to be required for cytokinesis but our results suggest a new function in spindle formation. The meiotic central spindle appears during prometaphase and includes passenger complex proteins such as AurB and Incenp. Unlike mitotic cells, the passenger proteins do not associate with centromeres prior to anaphase.

In the absence of Subito, central spindle formation is defective and AurB and Incenp fail to properly localize. We propose that Subito is required for establishing and/or maintaining the central spindle in Drosophila oocytes and this substitutes for the role of centrosomes in organizing the bipolar spindle.

Mitotic Spindle Assembly
Subito is a homolog of human MKLP2. Based on the requirement of MKLP2 for cytokinesis in mammalian cells, we investigated the function of Subito in mitotically dividing Drosophila cells. During metaphase, Subito localizes to microtubules at the center of the mitotic spindle, an area most likely composed of interpolar microtubules that originate at the poles and overlap in antiparallel orientation. Consistent with this localization pattern, subito mutants improperly assemble microtubules at metaphase, causing activation of the spindle assembly checkpoint and lagging chromosomes at anaphase. These results are the first demonstration of a Kinesin 6 family member with a function in mitotic spindle assembly, possibly involving the interpolar microtubules. On the other hand, the role of Subito during mitotic anaphase resembles other Kinesin 6 family members. Subito localizes to the spindle midzone at anaphase and is required for midzone localization of Polo, Incenp and Aurora B. Genetic evidence suggests that the effects of subito mutants is attenuated due to redundant mechanisms for spindle assembly and cytokinesis. For example, while the subito mutant defects are not severe enough to cause lethality, subito double mutants with ncd, polo, Aurora B or Incenp mutations are synthetic lethal. In some cases, these double mutants had severe defects in microtubule organization. And while we observed only a mild cytokinesis defect in subito mutants, the subito +/subito Incenp double mutant exhibited a higher frequency of cytokinesis failure.

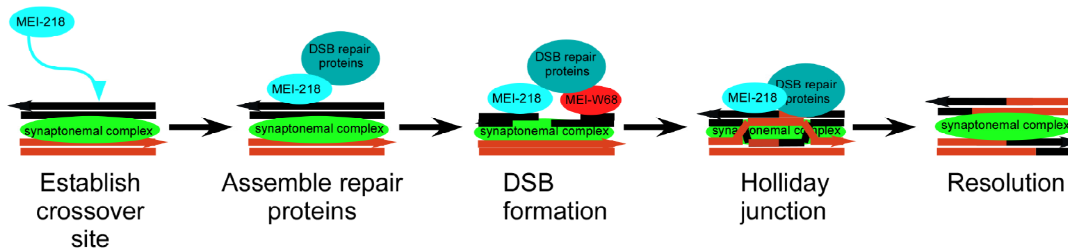


Figure 1: Gene required for crossing over and DSB repair may assemble with recombination sites prior to cutting of the two DNA strands. It is not known how the DSB sites are chosen, and they are different every meiosis.

Molecular Biology of Plant Development

Dr. Joachim Messing
University Professor of Molecular Biology

Dr. Charles Du
Visiting Professor

Galina Fuks
Research Associate

Dr. Jinsheng Lai
Research Associate

Dr. Arvind Bharti
Research Associate

Dr. Moises Cortes-Cruz
Research Assistant

Dr. Gabriel Romero
Visiting Scientist

Kathy Ward
Laboratory Researcher II

Amy Bronzino-Nelson
Senior Laboratory Technician

Gladys Keizer
Senior Laboratory Technician

Valerie Zohovetz
Laboratory Technician

Introduction

The Messing lab would like to contribute to the understanding of the regulation of gene expression in plants and how genes are organized within the chromosomal architecture. Recent studies have suggested that the grasses could be considered as a single genetic system because of the conservation of their genes and their order (collinearity) in many chromosomal locations. Therefore, one assumes that the grasses have diverged mainly due to the reshuffling of large chromosomal sections and the expansion of chromosomes by the insertion of repetitive DNA between genes. Alternatively, the smaller genomes may also have lost repetitive DNA, which would contribute to the enormous size differences between plant genomes. For instance, sorghum and rice are 3-fold and 6-fold smaller than maize. Consequently, it might be possible to take advantage of the smaller grass genomes as a surrogate genome for the larger ones. However, many of the comparisons between the smaller and larger grass genomes have been based on mapped markers and not on the DNA sequence level. To facilitate a more comprehensive analysis of these plant species at the genome level, we have embarked on the Plant Genome Initiative at Rutgers (PGIR). For a more complete description of PGIR visit the home page of PGIR (http://pgir.rutgers.edu).

The International Rice Genome Sequencing Project

Since rice is one of the smallest grass genomes (389 Mb) and also one of the most important staples in worldwide food supply, it was selected for complete sequencing. Although several groups have conducted genome-wide survey sequencing, the International Rice Genome Sequencing Project (IRGSP) sought to determine the entire sequence at high quality scores collinear with the genetic map. Such parameters are critical for cloning genes by traits, in particular quantitative traits, SNPs discovery, and evolutionary studies. PGIR sequenced 45 BAC clones from chromosome 10, 11, and 12. Each region is completely contiguous with sequences that have been determined by our collaborators, the Indian Initiative for Rice Genome Sequencing, New Delhi, India; The Institute for Genome Research, Rockville, MD; Genoscope, Evry, France, The Arizona Genome Institute, Tucson, Arizona, and the Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. Our consortium, which also includes the Rice Genome Projects in Japan, Taiwan, China, Korea, and Brazil, assembled all sequences into twelve chromosomes leaving only about 5% of the total genome in small gaps mainly in heterochromatic regions and analyzed the

organization of genes and repetitive elements. Using computational methods, 37,544 genes, 29% of them in gene families, have been predicted, more than in any animal genome sequenced so far. A detailed account of the analysis has been published in Nature last summer and has received broad press coverage.

Dr. Joachim Messing

Sequencing the Maize Genome

The next plant genome to be sequenced is the maize genome. To address the question of how to sequence the maize genome, we established the Sequencing the Maize Genome (STMG) Consortium. The STMG consortium consisting of the Plant Genome Initiative at Rutgers (PGIR), Arizona Genome Institute (AGI), Arizona Genomics Computer Laboratory (AGCoL) and the Broad Institute (BI) of MIT and Harvard was one of the two NSF pilot projects for genomic sequencing of maize. Data analysis was carried out in collaboration with the Munich Information Centre for Protein Sequences (MIPS), which was funded by other sources. The major components of this project included generation of genomic resources - fingerprinting and end sequencing of BAC clones, generation of high quality reference sequences from random regions, and sequencing of a large contig of BAC clones. Assemblies from deep shotgun sequence coverage of these BACs allowed the evaluation of various sequencing strategies including different coverage depths and the utility of Reduced Representational Sequencing (RRS) data.

Key conclusions from the STMG project studies are:

1) The HICF and BES resource led to an improved physical map of maize inbred B73 of a minimal set of 17,704 overlapping clones with a sequence tag of 650 base pairs every 6.2 kilobases.
2) The average gene density in maize is one gene every 43.5 kilo bases with a repeat content of about 70%.
3) The maize transcriptome is about 180 Megabases or 7.5% of total genome length. While exons have about the same length as in rice, introns are larger mainly due to the insertion or visitation of trans posable elements.
4) Sequencing all the genes of the maize genome by gene enrichment methods is not feasible because of cloning biases particularly in promoter regions and larger introns, but a map-based sequencing aproach is possible because of the divergence of repeat elements.
5) Shotgun sequencing of BAC clones was optimal at six-fold redundancy, at which level sequences could be assembled into a few contiguous sequences using an optimized computer program, called Arachne.
6) Two large contiguous genomic sequences exemplify homoeologous regions of maize that have undergone differential expansion compared to rice, thereby providing new insights into the evolution of plant genomes.

Steven Young
Senior Laboratory Technician

Wolfgang Goettel
Graduate Fellow

Stephanie Andino
Administrative Assistant

Haplotype Variability in Maize

In addition to the comparison of rice, sorghum, and maize chromosomal regions that we have conducted in the past, we also conducted comparisons of chromosomal regions of different maize inbred lines. This line of studies was prompted by our observation that storage protein genes differed in their copy number and protein products between inbred lines. While allelic variations of storage proteins have been known for a long time and used to map their loci in the genome, our studies showed that these loci represent haplotypes that not only differed in alleles but also in the presence and absence of genes. Interestingly, the expression of non-allelic gene copies diverged in respect to their transcriptional regulation, which also explains the penetrance of certain phenotypes like opaque kernel in different genetic backgrounds. Such variation of having non-allelic gene copies was surprising and previously thought to arise mainly during speciation.

Work in the Dooner laboratory and at Dupont also showed the same variation for other loci. Therefore, our laboratories at Waksman investigated possible mechanisms for the generation of haplotypes with extra genes. Based on the analysis of the bronze locus in maize, it appeared that parts of genes located in different locations of the genome got copied and their copies inserted at the bronze locus. The sequence features associated with the insertion of the gene fragment resemble the target site of helicases that promote a rolling circle-type replication as known from bacteriophage. Sequence elements like this have been found in plants and named helitrons. In contrast to the bronze locus, copies of storage protein genes seem to have arisen by a different copying mechanism.

Nevertheless, the appearance of non-allelic or even haplotype-specific gene copies in different maize lines illustrate the significance of hybrids that are likely the chief pathway of spreading haplotypes among maize lines because of recombination. Inbreds on the other hand can freeze unique haplotype combinations. For instance, while the z1C1 loci (storage protein genes) in B73 and BSSS53 are very different in content and size, their z1B loci (also storage protein genes, but different location) are not. Therefore, sequencing the B73 genome will provide the reference for one set of haplotypes that can then be compared with other inbreds and races. Such an alignment of related sequences could provide important insights into the molecular basis of hybrid vigor or heterosis.

Developmental and Molecular Genetics

Richard W. Padgett
Professor

Huang Wang
Laboratory Researcher

Tina Gumienny
Postdoctoral Fellow

Yi Sun
Postdoctoral Associate

Maocheng Yang
Postdoctoral Associate

Namrata Patel
Arresty Fellow

Shannon Smith
Undergraduate Researcher

Ying Li
Graduate Assistant

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

TGFβ is involved in many important developmental decisions in a wide variety of organisms, from sponges to vertebrates. Our main emphasis is on the identification of TGFβ signaling components and determining how downstream targets affect the eventual developmental state of cells. Because of the powerful genetics and molecular tools available, we are using both Drosophila and C. elegans as experimental systems to study components of TGFβ-like signal transduction pathways.

Studies of TGFβ-like Pathways in Drosophila

The Drosophila genome consortium has identified seven TGFβ-like ligands. These ligands represent two branches of the TGFβ superfamily—the *dpp*/BMP and the activin branches. Using a combination of genetic analysis and microarray analysis, we are focusing on elucidating the functions of the activin pathway in the imaginal wing and brain. Mutations have been generated in one of the putative activin ligands, *dActivin2*, and we are characterizing its role in the development of the animal. We have also performed microarray analysis of the *dpp* and activin pathways in fly imaginal disks to compare and contrast their downstream targets. We are focusing on three genes that are regulated by both TGFβ pathways in the fly brain. Each is highly conserved among phyla, but have not been mutated or studied in any organism. Hints of their importance come from RNAi studies of the nematode homolog in which all three show growth defects. Mutations have been generated in two of these genes and we will be characterizing their functions in the coming year.

In past years, we have completed a large genetic screen looking for modifiers of the *decapentaplegic* pathway, one of the TGFβ pathways in *Drosophila*. This screen is designed to identify genes that participate in the *decapentaplegic* pathway and/or genes that act in parallel to TGFβ signaling to modify its activity. One of our mutations fails to complement *pratfall*, an uncloned gene identified in a previous maternal effect screen. We have now cloned the gene and show that it encodes TBP, TATA binding protein. Mutations reside in the highly conserved C-terminus, and presumably, these mutations slow transcription sufficiently to suppress the activity of the activated form of *thick veins*.

Dr. Richward W. Padgett

Studies of TGFβ Signaling in C. elegans

We are also using genetic screens in C. elegans to identify additional components of TGFβ-like pathways. Three different screens have been carried out in C. elegans: 1) an F2 screen for small animals (a mutant phenotype exhibited by many genes in the pathway), 2) suppressors of lon-2, an upstream gene of the pathway, and 3) suppressors of lon-1, a downstream gene in the pathway. Several of these loci are being mapped for eventual cloning.

One locus, sma-10, has been molecularly cloned. It encodes a transmembrane protein that consists of leucine and immunoglobulin repeats, with a short cytoplasmic tail (19 aa). Genetic epistasis experiments place it between the ligand and the receptor, supporting the molecular data suggesting it is a transmembrane protein. Given that its mutant phenotype is identical to other members of the signaling pathway, it appears to be absolutely required for signaling in the body size pathway. However, the male tails of these mutants are normal, suggesting that sma-10 is a tissue specific factor. sma-10 is strongly conserved in Drosophila, and in vertebrates, further supporting an essential role in TGFβ signaling. Biochemical experiments are underway to determine if it interacts with the ligand or the type I/II receptors to increase signaling. Preliminary studies suggest it does not interact with the ligand.

We have examined lon-2, which mutates to a size that is 25% longer than wild type. Further analysis of this gene shows it is related to the glypican family of genes. Our genetic data suggests that lon-2 must be involved in attenuating the TGFβ signal and is genetically upstream of sma-10, but downstream of the ligand. We have determined that LON-2 interacts with ligand, and attenuates signaling.

Examination of MicroRNA Genes

Recently, we have undertaken a new project to study the developmental and growth roles of microRNAs. MicroRNA 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 sometimes affect mRNA levels.

To develop the necessary tools for studying microRNAs, we have developed microarrays for high throughput expression analysis (in collaboration with R. Hart). The regulation of microRNAs in animals is executed through imperfect binding of the microRNA to 3’ sequences in target genes. Since the core-binding domain of the microRNA is 6-7 nucleotides, identifying the real targets from possible targets is difficult. We have collaborated with H. Robins to develop computational methods to predict targets and to develop bioassays to validate putative targets. Using these approaches, we have identified bantam as a regulator of Mad, a signal transducer of TGFβ. Assays in Drosophila are underway to validate this finding. To further our studies of TGFβ in the brain, we have profiled microRNA 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 TGFβ. Mutations of some of these miRNAs are being generated in Drosophila.

As an extension of these projects revolving around growth control, we are looking at miRNA expression profiles of cells in various stages of breast cancer. We find that several microRNAs change expression in various stages of cancer progression. In addition, we find that more miRNAs are down regulated in more progressed cancers, suggesting that several proteins are up regulated. Little is known about these miRNAs, but some have been implicated in growth control and in cell death regulation. Current studies are aimed at identifying their regulatory roles in breast cancer cells.

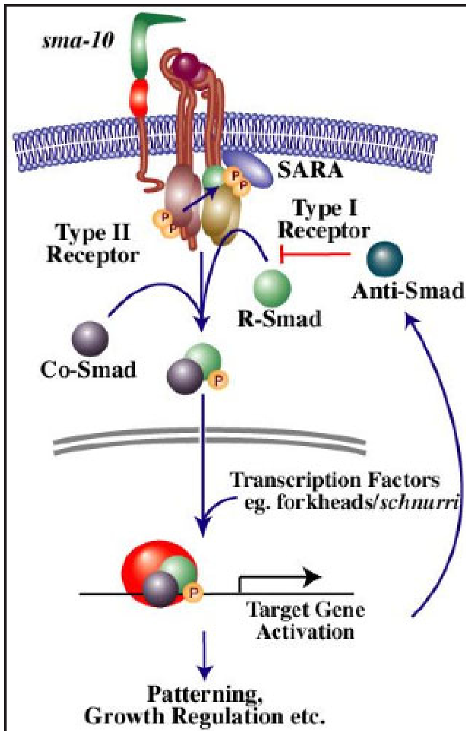


Figure 1. *Model for TGFβ Signaling*.

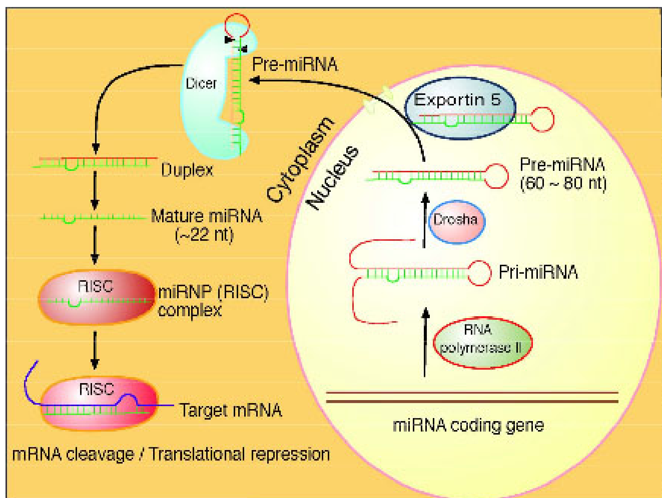


Figure 2. *MicroRNA biogenesis*.

Segmentation during Animal Development

Cordelia Rauskolb
Assistant Research Professor

A fundamental process in the development of many organisms, including annelids, arthropods, and vertebrates, is segmentation. Segmentation serves to subdivide tissues into a series of repeating building blocks along either the body axis, or in some cases, the appendage axis, whereupon each basic unit can then be further elaborated upon during development. In most cases of segmentation, the subdivision of a tissue into repeating units must occur repeatedly as the tissue grows in size. The molecular mechanisms involved in generating a repeating segmental pattern in growing tissues are not well understood.

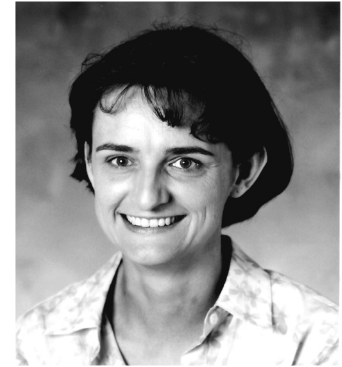
We use *Drosophila*, with its powerful genetics and its known genome sequence, as a model organism in which to study the segmentation of growing tissues. In particular our work focuses on identifying the molecules required for segmentation of the *Drosophila* leg, a tissue in which segmentation must repeat continuously and must be coordinated with tissue growth. The *Drosophila* leg develops during larval stages from a cluster of undifferentiated cells, the leg imaginal disc. It is during these larval stages of development that the leg tissue must be subdivided and borders made between different cell populations. Ultimately, adult *Drosophila* legs are composed of nine leg segments and each segment is separated from the next by a flexible joint.

Notch Signaling Controls Leg Segmentation and Growth

The Notch signaling pathway plays an essential role in the segmentation and growth of the *Drosophila* leg. Notch is a transmembrane receptor protein. There are two ligands for Notch in *Drosophila*, Serrate and Delta. The Notch signaling pathway is conserved amongst many animal species, and is fundamental to a wide range of developmental processes. Furthermore, mutations in human Notch signaling components have been implicated in leukemia (TAN-1), stroke and dementia (CADASIL), and Alagille syndrome, a childhood syndrome resulting in chronic liver disease and segmentation defects. Thus, results we obtain are likely to reveal developmental principles relevant to the biology of a wide variety of organisms. Moreover, because Notch signaling also controls the growth of tissues, and unregulated growth is a key feature of cancer, identifying genes downstream of Notch signaling may provide insights into how tissue growth is controlled.

During *Drosophila* leg development, Serrate and Delta are expressed in a segmentally repeated pattern; one ring of expression per future leg segment, just proximal to the cells destined to form the segment boundary.

This segmentally repeated pattern of expression results in the localized activation of Notch within each presumptive leg segment. Ultimately, upon activation and processing events, the intracellular domain of Notch translocates to the nucleus, resulting in transcriptional regulation of target genes. This local activation of Notch in each leg segment has two important morphological consequences: leg segmentation and growth. Our current aim is to identify the target genes regulated downstream of Notch in order to elucidate the molecular link between leg segmentation and tissue growth.



Dr. Cordelia Rauskolb

The Effectors of Leg Segmentation and Growth

Four-Jointed
four-jointed (*fj*) is regulated by Notch signaling in the leg, eye and wing of Drosophila and thus may be an important mediator of Notch function in a wide range of tissues. *Fj* is expressed in a series of concentric rings in all segments of the developing leg disc and is required for the growth of many leg segments. In addition, *fj* mutants lack a particular segment border, resulting in the fusion of two leg segments. We have previously found that the expression of *fj* is regulated downstream of Notch activation, and also that *fj* can induce expression of the Notch ligands. To further elucidate the mechanism by which *four-jointed* regulates Notch ligand expression, we are investigating the role of genes that participate with four-jointed in the patterning of other tissues, such as the wing; these include members of a Fat-signaling pathway *fat, dachsous, and dachs*. We have determined that mutant clones of the tumor suppressor gene fat and of *dachsous* result in ectopic Serrate (a Notch ligand) expression. Interestingly, dachs suppresses the ability of fat to induce Serrate expression. Hence, *dachs* acts as an important downstream effector of the Fat pathway. Importantly, *dachs* mutants have shortened legs, indicating that the Fat signaling pathway regulates growth during normal leg development. These studies have the potential to reveal a molecular link between leg segmentation and tissue growth, and to further our understanding of how different signaling pathways intersect to regulate development. Additional molecular components of the Fat signaling pathway are being characterized.

Identification of Novel Genes Involved in Leg Segmentation and Growth
To identify additional genes that may be important in leg development, we employed a genetic screen for genes that, when ectopically or over-expressed during leg development, result in abnormal leg segmentation or growth. We have identified a gene that, when over-expressed, results in strong ligand-independent activation of targets downstream of Notch signaling. Thus, a novel regulator of the Notch signaling pathway has been identified. Importantly, we have found that over-expression of this gene results in the accumulation of Notch protein. Given that some of the genes involved in protein ubiquination can give similar phenotypes, we are exploring the possibility that this unidentified gene affects Notch protein ubiquination, an emerging novel mechanism of Notch regulation.

Synapse Formation and the Central Nervous System

Dr. Christopher Rongo
Associate Professor

Dr. Lesley Emtage
Postdoctoral Associate

Dr. Doreen Glodowski
Postdoctoral Associate

Henry Schaefer
Graduate Fellow

Eunchan Park
Graduate Assistant

Lawrence Kramer
Graduate Assistant

Paris Rapp
Laboratory Researcher

John Lee
Undergraduate Student

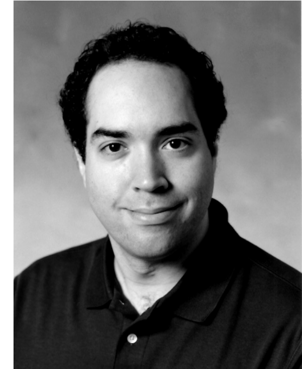
Rebecca Tiver
Undergraduate Student

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. Our research is focused on glutamate receptors (GluRs), which detect glutamate, the major excitatory neurotransmitter in our brain. We are particularly interested in how GluRs are localized to synapses because such glutamate receptor cell biology plays an important role in synaptic communication, synaptic plasticity, and learning and memory. In addition, glutamate receptors are implicated in several diseases of the nervous system, and are a primary neurodegenerative agent activated by mechanical damage (e.g., traumatic injury) and by oxygen deprivation (e.g., stroke). Thus, a better understanding of these receptors will facilitate the diagnosis, treatment, and prevention of diseases attributable to neurodegeneration, and help us better understand the mechanisms behind learning and memory.

Our focus has been to identify the factors that regulate GluR localization and function using a genetic approach in the nematode *C.elegans*. We use *C.elegans* because its simple nervous system, which is easily visualized through its transparent body, allows us to observe glutamate receptor trafficking within neurons in an intact and behaving animal. My lab has used the rich genetic and genomic tools of this organism, and both forward and reverse genetic approaches, to identify multiple genes that function in glutamate receptor biology. All of the genes we have identified have human equivalents that seem to be playing similar or identical roles in the human brain, suggesting that our findings are likely to be applicable to human health.

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

recycling, and finally degradation. We have obtained mutants for genes that regulate all of these steps in GLR-1 localization. Using additional subcellular markers, we showed that nearly all of the genes identified by this screen are relatively specific for GLR-1 localization, and do not impair protein trafficking or synapse formation in general. We have mapped and cloned many of these genes during the last few years, and describe several of them below.



Dr. Christopher Rongo

The Role Of Cytosolic Tail Sequences And Subunit Interactions
Many of the factors that are thought to facilitate glutamate receptor localization do so by interacting with carboxy-terminal tail sequences of these receptors. However, this hypothesis had been difficult to test for AMPA-type glutamate receptors, which can form heterotetrameric complexes with other subunits, making it difficult to assay the contribution of a single subunit. Our work with GLR-1 and a related subunit, GLR-2, indicated that subunit interactions can mediate glutamate receptor localization, and can mask the loss or removal of any one subunit within a channel. A subunit expressed in the absence of other subunits can become localized; moreover, it requires its carboxy-terminal tail sequences for this localization. In the case of GLR-2, we showed that these sequences, when placed on a heterologous transmembrane protein, are sufficient to confer localization. We are currently characterizing proteins that bind to the GLR-1 and GLR-2 tail sequences.

KEL-8, a Ubiquitin Ligase That Regulates GLR-1 Synaptic Abundance
Mutants that lack the BTB-Kelch protein KEL-8 have increased GLR-1 levels at synaptic clusters, whereas the levels and localization of other synaptic proteins appear normal. We cloned KEL-8, and found it to be a strictly neuronal protein that is localized to sites adjacent to GLR-1 postsynaptic clusters along the ventral cord neurites. KEL-8 is required for the ubiquitin-mediated turnover of GLR-1 subunits, and kel-8 mutants show an increase in GLR-1-mediated behaviors (i.e., increased frequency of spontaneous reversals in locomotion), suggesting increased levels of GLR-1 are present at synapses. We found that KEL-8 binds to CUL-3, a Cullin 3 ubiquitin ligase subunit, which we also found to mediate GLR-1 turnover. Our findings indicated that KEL-8 is a substrate receptor for a Cullin 3 ubiquitin ligase that is required for the proteolysis of GLR-1 receptors, and suggested a novel postmitotic role in neurons for Kelch/CUL3 ubiquitin ligases.

Proteins that Regulate GLR-1 Recycling
This year we examined the dynamic trafficking of GLR-1 receptors in vivo. Our findings suggested that GLR-1 is localized not only at synapses but also on early endosomes in neurons. We showed that animals lacking LIN-10, a PDZ-domain containing protein, accumulate GLR-1 primarily in early endosomes, and display a decreased frequency of reversals. Both phenotypes are suppressed by a mutation in unc-11 (AP180), which

reduces endocytosis, suggesting that LIN-10 functions at a step after endocytosis. In addition, we showed that LIN-10 associates with early endosomes, since GFP-tagged LIN-10 colocalized with RFP-tagged RAB-10, an early endosomal protein that mediates receptor recycling. Interestingly, we also found that rab-10 mutant animals display behavioral and GLR-1 localization phenotypes similar to those of lin-10 mutants. Thus, we have identified a novel regulator of AMPAR trafficking, RAB-10, which may work together with LIN-10 to mediate transport of AMPARs out of early endosomes. Taken together, our data indicated that GLR-1 glutamate receptors actively traffic between synapses and endosomal compartments within neurons, and that such receptor dynamics are important for GLR-1 function. Our findings highlighted the importance of glutamate receptor trafficking in nervous system function and behavior in intact animals.

Future Studies

Only by identifying all of the factors that regulate GluR localization can we begin to have a complete understanding of this process. Thus, our current work focuses on mapping and characterizing the remaining genes identified in our screens. So far we have identified molecules that function intrinsically to regulate GluR localization. We are now beginning to focus on characterizing molecules that function extrinsically (e.g., cell-cell signaling molecules), with an emphasis on understanding how intrinsic factors respond to extrinsic signaling to regulate GluR localization. Finally, we are beginning to apply newly developed tools for analyzing GluR dynamics to our collection of mutants. Understanding these dynamics will provide critical insight to synaptic regulation.

Mechanisms of Transcription in Microorganisms

Dr. Konstantin Severinov
Professor

Dr. Konstantin Kuznedelov
Research Associate

Dr. Leonid Minakhin
Research Assistant

Dr. Ekaterina Semenova
Research Assistant

Yulia Yuzenkova
Research Assistant

Nikolas Zenkin
Research Assistant

Dr. Tatyana Naryshkina
Postdoctoral Associate

Dr. Andrey Kulbachinskiy
Postdoctoral Fellow

Dr. Anatoly Vassilov
Postdoctoral Fellow

Ekaterina Bogdanova
Visiting scientist

Transcription is the central step, and a major regulatory checkpoint of gene expression. Defective transcription regulation is the common cause of aberrant growth and development and may result in malignant transformation. Transcription is carried out by DNA-dependent RNA polymerases– large, multisubunit molecular machines. Understanding RNA polymerase (RNAP) structure and function is a key to understanding gene expression in molecular detail. The long-term objective of our research is to uncover the molecular basis of the transcription mechanism and regulation through structure-functional analysis of RNAP and associated proteins. In addition, we use bacteriophage development as a model system to study temporal regulation of gene expression and to uncover novel mechanisms of transcription regulation. As experimental systems we use bacterial RNAP from *Escherichia coli*, thermophilic *Thermus aquaticus* and *T. thermophilus*, opportunistic human pathogen *Pseudomonas aeruginosa*, and plant pathogen *Xanthomonas oryzae*.

The following research projects were actively pursued during the last year.

1. Studies of bacteriophage development

We analyzed the process of E. coli RNA polymerase-catalyzed synthesis of replication primer from single-stranded origin of replication of bacteriophage M13. The results define a novel conformation of transcription elongation complex that is formed due to formation of overextended RNA-DNA hybrid during transcription of single-stranded DNA. Similar conformations may arise during transcription of double-stranded cellular DNA and may affect the rate of elongation and be subject to regulation.

We completed genomic sequencing and determined the temporal transcription pattern of B. anthracis typing phage Fah. The results showed that the late genes of the phage are expressed with the help of phage-encoded RNA polymerase σ factor that is very similar to B. anthracis σ that initiates sporulation. Thus, the Fah phage may be able to change its gene expression strategy depending on the physiological state (exponential growth versus sporulation at nutrient limitation) of the host cell.

In collaboration with the group of Dr. Alexander Solonin, Puschino, Russia, we continued to analyze temporal regulation of transcription during the establishment of mobile restriction-modification systems in naïve bacterial hosts. Since restriction endonucleases encoded by such systems will kill the host cell unless enough methyltransferase that methylates host DNA and prevents endonuclease function (and thus protects the cell) is produced, different restriction-modification systems have evolved

elegant means to ensure that there is a time delay in endonuclease synthesis. We characterized the molecular mechanism that ensures such a delay in a class of systems that are controlled by specialized C (control) proteins, which are homologous to phage λ repressor protein.

Dr. Konstantin Severinov

2. Structure-functional analysis of RNAP

Studies of RNAP-binding transcription inhibitor Microcin J25 (MccJ25) and Streptolydigin (Stl) were performed. Research on MccJ25, which functions by binding to RNAP and blocking the secondary channel--a conduit for NTP substrates to the catalytic center--focused on structure-activity analysis of this peptide antibiotic, using partially proteolyzed MccJ25 derivatives as well as genetically engineered mutants. The results allowed to delineate MccJ25 determinants responsible for cell entry and RNA polymerase binding. Research on Stl involved structural analysis (in collaboration with Dr. Dmitriy Vassylyev's laboratory, University of Alabama, Birmingham) as well as mutational, biochemical and kinetic analysis of the inhibition process. The results lead to a view that binding of Stl greatly reduces the rate of RNA polymerase isomerization that is required to load the NTP substrate from a preinsertion site to an insertion site from which catalysis of phosphodiester bind synthesis can occur.

In collaboration with Seth Darst, Rockefeller University, we developed a genetic system that allows us to produce recombinant T. aquaticus RNA polymerase for structural studies. This is a major advance as it will allow us to perform structural analysis of RNA polymerase mutants, which heretofore was impossible.

As part of a long-standing collaboration with the laboratory of Dr. Martin Buck, Imperial College, London, UK, we continued the analysis of the contribution of individual RNAP core domains to transcription initiation by E. coli RNAP σ^{54} holoenzyme. We analyzed the role of the downstream jaw of the β' subunit in the izomerization of the σ^{54} holoenzyme from closed to open complex.

Timur Kazakov
Visiting Scientist

Dhruti Savalia
Graduate Fellow

Olga Pavlova
Visiting Undergraduate Student

Reproductive Biology, Cell-Cell Interactions

Dr. Andrew Singson
Associate Professor

Indrani Chattergee
Graduate Student

Pavan Kadandale
Graduate Student

Julie Hang
Graduate Student

Jean Parry
Graduate Student

Dr. Rika Maruyama
Postdoctoral Fellow

Fertilization is a biological process that has important social, economic and medical implications. Our primary research interest is the mechanisms of sperm-egg interactions. This includes understanding the molecular events that mediate gamete recognition, adhesion, signaling and fusion. 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.

We are pioneering the use of the nematode worm *Caenorhabditis elegans* for addressing the mechanisms of sperm-egg interactions. The powerful tools of classical and molecular genetics developed for the worm are not available or are very difficult to utilize in the other organisms traditionally used for studying fertilization. We have had great success taking advantage genome analysis combined with powerful forward and reverse genetic approaches to identify important new molecules of fertilization.

The ameboid sperm of *C. elegans*, despite lacking an acrosome and flagellum, carry out the same basic functions common to all spermatozoa. The reproductive biology of *C. elegans* facilitates the identification of mutations that affect sperm and no other cells. The worm exists as a hermaphrodite that makes both sperm and oocytes or as a male that makes only sperm. Mutant hermaphrodites that are spermatogenesis-defective (*spe*) are self-sterile and lay unfertilized oocytes. However, when these otherwise healthy worms are crossed to wild type males (a source of sperm) they can produce outcrossed progeny.

Mutants that Affect Sperm Activation

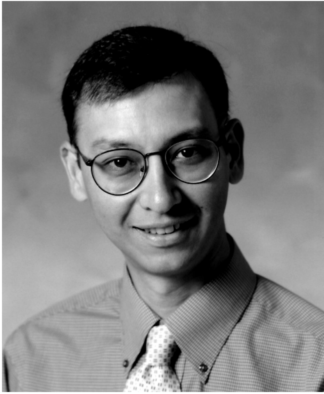
Reproductive success requires that two haploid cells – sperm and egg – unite to form a diploid zygote. Both sperm and egg cells must differentiate into forms that are highly specialized for their specific roles in fertilization and the environment in which they function. This is especially important for sperm that must differentiate to form polar and motile cells capable of locating, moving towards and fusing with an egg. In *C. elegans*, the late steps of spermatogenesis that lead to the conversion of round non-motile spermatids into polar and motile spermatozoa are triggered by a poorly understood signaling pathway that is activated by factors in seminal fluid and/or the reproductive tract. From genetic screens for sterile mutants, we have identified three genes (*spe-19, spe-24, and spe-43*) required by sperm to respond to these seminal fluid and/or reproductive tract signals. We have cloned one of these genes (*spe-19*) and find that it encodes a candidate sperm receptor required to receive the sperm differentiation or

"activation" signal.

Mutants that Affect Sperm Function at Fertilization

We have been examining a set of *spe* genes that produce sperm with normal morphology and motility that cannot fertilize eggs even after contact. From this phenotype we infer that these mutants disrupt sperm-egg recognition, adhesion, signaling or fusion. The characterization of these genes is a critical step in formulating a model concerning their role in wild-type fertilization.

Our collection of sperm function mutants includes the *spe-9, spe-13, spe-36* and *spe-38* genes. We have determined the molecular nature of the proteins encoded by the *spe-9* and *spe-38* genes. Both genes encode sperm surface transmembrane proteins that localize to the pseudopd of mature sperm (Figure 1). This places both proteins in a subcellular location where they could function directly in sperm-egg interactions. SPE-9 is an EGF-repeat containing protein that may function as a sperm ligand for an egg surface receptor. SPE-38 is a novel tetraspan integral membrane protein. Other structurally similar tetraspan molecules have been implicated in processes such as gamete adhesion/fusion in mammals, membrane adhesion/fusion during yeast mating, and the formation of tight-junctions in metazoa. This suggests that the SPE-38 protein is required for gamete adhesion and/or fusion at fertilization.



Dr. Andrew Singson

We are also working to determine the molecular nature of the *spe-13* and *spe-36* genes. As we determine the function of these genes we will gain a better understanding of how all of these genes cause similar "sperm sterile" phenotypes and function together at fertilization.

Mutants that Affect Egg Function at Fertilization

In order to fully understand the molecular mechanisms of fertilization, we must also examine the egg's role in this process. From the worm genome project, we have identified a number of egg enriched/egg specific cell surface molecules that could potentially be required by the oocyte at fertilization. In order to determine the function of these molecules at fertilization we have obtained worm strains where these genes have been disrupted. These worms all display an "egg sterile" (*egg*) mutant phenotype consistent with our hypothesis that they function at fertilization. Two of these genes, *egg-1* and *egg-2*, encode semi-redundant low density lipoprotein receptor (LDL-R) related egg surface molecules. This is the first time that LDL-R class molecules that are best know for their role in fat and cholesterol metabolism have been linked to the process of fertilization.

Mutants that Affect Egg Activation

We have also identified another egg sterile gene, egg-3, that is required for egg activation after sperm entry. This mutant represents the first genetic uncoupling of sperm entry and the start of the developmental program of a new individual. Our analysis of these egg genes compliments our work with sperm mutants and should eventually allow us to make direct

connections between molecules on opposing gametes.

Our research program has led to the first clues concerning the molecular mechanisms of C. elegans sperm-egg interactions. This work both compliments studies of fertilization in other organisms and provides insights relevant to our understanding of cell-cell interactions in general.

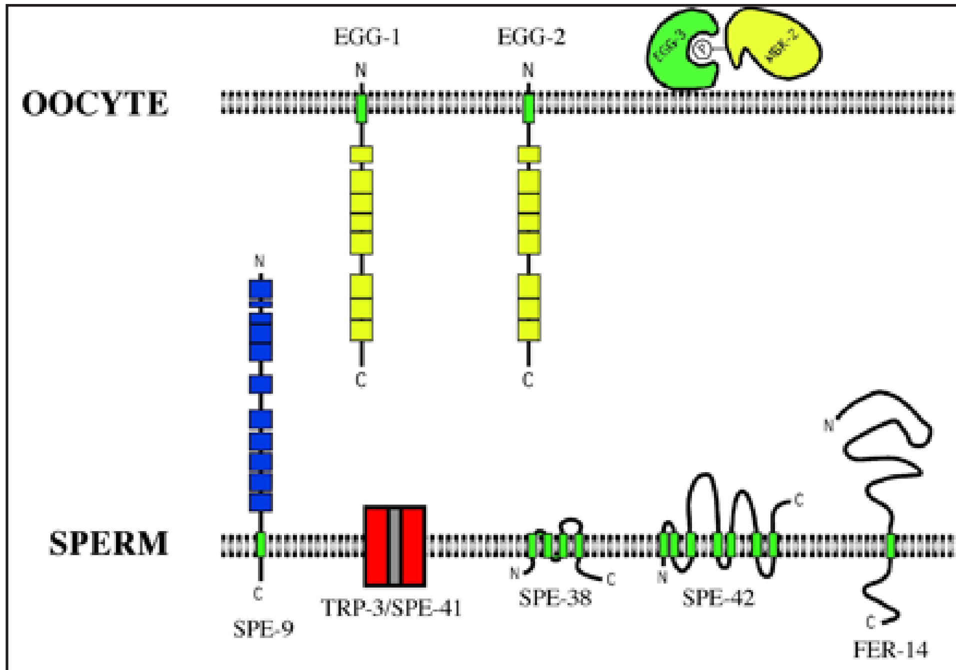


Figure 1. Schematic representations of sperm and egg molecules required for fertilization in C. elegans.

Outreach Activities

Dr. William Sofer
Director, Professor

Dr. Andrew Vershon
Director, Professor

Susan Coletta
Educational Director

Martin Nemeroff
Laboratory Director

There is a gap that separates contemporary research findings (as exemplified by the articles in this Report) from the subject matter that students study in schools and that the public sees in the mass media. This gap is widening as the pace of scientific discovery, especially in the molecular biosciences, increases. It means that we face an ever-worsening problem. People will increasingly have to make critical political decisions about the technologies born of our biological research, but they will lack an understanding of the underlying science that is required to make informed choices.

For over a decade, the Waksman Institute (in collaboration with GE Healthcare, the Rutgers Division of Life Sciences, and the Center for Mathematics, Science and Computer Education at Rutgers University) has been collaborating closely with high school teachers and administrators, in an effort to address this problem. Our strategy has been to engage high school students and their teachers in scientific research, in an effort to bridge the gap between the manner in which science is practiced and the way in which science is taught in the schools. We've developed three programs. The first, the Waksman Student Scholars Program (WSSP), is entering its 14th year. The second, BRITE (Bioinformatics – the Rutgers Initiative in Teacher Enhancement), began in September, 2004 with a grant from the National Science Foundation. The third, the "Waksman Challenge", is in its tenth year.

WSSP

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

The WSSP consists of two interrelated parts: the Waksman Student Scholars Summer Institute (WSSSI) and the Academic Year Program. The WSSSI brings teams of local high school students and their teachers to the Waksman Institute for four weeks in July. Twenty teams joined us this past summer, each team consisting of two students and a teacher. The WSSSI consists primarily of daily seminars and laboratory activities that focus on molecular biology and bioinformatics. The laboratory sessions introduce students and teachers to the tools and procedures needed to carry out the

research project in the schools during the academic year. Both students and teachers also learn to use computers to process and analyze their data. There are additional sessions that deal with bioethical issues and career opportunities. Participants also meet with scientists to discuss recent research developments in the field of molecular biology.

Schools participating in the Academic Year Program are encouraged to develop yearlong research courses organized around teams of students and teachers who participated in the WSSSI. Such courses provide students with opportunities to advance their knowledge of science by conducting research in collaboration with scientists at the University. Students work in teams, discussing findings, trouble-shooting problems with their teachers, and raising additional questions that can be directed at scientists at the University. High school students who successfully complete the Academic Year Program are eligible to receive three units of college credit in the course "Introduction to Research in Molecular Biology", 01:119:105. Over 430 students have enrolled in the course since it first began in spring, 1998.

Dr. William Sofer

The Research Question

This year's WSSP asked the following research question: What can we learn about the genes and proteins of the brine shrimp, *Artemia franciscana*?

In order to answer the question, Dr. Nemeroff prepared a cDNA library from adult brine shrimp. During the summer, the students did minipreps from individual clones of the library, cut DNA with restriction endonucleases, performed polymerase chain reactions, analyzed DNA electrophoretically, sequenced inserts from their clones, and analyzed these sequences using the tools of bioinformatics.

The Academic Year

During the school year, students and teachers returned to the Waksman Institute for six workshops. In these sessions, they discussed problems encountered with their research programs in the schools. They also presented results from their research efforts. While at the University, they used equipment and materials not generally available in the schools. Many students had DNA sequences accepted in GenBank, one of the world's repositories of DNA sequence data.

Near the end of the academic year, each school team presented its research findings at a poster session held at GE Healthcare to which scientists, school administrators, and parents were invited. Each poster was carefully reviewed by scientists from Rutgers and GE, each student team received feedback on their poster, and a plaque was awarded to each participating school.

BRITE

BRITE began last fall. Its goal is to help teachers use information technology, specifically bioinformatics and the computer analysis of protein and

nucleic acid three dimensional structures, and work with teachers to develop strategies that can be introduced into existing high school programs that reflect the way that science is actually practiced. To acheive this goal, teachers work with project faculty to develop, mentor students, and evaluate responses to Waksman Challenges (see below).

Waksman Challenge

Students in the WSSP joined others from around the world in responding to a set of bioinformatics research problems ("Waksman Challenge") presented over the Internet (http://morgan.rutgers.edu). First introduced in the autumn of 1996, Challenge problems have been drawn from the areas of molecular biology, structural biology, genomics, and bioinformatics. The first Challenge of this year began with the online Amino Acid Challenge Game, which was specifically developed for this Challenge. Investigations in protein structure and folding followed the Game. The second Challenge was developed in collaboration with faculty at the Milwaukee School of Engineering (and others), and explored how some mutations lead to structural changes in proteins. The final Challenge focused on the evolutionary pressures to conserve protein structure and function. Student responses to the Waksman Challenge were submitted via the Internet. Each student team received feedback concerning their research. The most creative and substantial responses were posted on our web site.

Embryonic Patterning, Immune Response and Epigenetic Control of Gene Expression in Drosophila

Dr. Ruth Steward
Professor

Dr. Svetlana Minakhina
Research Assistant

Dr. Dmitry Karachentsev
Postdoctoral Associate

Dr. Ayako Sakaguchi
Postdoctoral Associate

Dr. Changjian Wu
Research Associate

Le Nguyen
Laboratory Technician

Marina Druzhinina
Laboratory Technician

Diya Harjani
Undergraduate Student

The Rel Pathway, dorsal-ventral Patterning, and Immune Response in Drosophila

The Toll-Dorsal (NF-κB/Rel) pathway functions in establishing dorsal-ventral polarity in the early Drosophila embryo and in the innate humoral and cellular immune response of larvae and adults. The pathway is conserved in flies and vertebrates. It functions in mammals in the immune and inflammatory responses and is critical for cell growth and survival. Moreover, a large number of mammalian tumors are associated with mis-regulation of the NF-κB/Rel proteins.

Dorsal, like all other Rel proteins, is retained in an inactive state in the cytoplasm through direct interaction with an IκB protein, Cactus. The ventral signal, transmitted through the transmembrane receptor Toll, destabilizes Cactus and controls the formation of a ventral-to-dorsal nuclear Dorsal gradient. This results in the formation of the dorsal-ventral axis through the specific activation of zygotic genes.

The induction of immunity in the Drosophila larval fatbody is dependent on the NF-κB/Rel proteins, Dorsal, Dif, and Relish. In response to microbial infection these proteins are released from their inactive state in the cytoplasm and enter nuclei where they activate the transcription of antimicrobial peptide genes.

In *Drosophila*, mis-regulation of the NF-κB/Rel pathway also results in overgrowth of hematopoetic cells and melanotic tumors. We are studying two genes involved in the formation of melanotic tumors. One, cactin, was initially identified through its interaction with the IκB protein, Cactus. We produced transgenic flies expressing a cactin-RNAi construct in hematopoetic cells and fat body and we find that their larvae produce melanotic tumors similar to cactus loss-of-function larvae. Most recently we are studying the function of the Cactin protein in detail to determine the underlying cause for the formation of the melanotic tumors and are now inducing a targeted mutation in the gene.

The other gene is *zfrp8*; it is highly conserved in humans and Drosophila. We found that in Drosophila the *PDCD2/zfrp8* gene (zinc finger RP8) functions in blood cell differentiation and proliferation. Several loss of function alleles in zfrp8, created in our laboratory, cause enormous hyperplasia of the hematopoetic organs, the lymph glands, and over-proliferation of immature blood cells. The function of PDCD2 in humans is unknown, but our analyses of expression profiles in large scale DNA microarray databases currently available online suggest that PDCD2 is differentially expressed in hematological malignancies, including Acute

Myelogenous Leukemia (AML), while its expression is not changed dramatically in other cancers. The PDCD2/Zfrp8 proteins contain a zinc-finger domain, often found in DNA binding proteins, and a PDCD domain found in only two proteins in vertebrates and flies.

The proteins are 38% identical and both contain a zinc-finger DNA binding domain and a pdcd-2 domain. Our experiments show *PDCD2/zfrp8* does not function in programmed cell death, contrary to expectation based on results in vertebrates. Rather, it appears to regulate tissue and cell growth, functions attributed to cytokines.

Dr. Ruth Steward

Epigenetic Control of Gene Expression

The development of a single cell into an embryo consisting of specific tissues is dependent on cascades of cell-to-cell signaling events that activate transcription factors controlling the expression of specific genes. Control of expression of these genes is regulated at two levels, the interplay of transcription factors that bind DNA directly, and the conformation of chromatin that controls the access of the transcription factors to the DNA. Chromatin organization is controlled at least in part by post-translational modification of the four histone proteins that organize the packaging of DNA into the nucleosome, the basic unit of chromatin.

We are studying the distribution and function of the PR-Set-7 histone methyl transferase (HMT) in *Drosophila* development. We have produced a complete loss of function mutation in the *Pr-Set7* gene. In homozygous mutants, the maternally deposited PR-Set7 protein does not perdure into the first instar larval stage, but mono-, di-, and trimethylation of H4-K20 is detectable into late larval stages, when all three methyl marks are reduced compared to wild-type, and then disappear.

PR-Set7 mutants suppress variegation, confirming that PR-Set7 functions in silencing gene expression. The mutants die at the larval-to-pupal transition and show strong phenotypes in their imaginal discs; the number of cells in the discs is reduced and the content of DNA increased, suggesting a failure to complete cell division. To further investigate the role of histone H4-K20 mono-methylation in the progression through the cell cycle, we used Drosophila neuroblasts from third-instar larvae. We find that mono-methylation of H4-K20 is detected on condensed chromosomes like phosphorylation of histone H3 Ser 10, throughout mitosis. In PR-Set7 mutants progression through early mitosis is delayed and chromosome condensation and spindle formation appear uncoupled in the delayed cells. Mutant metaphase chromosomes show a defect in both sister chromatid resolution and chromosome axis shortening. Interestingly, cyclin B fails to accumulate in the mutant, apparently because the DNA damage checkpoint is activated.

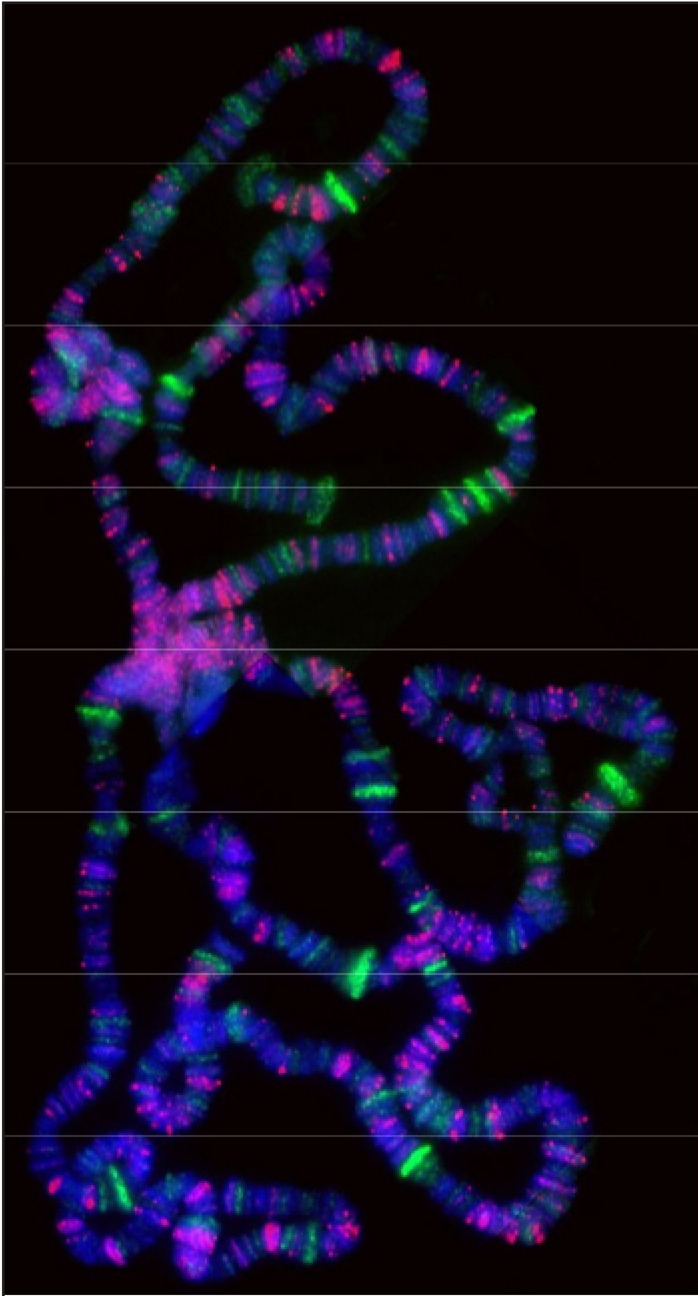


Figure: Drosophila salivary gland chromosome stained with anti-mono-methyl Histone H4-K20 (red) and with antibody specific for the catalytic subunit of RNA polymerase II (green).

Transcriptional Regulation in Yeast

Andrew K. Vershon
Professor

Adrian Bruning
Research Associate

Janet Mead Parent
Research Associate

Brian Gelfand
Graduate Assistant

Harmeetinder Bassi
Undergraduate Student

Jennifer Chen
Undergraduate Student

Shira Eytan
Undergraduate Student

The main focus of our research is on the regulation of transcription in the yeast *Saccharomyces cerevisiae*. Specifically, we are investigating how different regulatory proteins interact to control gene expression and how these interactions influence the regulatory function of these proteins. We have chosen three systems to study this problem: 1) examining the mechanism of repression by the yeast Matα2 protein, a cell-type specific repressor involved in mating-type regulation, 2) examining the molecular interactions of the Mcm1 protein, a MADS-Box protein that is involved in the transcriptional regulation of cell type, cell-cycle and metabolic pathways, and 3) investigating factors involved in the regulation of transcription at the middle stages of meiosis.

Transcriptional Regulation by the Yeast α2 Repressor

The yeast α2 and a1 proteins, members of the homeodomain family of DNA-binding proteins, bind in combination to specific DNA sites to repress haploid-specific genes in the diploid yeast cell type. To determine which genes are regulated by the a1/α2 repressor complex, we have collaborated with the lab of Anirvan Sengupta, who developed a computational algorithm that combines binding site preference data with microarray analysis to identify potential target sites in the yeast genome. In addition to known genes, or genes that are suspected to be repressed by a1/α2, we identified several new targets of the complex. Interestingly, we have also found a binding site near a gene that requires a1/α2 for expression in diploid cells. We have shown that this site regulates transcription of an anti-sense RNA which interferes with expression of a target gene. We are currently investigating how this anti-sense transcript affects expression of the sense transcript.

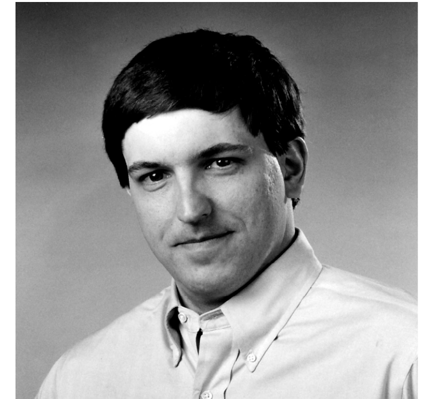
Protein-Protein and Protein-DNA Interactions of the Yeast Mcm1 MADS-Box Protein

Mcm1 is an essential protein in yeast that, in complex with several cofactors, is involved in the regulation of cell-type specific and cell-cycle genes. To investigate the mechanism of how Mcm1 activates these different sets of genes we have systematically screened the yeast genomic deletion library for mutants that fail to fully activate Mcm1-mediated transcription of different target genes. We have identified deletion mutants that affect the expression of one set of Mcm1-regulated genes but not another. This result suggests that Mcm1 uses different mechanisms to activate transcription at the different promoters it regulates. Many of the mutants we have

identified are in components of complexes associated with RNA polymerase II. We are now in the process of testing if these proteins directly interact with Mcm1.

Mcm1 forms a dimer in most of its different regulatory complexes. However, we have shown that Mcm1 is also able to form a heterodimer with Arg80, another MADS-box protein, to regulate genes involved in arginine metabolism. This heterodimer has different DNA-binding specificity than the Mcm1 homodimer and is able to bind DNA cooperatively with Arg81, another transcription factor. This work shows that Mcm1 can function as both a homodimer and heterodimer and how different combinations of MADS-box proteins result in different regulatory activities.

A deletion of the N-terminal arm of Mcm1 results in sensitivity of the cells to high levels of salt. We have used a bioinformatics approach to identify Mcm1 target genes that are sensitive to deletion of the N-terminal arm. A number of the genes identified in this search also appear to have a role in synthesis and maintenance of the yeast cell wall. In support of this finding, we have shown that deletion of the Mcm1 N-terminal arm makes the cells sensitive to drugs, such as calcufluor white, that target the cell wall components. This work has uncovered a new role for the Mcm1 protein. We have shown that the N-terminal deletion does not affect expression, DNA binding in vitro or in vivo or DNA bending by the Mcm1 protein. This suggests that the N-terminal arm region may be important for interaction with a cofactor that is required for expression of these cell wall genes. We are in the process of identifying this potential cofactor.



Dr. Andrew Vershon

Transcriptional Regulation of Meiosis-Specific Promoters in Yeast. Meiosis and sporulation in *Saccharomyces cerevisiae* are characterized by the specific and sequential expression of large numbers of genes. The MSE is a regulatory element found in the promoters of many middle-sporulation genes that sets the timing of their expression during meiosis. The Ndt80 protein binds to MSEs during middle sporulation to activate gene expression. We have determined through DNA microarray analysis that the Sum1, Rfm1, and Hst1 proteins are required to repress transcription of middle-sporulation genes during both vegetative growth and early meiosis. Sum1 binds MSEs of a large number of middle sporulation genes. At a subset of these promoters it interacts with Rfm1, which in turn recruits Hst1 to repress transcription. However, Hst1 is not required to repress transcription of some middle-sporulation genes, which suggests that Sum1 uses a different mechanism to repress these promoters. We are currently investigating how Sum1 represses these promoters.

Sum1 represses middle-sporulation genes during mitotic growth of the cell. We have shown that Sum1 is degraded during the middle-sporulation, allowing expression of the genes it represses. We have mapped the region of the protein that is required to signal this degradation and in collaboration with Ed Winter's lab have shown that this region of the protein is specifically phosphorylated. We are investigating if this phosphorylation has an important role in signaling degradation of the protein. We are also

investigating the mechanism of how Sum1 binds DNA and interacts with its cofactors.

The Hst1 protein shares very strong sequence similarity with Sir2, a histone deacetylase that is required for transcriptional silencing of several different loci in yeast and involved in determining the life span of the cell. Under normal conditions these proteins do not work with each other's co-factors. To determine how these proteins recognize their specific cofactors, we have created a series of domain swaps and amino acid substitutions in these proteins. We have determined that a transfer of the N-terminal region of Sir2 onto Hst1 allows the protein to interact with Sir2 cofactors and function as a transcriptional silencer. In contrast, we have found that a few specific amino acid substitutions in the enzymatic core region of Sir2 will allow it to function as a repressor of middle-sporulation genes in complex with Sum1 and Rfm1. This work shows that the interactions with the different cofactors has a very important role in determining whether members of this class of histone deacetylases function as global silencers or gene-specific repressors.

Cell and Cell Products Fermentation Facility

Kenneth Callanan
Director

Amanda Harford
Senior Laboratory Technician

Erica Foote
Laboratory Technician

The Cell and Cell Products Fermentation facility provides affordable fermentation services to a highly diverse client base.

The facility staff conduct research and development; primarily process scale-up, optimization, characterization and purification for academic institutions, start-up biotech firms as well as the leaders in the pharmaceutical industry. The facility has also served as a production unit and incubator for virtual companies.

Designated BL2-LS for containment of recombinant organisms,and licensed by the EPA, the facility is validated, certified to NIST standards and follows cGMP requirements for the production of, 'Preclinical Biologics'.

During the past calendar year, more than 125 fermentations were performed producing proteins, enzymes, growth factors, antibiotics, and bioremediation agents.

Benedict Michael Fellow

Dragana Rogulja

Dragana Rogulja – *Predoctoral Fellow*

For a body to be correctly proportioned, organ growth and patterning must be linked during development. The ancient Roman architect Vitruvius advocated building temples based on the "perfect proportions of the human body". This concept inspired Leonardo da Vinci's Homo Vitruvius, arguably worlds' most famous drawing. But what links patterning and growth during development?

If normally nonadjacent pieces of tissue from insect or amphibian limbs are surgically juxtaposed, intercalative proliferation can replace the missing tissue. Importantly, the appropriate patterning information is also restored, coupled to the new growth. These regeneration experiments from four decades ago anticipated the existence of morphogen gradients, and led to "gradient models" linking growth and patterning during development, positing that: (A) cells assume distinct "positional values", reflecting their location within the morphogen gradient. (B) Neighboring cells compare their values. (C) Meaningful discrepancies in neighboring values lead to proliferation. (D) Daughter cells assume intermediate positional values, flattening the gradient until further proliferation ceases. It has been clear that slopes of morphogen gradients regulate patterning during development. We now provide the first evidence for a morphogen gradient slope being crucial in growth regulation as well.

Decapentaplegic (Dpp) forms a gradient in the fly wing with its peak at the Anterior-Posterior boundary. Without Dpp, wings do not grow; ectopic Dpp can cause wing overgrowth. In the wild type situation however, more Dpp does not mean more growth: all wing cells proliferate uniformly. How can the *gradient* of Dpp ensure *uniform* proliferation? The gradient model could come to rescue: if Dpp specifies a gradient of positional values in the wing, all cells are different from their neighbors, and thus guaranteed proliferation. A prediction of this model is that juxtaposition of cells with disparate levels of Dpp pathway activation should stimulate proliferation non-autonomously. However, clones expressing a constitutively activated Dpp receptor (Tkv*) have been reported not to promote proliferation non-autonomously. In this case, only a long-term response to Tkv activation was examined. We reasoned that early and late events after pathway activation might differ. This is not possible to examine with the conventional method, as the "young" clones are too small. Therefore, we created a new technique for targeted gene expression: a Flp-out version of a progesterone-inducible Gal4 driver. Without RU486, ectopic clones are made and express Gal4, but it is inactive. Feeding animals RU486 activates Gal4 and consequent UAS-regulated gene expression. This makes it possible to exercise both quantitative and temporal control of gene

expression in large clones of cells.

We found that either activating or inhibiting the Dpp pathway at levels distinct from those in the surrounding cells indeed stimulates strong non-autonomous proliferation. However, this effect is transient. Lateral, but not medial Tkv* clones, also stimulate autonomous proliferation. Abolishing the Dpp gradient by expressing Tkv* throughout the disc obliterates proliferation in the medial parts of the wing, while promoting it laterally. Thus, the gradient of Dpp is needed in the medial region, but Dpp can activate proliferation cell-autonomously in lateral wing cells. The observation that distinct responses to Dpp exist in different aspects of the gradient provides an explanation for how a non-uniform gradient can affect uniform growth: medially, where the gradient is steep, cells rely on its slope for proliferation. Laterally however, the gradient is shallow and cells use the absolute levels of Dpp for proliferation.

We are now studying the mechanism that converts the gradient of Dpp into growth. We expect that cell-surface molecules will be involved, as this would provide the easiest means for cells to communicate with their neighbors. The propagation of the proliferative effect into the wt tissue surrounding the clones in our system is reminiscent of the "domineering non-autonomy" seen with clones that disrupt the establishment of tissue polarity. This process indeed relies heavily on proper expression and localization of cell-surface molecules. Importantly, the same signaling pathway, the Fat tumor-suppressor pathway, regulates both tissue polarity and growth during development. We are now examining the relationship between the Dpp and Fat signaling pathways, and have found evidence for their genetic interaction.

Charles and Johanna Busch Fellows

Doreen Glodowski

Doreen Glodowski – *Postdoctoral Fellow*

In the Rongo lab, we study the molecular mechanisms that direct trafficking and localization of AMPA-type glutamate receptors (AMPARs) in neurons. AMPARs are ion channels that reside in postsynaptic densities, where they transduce most of the excitatory signal in the central nervous system. The strength of signal transduced at individual synapses can increase or decrease, based on the number of AMPARs present at the postsynaptic membrane. Such changes in synaptic strength provide a molecular basis for current models of learning and memory. Thus, by understanding the regulated trafficking of AMPARs in neurons, we are likely to gain insight into complex brain functions like learning and memory.

The genetically tractable model organism, *Caenorhabditis elegans*, which contains two conserved AMPAR subunits (GLR-1 and 2), is an excellent system with which to study the trafficking of AMPARs. *C. elegans* has a relatively simple (only 302 neurons), well-defined nervous system with an invariant structure wherein every chemical and structural synapse has been identified. In addition, the entire genome has been sequenced, and it is relatively easy to develop stable animal lines expressing a desired transgene. Finally, the transparent cuticles of these animals make it possible to observe trafficking and localization of fluorescently tagged neuronal proteins in an intact, living organism.

The LIN-10 protein was identified as a regulator of AMPAR trafficking by results from a genetic screen performed in *C. elegans*. This screen was based on observing the punctate postsynaptic localization of GLR-1::GFP, a chimeric protein containing the AMPAR subunit, GLR-1, fused to GFP. In lin-10 null mutants, GLR-1 accumulates in large patches throughout the ventral nerve cord. To determine which step in the trafficking of GLR-1 requires LIN-10, we asked which subcellular compartment is the site of GLR-1 accumulation in mutant animals. We addressed this question by expressing RFP-tagged versions of proteins that reside in different endosomal compartments, together with GLR-1::GFP, in lin-10 mutant animals. Interestingly, the large patches of GLR-1::GFP colocalized with RFP-tagged RAB-10, a resident of early endosomes. By contrast, GLR-1::GFP-containing patches did not colocalize with RFP-tagged RME-1, a resident of recycling endosomes. Thus, we conclude that, in the absence of the LIN-10 protein, GLR-1 accumulates in early endosomes.

This data led us to hypothesize that LIN-10 might function at early endosomes to direct the trafficking of GLR-1 after endocytosis from the postsynaptic membrane. Consistent with this hypothesis, we found that GFP-tagged LIN-10 colocalized with RFP-tagged RAB-10 when these proteins were coexpressed in *C. elegans*, suggesting that LIN-10 is present

at early endosomes. We next asked if RAB-10 might also play a role in directing the trafficking of GLR-1, perhaps working together with LIN-10. To address this question, we examined the localization of GLR-1::GFP in rab-10 mutant animals, and found a GLR-1 localization defect very similar to that in lin-10 mutant animals. Therefore, we have identified RAB-10 as a novel regulator of GLR-1 trafficking.

To determine if LIN-10 and RAB-10 function together to direct GLR-1 trafficking, we looked at GLR-1::GFP localization in lin-10;rab-10 double mutant animals. Here, we found significantly greater accumulation of GLR-1 in neurites, as compared to the accumulation in either single mutant. This result suggests that LIN-10 and RAB-10 function in distinct pathways to direct GLR-1 trafficking. In addition, we found that UNC-11, which regulates clathrin-mediated endocytosis of GLR-1, functions upstream of LIN-10, but not RAB-10. Thus, we hypothesize that GLR-1 trafficking at synapses involves both clathrin-dependent and clathrin-independent endocytosis from the postsynaptic membrane. Subsequently, distinct endosomal sorting events, depending upon how GLR-1 was endocytosed, would be regulated by either LIN-10 or RAB-10. Current work is aimed at identifying additional factors involved in the distinct pathways directing endosomal trafficking of GLR-1 at synapses.

Jun Huang - *Predoctoral fellow*

Jun Huang

stk1 is the proximalmost gene in the *bz* gene island on chromosome 9. Its predicted protein has high similarity to serine/threonine protein kinases, hence its name. Both *stk1* and *stk2*, a closely related gene on a different chromosome, show a highly specific pattern of expression: they are expressed only in pollen and the mature tassel and not at all in other tissues. Transmission of genes by pollen, the haploid male gametophyte, involves pollen development in the anthers, hydration and germination in the stigma of the female parent, pollen tube growth in the style, and delivery of the sperm to the embryo sac in the ovary. The stk genes likely function in pollen tube growth since mutations of *stk1* show reduced pollen transmission only when competing with wild type pollen.

To facilitate study of their function, I have characterized *three stk1* mutants that arose from Ac transposition events. Two lines (*trAc1355* and *trAc2151*) carried Ac at different positions in the third exon of *stk1*. The Ac in the third line, bz-s39.8 Ac, caused a 1.8-kb adjacent deletion that includes the divergent *bz* and *stk1* promoters and extends into the second exon of *stk1*. These mutations reduce pollen transmission efficiency by as much as 40%. Possibly, *stk1* and *stk2* perform redundant functions. To isolate mutants of *stk2*, we are utilizing the MTM screening service for *Mu*-induced mutations available at Cold Spring Harbor Laboratory. The F1 screening is finished and the F2 confirmatory screening is in progress. Putative mutants should be soon made available to us.

Maize is considered to be an allotetraploid that arose not more than 10 MYA. With 86% nucleotide identity and 68% amino acid identity throughout the entire sequence, one would reasonably assume that stk1 and stk2 are orthologous genes derived one each from the diploid ancestors of maize. However, phylogenetic analysis shows that each of these two genes has a remarkably closer counterpart in *Oryza sativa*, which diverged from maize 50 MYA. The respective STK proteins in rice are 83% identical to STK1 and 78% identical to STK2. Hence the gene duplication happened before the maize-rice speciation event. Therefore the *Zmstk1* and *Zmstk2* are paralogs, not orthologs.

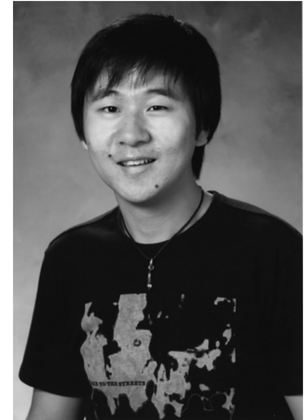
Further study, for instance, analysis of the subcellular localization of the STK1 and STK2 protein, mutants characterization and utilization, will be conducted in the future.

Tengbo Huang — *Predoctoral Fellow*

The leaves of most plants exhibit differences between their adaxial (dorsal) and abaxial (ventral) surfaces and internal tissues of the leaf blade are polarized along this adaxial-abaxial axis. The Kerstetter Lab is working on defining the molecular mechanism involved in this adaxial-abaxial patterning.

Leaf polarity is controlled by several groups of genes. Among these genes, the *KANADI* (*KAN*) genes are key promoters of abaxial leaf fate. *KANADI* encodes a putative transcription factor belonging to the *myb*-related GARP family. To further characterize its role, the lab has pursued the identification of the downstream targets of *KAN1*. I have tested the DNA binding properties of *KAN1* both in vitro and in vivo. Using PCR-assisted DNA binding site selection, I identified a core DNA sequence **WTATXC** (W: A/T; X: any base) to which *KAN1* GARP binds in vitro. This consensus sequence was confirmed by gel-shift assay in which *KAN* GARP protein exhibited higher affinity to the core sequence than to mutant variants. To investigate the binding of *KAN1* to putative targets in vivo, I performed chromatin immunoprecipitation (ChIP) experiments. Quantitative assay of ChIP products has so far revealed 6 target gene promoters that are bound by *KAN1* protein. This set of putative targets include genes involved in plant hormone metabolism, transport and response, protein kinases and transcription factors. Interaction of *KAN1* with these promoters is beginning to provide insight into the transcriptional network that specifies abaxial leaf fate.

Although *KAN* genes are essential promoters of abaxial leaf identity, we are working to find additional factors critical for specifying leaf polarity and subsequent events in leaf blade outgrowth. The *ARROW1 (ARO1)* gene was identified as an enhancer of kan. Combining aro1 with *kan1 kan2* enhances the *kan1 kan2* mutant phenotype, which indicates that *ARO1* plays a significant role in leaf polarity. I mapped *ARO1* to the bottom of chromosome I and confirmed its molecular identity by identifying additional T-DNA insertion alleles and transgene complementation. *ARO1* is annotated as a Pumilio/PUF RNA-binding domain containing protein. It belongs to a conserved family of RNA-binding proteins. *Drosophila Pumilio* is a founding member of this family and is required for establishment of anterior-posterior polarity and stem cell maintenance through translation inhibition. The sequence similarity between *ARO1* and *Pumilio* indicate that *ARO1* may function as a translational inhibitor in a manner similar to Pumilio. The aro1 single mutant displays a variety of defects in shoot and root development. It shows synergistic genetic interactions with mutants in *ASYMMETRIC LEAVES1 (AS1), ASYMMETRIC LEAVES2 (AS2)* and *REVOLUTA (REV)*, genes involved in adaxial leaf polarity and meristem development. The pleiotropic aro1 mutant phenotype and its interaction with other leaf development mutants indicate that *ARO1* plays important roles in multiple pathways during plant development.



Tengbo Huang

KAN1 and *ARO1* are both important regulators of leaf development. In future studies, I plan to test additional targets of *KAN1* using ChIP and characterize the *KAN1* binding sequence fused to a *green fluorescent protein* reporter in transgenic plants. I will also continue to study the pleiotropic roles of *ARO1* in plant development and use immunoprecipitation combined with microarray analysis to identify specific targets of *ARO1* translational inhibition. We hope the elucidation of functions of *KAN* and *ARO1* will provide novel insights into the transcriptional and translational network that regulates leaf development.

Andrey Kulbachinskiy - *Postdoc Fellow*

The laboratory of K. Severinov is focused on studies of the molecular basis of transcription and its regulation through structure-functional analysis of bacterial RNA polymerase (RNAP) and associated protein factors. Initiation of transcription in a bacterial cell is carried out by RNAP holoenzyme, a complex of the core enzyme ($\alpha 2\beta\beta'\omega$) and a specificity factor, σ. Recognition of bacterial promoters occurs through interactions of the σ subunit with specific promoter elements. Most promoters contain two elements with consensus sequences TTGACA and TATAAT that are centered, respectively, 35 and 10 base pairs upstream of the transcription start site. The so-called "extended -10" promoters contain an additional TG motif located one base pair upstream of the -10 promoter element; these promoters do not require the -35 element for their activity. The -10, -35, and extended -10 motifs are recognized by regions 2.4, 4.2, and 2.5 of the σ subunit, respectively. During transcription initiation, contacts of σ with promoter DNA and core RNAP must be broken to allow promoter clearance and transition to productive elongation of transcription. During my stay in the Severinov laboratory, I analyzed mechanisms of promoter recognition in diverse bacteria and studied the role of the σ subunit in the process of promoter clearance.

Andrey Kulbachinskiy

Previously, we selected single-stranded DNA aptamers that specifically interact with the σ subunit from T. aquaticus (Taq). All aptamers contained a sequence similar to the -10 promoter consensus element wich was a part of a larger conserved motif, TGTATAATGGGA. Double-stranded DNA fragments based on aptamer sequences served as strong promoters for Taq RNAP but were not recognized by the E. coli RNAP. In contrast to other known promoters, aptamer-based promoters lack either the -35 or the extended -10 motifs. The goal of my work in the Severinov lab during last year was to characterize this novel class of promoters and to study the mechanism of their recognition. The main results of my work are summarized below.

1) Motifs within the aptamer sequence that are important for promoter recognition.

I obtained several mutant variants of aptamer-based promoters with changes in various parts of the sequence and studied their transcription properties. It was found that the GGGA motif located downstream of the -10 sequence is critical for aptamer-based promoters' function and that recognition of the promoters does not depend on specific sequences located upstream or downstream of the conserved aptamer motif. The first three nucleotides from the GGGA motif were the most important for promoter function.

2) Regions of Taq RNAP that are important for the recognition of the aptamer promoters.

By using mutant variants of Taq RNAP bearing changes in several domains which could potentially be involved in the interactions with promoter DNA, I demonstrated that sequence-nonspecific interactions of the

C-terminal domain of the α subunit, the flap domain of the β subunit of core RNAP and region 4.2 of the σ subunit with upstream promoter DNA are important for the activity of the aptamer-based promoters.

3) Structure of the complexes formed by Taq RNAP on aptamer-based promoter.

Complexes between RNAP and aptamer-based promoters were studied using KMnO4 probing. It was demonstrated that the size of transcription bubble formed by RNAP on this type of promoters does no differ from other studied promoters. The GGGA motif from the conserved aptamer sequence was critical for open complex formation since its mutations impaired promoter opening.

4) The basis of the promoter specificity.

To reveal why aptamer-based promoters are not recognized by the E. coli RNAP, I studied several variants of aptamer-based promoters containing the -35 and/or lacking the GGGA motif. It was found that introduction of the -35 element make the aptamer-based sequence a strong prtomoter for E. coli RNAP. At the same time, the GGGA motif was not important for promoter recognition by the E. coli enzyme. Using site-specific DNA-protein cross-linking, it was demonstrated that the GGGA motif is recognized by region 1.2 of the Taq σA subunit.

5) Analysis of natural Taq promoters.

Analysis of known Taq promoters and closely related T. thermophilus promoters revealed that some of them contain GGGA-like motifs. I studied the properties of these natural promoters and their mutants with changes in the GGGA motif. The results of this work demonstrated that in natural Thermus promoters the GGGA motif has a function similar to that in the aptamer-based promoters.

In summary, a novel class of bacterial promoters has been comprehensively analyzed. The results of the work are important for understanding of species-specificity of transcription initiation in bacteria. In addition, this work may have important practical implications for designing efficient expression vectors to produce biotechnologically important proteins in Taq and related bacteria.

microRNAs (miRNAs) are a newly identified and abundant class of small non-coding RNAs, which are about 22nt in length, processed from single strand stem loop precursor RNA by Dicer. Two mechanisms for post-transcriptional gene regulation by miRNAs have been reported so far. In plants, mRNAs containing sequences perfectly complementary to miRNAs are cleaved by ribonucleases in RISC complex, thus degrading the mRNAs. In animals, miRNAs repress translation by imprecise complementary to the 3' UTR of their target mRNAs. In addition, recent reports show that miRNAs can affect message levels in animals. Hundreds of microRNAs have been found in different organisms, now establishing that they are universal in higher organisms. However, the functions of only a few are known, and the cellular functions of genes that are regulated by microRNAs are only beginning to be revealed. In the Padgett lab, we focused on studying microRNAs related to TGFβ signaling, and their roles in development.

Ying Li

bantam, a *Drosophila* miRNA, has been shown to prevent apoptosis through regulation of the pro-apoptotic gene *hid*, and to stimulate cell proliferation by regulation of an unknown pathway. Through computational algorithm predictions, we found that *Mad*, a key signal tranducer in TGFβ pathway, contains three possible bantam binding sites in its 3'UTR. I tested this hypothesis by creating a *Mad* sensor. The *Mad* 3'UTR is placed under control of the *Drosophila actin* promoter, and transfected into S2 cells. I found that expression was greatly decreased by the over expression of *bantam*. This inhibitory effect of bantam on *Mad* can be abolished by mutating two out of three bantam binding sites in the *Mad* 3'UTR. Using similar sensor construct in animals, (instead of firefly luciferase, I used EGFP as reporter) I examined *bantam* expression patterns in *Drosophila* third instar wing discs. The *bantam* sensor has higher expression in cells near the anteroposterior and dorsoventral boundaries, indicating lower levels of bantam in these regions. I found that the wild-type *Mad* sensor has a similar pattern to the bantam sensor, but a mutated *Mad* sensor shows high expression of EGFP all over the wing disc. This suggests that some miRNA regulates *Mad*, and my hypothesis is that it is bantam. To further show a connection between *bantam* levels and the *Mad* reporter expression, I over expressed *UAS-bantam* with a patched-Gal4 driver, which expresses along A-P boundary. In this experiment, the Mad sensor expression was significantly decreased at the A-P boundary. To further test the hypothesis that bantam could affect DPP signaling, I checked omb, a DPP target gene. When bantam was over expressed along A-P boundary, omb expression was greatly decreased. To get deep insight of function of interaction of bantam and DPP signaling during development, I generated transgenic flies, *ubi-Mad-w3'UTR*, which is ubi-Mad with its wild type 3'UTR, and *ubi-Mad-m3'UTR*, which is ubi-Mad with bantam binding sites mutated in its 3'UTR. I am going to compare their ability to rescue the dpp61 haplo-insufficiency.

Kerry Lutz

Kerry Lutz – *Predoctoral Fellow*

For my Ph.D. thesis I am developing a reproducible system for plastid transformation in *Arabidopsis thaliana*. The ability to reproducibly transform plastids is dependent on a reliable tissue culture system. Currently plastid transformation uses leaf tissue for plant regeneration. *Arabidopsis* leaves placed on regeneration medium form polyploid plants, which are sterile at a high frequency. Therefore, I have been working on developing a novel system to provide an abundant source of totipotent embryogenic cells to be used as recipients for plastid transformation. The plant regeneration system utilizes a steroid inducible *BABY BOOM (BBM)* gene that will trigger embryo differentiation from somatic cells when dexamethasone is added to the plant medium.

The *BBM* gene encodes a transcription factor promoting embryogenesis in somatic cells. I prepared constructs that make import of BBM into the plant nucleus steroid dependent by fusing it with a glucocorticoid-receptor (GR). In the absence of the steroid inducer, BBM is localized to the cytoplasm. Treatment of the plant with dexamethasone triggers import of the BBM transcription factor into the nucleus, where it activates its target promoters. Earlier, I successfully transformed the constructs into the nuclear genome of *Arabidopsis thaliana*. Currently, I am testing the utility of the BBM regeneration system by selection of spectinomycin resistant mutants, a protocol that is also used to recover transplastomic plants. By now I have identified five spectinomycin resistant mutants, of which two contain a restriction site change identifying mutations in the plastid *rrn16* gene. Plant regeneration from the mutant cell lines and identification of the nucleotide changes that confers spectinomycin resistance are in progress. Simultaneously, I initiated experiments to obtain transplastomic plants that I expect to complete in the next year.

Rika Maruyama – *Postdoctoral Fellow*

Rika Maruyama

Upon being fertilized by a sperm, the egg undergoes activation and starts development as a new individual. However, in spite of intense study, the oocyte factors that detect sperm entry and trigger egg activation still remain largely unknown.

The Singson lab uses the nematode worm *Caenorhabditis elegans* as a model system to understand the molecular mechanism of fertilization. *C. elegans* exist as self-fertile hermaphrodites that produce both sperm and eggs or as males that produce only sperm. The hermaphrodites that have defects in the process of fertilization are easily identified because they produce no progeny and lay eggs without egg shells.

Using these phenotypes as indicators, *egg-3* was identified in the large-scale phenotypic analysis by RNA interference. The *egg-3* gene encodes as a tyrosine-phosphatase-like protein but some catalytic residues are not conserved. Therefore, EGG-3 is predicted to be an anti-phosphatase, which might function as a competitor to active phosphatases for substrate or as an adaptor/scaffold module that bind phosphorylated targets.

The *egg-3(tm1191)* hermaphrodites isolated by National Bioresource Project also produced no progeny and their eggs had no eggshells. The lack of progeny production was not rescued by wild-type sperm. A single sperm entered each *egg-3* mutant oocyte, which indicated that fertilization and the block to polyspermy occurred normally. And *egg-3* embryos exhibited individualized chromosomes at meiosis I, however underwent meiosis I and meiosis II without polar body formation. These phenotypes are similar to the phenotypes caused by the depletion of actin filaments. Thus, *egg-3* mutants were defective in egg activation following fertilization.

GFP-EGG-3 driven by the germline specific promoter was localized at the oocyte cortex, which is similar to the localization of GFP-MBK-2. MBK-2 is required for the transition from oocytes to embryos. We found that GFP-MBK-2 was not localized at the oocyte cortex in *egg-3* mutants, on the other hand, the localization of GFP-EGG-3 was identical to wild-type in mbk-2(RNAi) worms. However, OMA-1-GFP and GFP-MEI-1, which are phosphorylated by MBK-2, were degraded in egg-3 mutants similar to wild-type. These data suggest that the oocyte cortex localization of MBK-2 that is dependent on *egg-3* might not be essential to the MBK-2 activity.

Taken together, we speculate that EGG-3 might be one of the essential oocyte factors that detect the signal of the sperm entry and trigger egg activation after fertilization and might also function as an scaffolding protein for MBK-2 at the oocyte cortex. We are currently collaborating with Fabio Piano's lab in NYU, to investigate the cortical actomyosin dynamics during meiosis in *egg-3* mutant.

Ayako Sakaguchi – *Postdoctoral Fellow*

Ayako Sakguchi

The Steward laboratory has been studying the role of the PR-Set7 histone methyl transferase (HMT) in *Drosophila*. This enzyme mono-methylates lysine 20 (K20) of histone H4, contains conserved sequence motif called SET domain, found in HMTs from plants and vertebrates. A complete loss of function allele of *PR-Set7* was isolated in the Steward lab by P-element mediated mutagenesis. Homozygous mutant animals die at the larval-to-pupal transition, demonstrating that *PR-Set7* is essential for viability. Mutant animals showed a decrease in mono-methylation and a mitotic phenotype. The number of cells in the larval discs is reduced about four times. However, many aspects of *PR-Set7* function and the importance of methylation of histone H4K20 still remain to be elucidated.

To identify the precise role of PR-Set7 and histone H4K20 methylation in the progression through the cell cycle, we examined mutant neuroblasts from third-instar larvae and found:

1. Mono-methylation of histone H4K20 was strongly reduced.
2. Both the mitotic and the S phase indexes were reduced.
3. Progression through early mitosis was delayed and chromosome condensation and spindle formation appeared uncoupled.
4. The protein level of cyclin B was reduced by APC/C dependent proteolysis.
5. Mutant metaphase chromosomes showed a defect in both sister chromatid resolution and chromosome axis shortening.

To investigate if these phenotypes are caused by the activation of the DNA damage checkpoint, we made a homozygous double mutant of *PR-Set7* and the *Drosophila ATR orthologue, mei-41*. The *mei-41*^{D3} allele used here has a defect in the checkpoint allowing cells with damaged DNA to enter mitosis. In the *mei-41;PR-Set7* double mutant, both the mitotic and S phase indexes became similar to those observed in the homozygous *mei-41* mutant. The number of prophase cells was reduced compared to *PR-Set7* mutant and became similar to wild type and *mei-41*, showing that the double mutant suppressed abnormalities of cell cycle progression. The protein levels of cyclin B also recovered. These results show that in *PR-Set7* the DNA damage checkpoint is activated and that it induces the mitotic phenotypes. However, the ratio of anaphase/telophase cells with lagging chromatids was increased when the checkpoint-arrest was abolished in the double mutant, suggesting that the defect in chromosome condensation is independent of the checkpoint.

Little is known about how sensors for the DNA damage checkpoint work. Although it was reported that abnormal chromatin structure activates spindle checkpoint in yeast, it remains to be determined if the DNA damage checkpoint proteins sensor abnormal nucleosome structure or abnormal chromatin structure. Our results show that the aberrant mono-methylation state of histone H4K20 somehow causes abnormal higher order structure of DNA, which activates the DNA damage checkpoint. If the loss of mono-methylated H4K20 affects the DNA structure, mono-methylation could be indispensable for proper mitotic chromosome architecture.

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Faculty

Dr. Joachim Messing, Director

Dr. Helen Berman
Dr. Hugo Dooner
Dr. Richard H. Ebright
Dr. Robert Goodman
Dr. Kenneth Irvine
Dr. Randall Kerstetter
Dr. Casimir Kulikowski
Dr. Pal Maliga
Dr. Kim McKim
Dr. Richard W. Padgett
Dr. Cordelia Rauskolb
Dr. Christopher Rongo
Dr. Konstantin Severinov
Dr. Aaron Shatkin
Dr. Andrew Singson
Dr. William Sofer
Dr. Ruth Steward
Dr. Andrew K. Vershon

Professors Emeriti

Dr. Carl Price
Dr. Carl P. Schaffner
Dr. Robert W. Simpson
Dr. Evelyn Witkin

Administrative, Secretarial, Support and Computer Staff

Robert Rossi, *Executive Officer*

MaryLynn Bianca
Administrative Assistant

Attilio DeFalco
System Administrator

Ken Doran
Maintenance Mechanic

Michelle Holmes
Senior Administrative Assistant

Reuben F. Johnson
Unit Coordinator

Rose Keresztes
Admistrative Assistant

Judy Kopchala
Administrative Assistant

Greg Listner
System Administrator

Eileen McLaughin
Head Stock Clerk

Avonelle McKnight
Principal Laboratory Technician

Alberta Molnar
Business Manager

Ted Przybylski
Instrument Maker/Repairer

Michael Reves
Maintenance Mechanic

Steve Richards
Maintenance Mechanic

Doug Rosso
Unit Computing Specialist

Albin Schab
Regional Engineer

Laszlo Varga
Administrative Assistant

Faculty Addresses

Dr. Helen Berman
Professor
Department of Chemistry &
Chemical Biology
Wright and Rieman Laboratories
Rutgers, the State University of
New Jersey
610 Taylor Road
Piscataway, New Jersey 08854
(732)445-4667/FAX (732)445-4320
Berman@rcsb.rutgers.edu
Web URL:http://waksman.rutgers.
edu/Waks/Berman/berman.html
http://rutchem.rutgers.
edu/faculty/berman/berman.html

Dr. Hugo Dooner
Professor
Waksman Institute
Rutgers, the State University of
New Jersey
190 Frelinghuysen Road
Piscataway, New Jersey 08854-
8020
(732)445-4684/FAX (732)445-5735
Dooner@waksman.rutgers.edu
Web URL:http://waksman.rutgers.
edu/Waks/Dooner/dooner.html

Dr. Richard H. Ebright
Professor
Howard Hughes Medical Institute
Waksman Institute
Rutgers, the State University of
New Jersey
Piscataway NJ 08854-8020
PH: 732-445-5179
FAX:732-445-5735
Ebright@waksman.rutgers.edu
Web URL:http://www.hhmi.org/re-
search/investigators/ebright.html
Web URL:http://rutchem.rutgers.
edu/faculty/ebright.html
Web URL:http://waksman.rutgers.
edu/Waks/Ebright/ebright.html

Dr. Kenneth Irvine
Associate Professor
Howard Hughes Medical Institute
Waksman Institute
Rutgers, the State University of
New Jersey
190 Frelinghuysen Road
Piscataway, New Jersey 08854-
8020
(732)445-2332/FAX (732)445-5735
Irvine@waksman.Rutgers.edu
Web URL:http://waksman.rutgers.
edu/Waks/Irvine/irvine.html

Dr. Randall Kerstetter
Assistant Professor
Waksman Institute
Rutgers, the State University of
New Jersey
Piscataway NJ 08854-8020
PH: 732-445-4737
FAX:732-445-5735
Kerstter@waksman.rutgers.edu
Web:RL:http://waksman.rutgers.
edu/Waks/Kerstetter/kerstetter.html

Dr. Casimir Kulikowski
Professor
Computing Research and Education
Building (CoRE)
Rutgers, the State University of
New Jersey
110 Frelinghuysen Road
Piscataway, New Jersey 08854
(732)445-2006/FAX (732)445-0537
Kulikows@cs.rutgers.edu

Dr. Pal Maliga
Professor
Waksman Institute
Rutgers, the State University of
New Jersey
190 Frelinghuysen Road
Piscataway, New Jersey 08854-
8020
(732)445-5329/FAX (732)445-5735
Maliga@waksman.rutgers.edu
Web URL:http://waksman.rutgers.
edu/Waks/Maliga/maliga.html

Dr. Kim McKim
Associate Professor
Waksman Institute
Rutgers, the State University of New Jersey
190 Frelinghuysen Road
Piscataway, New Jersey 08854-8020
(732)445-1164/FAX(732)445-5735
McKim@waksman.rutgers.edu
Web URL:http://waksman,rutgers.edu/Waks/McKim/mckim.html

Dr. Joachim Messing
Director, University Professor
Waksman Institute
Rutgers, the State University of New Jersey
190 Frelinghuysen Road
Piscataway, New Jersey 08854-8020
(732)445-4257/FAX (732)445-0072
Messing@waksman.rutgers.edu
Web URL://waksman.rutgers.edu/Waks/Messing/messing.html

Dr. Richard Padgett
Professor
Waksman Institute
Rutgers, the State University of New Jersey
190 Frelinghuysen Road
Piscataway, New Jersey 08854-8020
(732)445-0251/FAX (732)445-5735
Padgett@waksman.rutgers.edu
Web URL:http://waksman.rutgers.edu/Waks/Padgett/padgett.html

Dr. Cordelia Rauskolb
Assistant Research Professor
Waksman Institute
Rutgers, the State University of New Jersey
190 Frelinghuysen Road
Piscataway, New Jersey 08854-8020
(732)445-2872/FAX (732)445-5735
Rauskolb@waksman.rutgers.edu
Web URL:http://waksman.rutgers.edu/Waks/Rauskolb/rauskolb.html

Dr. Christopher Rongo
Associate Professor
Waksman Institue
Rutgers, the State University of New Jersey
190 Frelinghuysen Road
Piscataway, New Jersey 08854-8020
(732)445-0955/FAX (732)445-5735
Rongo@waksman.rutgers.edu
Web URL:http://waksman.rutgers.edu/Waks/Rongo/rongo.html

Dr. Konstantin V. Severinov
Professor
Waksman Institute
Rutgers, the State University of New Jersey
190 Frelinghuysen Road
Piscataway, New Jersey 08854-8020
(732)445-6095/FAX (732)445-5735
Severink@waksman.rutgers.edu
Web URL:http://waksman.rutgers.edu/Waks/Severinov/severinov.html

Dr. Andrew Singson
Associate Professor
Waksman Institute
Rutgers, the State University of New Jersey
190 Frelinghuysen Road
Piscataway, New Jersey 08854-8020
(732) 445-0836/FAX (732)445-5735
Singson@waksman.rutgers.edu
Web URL:http://waksman.rutgers.edu/Waks/Singson/singson.html

Dr. William Sofer
Professor
Waksman Institute
Rutgers, the State University of New Jersey
190 Frelinghuysen Road
Piscataway, New Jersey 08854-8020
(732)445-3052/FAX (732)445-5735
Sofer@waksman.rutgers.edu
Web URL:http://waksman.rutgers.edu/Waks/Sofer/sofer.html

Dr. Ruth Steward
Professor
Waksman Institute
Rutgers, the State University of New Jersey
190 Frelinghuysen Road
Piscataway, New Jersey 08854-8020
(732)445-3917/FAX (732)445-5735
Steward@waksman.rutgers.edu
Web URL:http://waksman.rutgers.edu/Waks/Steward/steward.html

Dr. Andrew Vershon
Professor
Waksman Institute
Rutgers, the State University of New Jersey
190 Frelinghuysen Road
Piscataway, New Jersey 08854-8020
(732)445-2905/FAX (732)445-5735
Vershon@waksman.rutgers.edu
Web URL:http://waksman.rutgers.edu/Waks/Vershon/vershon.html