

Peter Schweitzer, New Director of High-Throughput Sequencing and Genotyping Unit of the W.M. Keck Center for Comparative and Functional Genomics

Peter Schweitzer and his family arrived just in time to celebrate the holiday season in the Midwest. He comes with superb credentials. After completing his undergraduate degree in biology at Washington University, he started his graduate training in immunology and immunochemistry at Johns Hopkins University and at the University of Pennsylvania in Philadelphia. His postdoctoral studies took him to the Jackson Laboratory (JAX) in Bar Harbor, Maine, where he pursued his research interests in the synthesis and secretion of immunoglobulins. Upon returning to Johns Hopkins for a

second postdoctoral position, he became interested in the molecular biology of DNA replication and hypermutation of immunoglobulin genes. The Jackson Laboratory recruited him as a research associate in 1991, where he first continued his studies in molecular genetics and genomics, later became the manager of the Induced Mutant Resource Typing Laboratories, and for the past five years has been managing the Molecular Biology Core facility for the Genetic Resources program.

continued on page 7



Peter Schweitzer

Post-Genomic Institute: Update

Preliminary programming has been completed by the selected architect/engineering team, CUH2A, working with a staff and faculty committee headed by Professors Harris Lewin and Charles Miller. Programming meetings and interviews have been conducted with key faculty, deans and department heads to determine the needs and requirements of the users for this new facility. The building design was approved at the September BOT meeting. Bids are expected to be requested in April 2002, accepted in May 2002, with substantial completion by May 2004.

The building design will facilitate collaboration between PGI research teams and provide space to advance technology transfer, education, and outreach in the field of genomic biology. Space will be provided for both animal resources and plant facilities. Each thematic research area will be housed in a "Thematic Lab Module" providing laboratory facilities for Biology, Chemistry and Bioinformatics. There will be six multidisciplinary research themes, each con-

taining five research teams (including one for bioinformatics). The PGI will also house a large technology core, which can be considered as a seventh research theme. Research on new technology will be conducted within this area in addition to providing service functions for the institute and the campus.

In addition to the PGI planning monies, the campus has also received \$3.2 million recurring funds from the State of Illinois to hire faculty and staff associated with the programmatic themes of the Post Genomic Institute.

A search is underway for the Director of the PGI. A faculty hiring initiative to fill the funded PGI lines has begun, and four new faculty members have been hired. Two faculty excellence hires have also added faculty to the PGI. Strong candidates for faculty positions in post-genomic biotechnology continue to visit the campus for interviews and seminars. In this newsletter, we will highlight several new PGI faculty hires.

M. Loots, Associated Vice Chancellor for Research

Spring 2002

IN THIS ISSUE

2

Studying Molecular Recognition by X-ray Crystallography

3

Stress Tolerance in Crops

4

Organic Molecules: Tools to Modulate Biochemical Pathways

5

How Does Chromatin Structure Regulate Gene Expression?

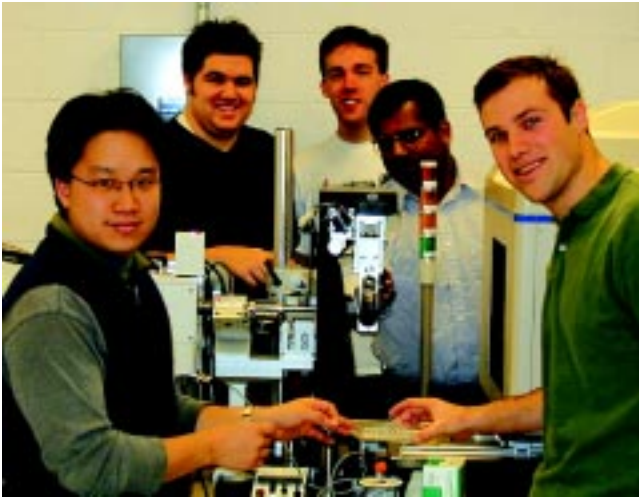
6

Genomic Imprinting: How does it work?

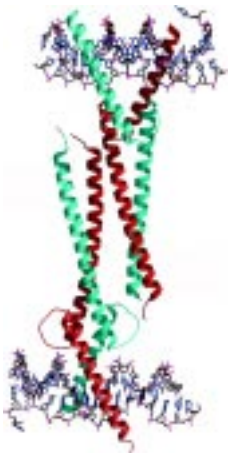
7

Facility Updates

Studying Molecular Recognition by X-ray Crystallography



Satish Nair and his graduate students, John Paul Yu, Michael Lasker, Jake Vick and Patrick Miller in front of their X-ray diffraction machine. The unit is a Rigaku H3R rotating anode with Osmic confocal mirrors. The detector for the system is a RAXIS IV++ Image Plate. The system is equipped with an EXTREME cold air delivery system which bathes the crystal in a stream of liquid nitrogen and slows down radiolysis.



The crystal structure of the proto-oncogene c-Myc bound to DNA was determined by Nair as a postdoctoral fellow. This structure demonstrates how two molecules of Myc can interact and allow sequentially distant regions of DNA to come together, possibly by DNA looping. This type of interaction is believed to be a scaffold for the formation of large multi-protein complexes such as the enhanceosome.

very interested in helping build the structural biology program at this campus. The department has been very supportive in setting up my laboratory. The School of Molecular and Cellular Biology has purchased brand new diffraction equipment and this shows their commitment to structural biology. The new X-ray diffraction machine enables us to complete about 80% of our work right here in my lab. It will be our “workhorse” to complete all preliminary work for optimizing and characterizing protein crystals. In addition, the University has committed to joining a consortium of Midwestern Universities who have bought a dedicated synchrotron beamline at Argonne National Laboratory. This allows my lab to complete X-ray structure determinations, to very high resolutions, in as little as an hour, once we have an ideal crystal characterized on our home X-ray source.”

Nair’s lab got off to a good start. With four graduate students already working on his projects, his research is in full swing. The main focus of his group is the study of signal transduction systems in eukaryotic cells that couple detection of changes outside the cell to appropriate cellular responses. His interest is in the characterization of these signaling molecules by high resolution X-ray crystallography, with the aim to discover malfunctions

and/or genetic defects that may cause human diseases. For example, John-Paul Yu, a first year Biophysics MD/PhD student in the Nair lab, is studying molecules that regulate the concentration of calcium ions inside the cells, and ultimately affect such processes as protein secretion, muscle contraction and cell death.

Another research focus is on how viruses and microbes can exploit these signaling pathways to manipulate the host’s cellular physiology for the benefit of the pathogen. “These pathogens have convergently evolved to produce a battery of virulence factors that mimic the three-dimensional structures of the host’s signaling molecules. This allows these pathogens to subvert normal cellular functions for their own benefit,” says Nair. Michael Lasker, a first year MD/PhD student in Biochemistry, is studying the structure of proteins in poxvirus which, through mimicry of molecules in the host signaling systems, allows for effective evasion of anti-viral response. Jake Vick, a first year Biochemistry PhD student, is also looking at molecular mimicry in pathogens but from an enzymatic view. “Bacteria, viruses and fungi use very unique enzymatic pathways to synthesize essential molecules. These enzymes are very attractive pharmaceutical targets and the structures of these enzymes will prove useful for drug design.”

A third area of research is on chromatin structure and its effects on transcription. “I did my postdoctoral work in a transcription factor lab. Old habits die hard, I guess,” Nair comments. Patrick Miller, a first year Biophysics student, is studying the structures of methyltransferases and ubiquitin ligases that modify the amino termini of histone protein. Such small modifications can affect how histones can engage in forming chromatin structure and ultimately regulate the access of transcriptional machinery.

“This campus offers numerous opportunities for me to collaborate with other faculty and enhance my research efforts. Getting things done is easy on this campus, because we have a great research infrastructure in place, excellent graduate students and extremely supportive senior faculty. Of course, being within a two hour drive to the brightest synchrotron source in the world is another big plus.”

S. Nair
Edited by B. Whitmarsh

Stress Tolerance in Crops

After 18 years in Tucson, Arizona, in the university's Department of Biochemistry, it seemed appropriate for someone originally from Germany to look for work in a less extreme climate. Hans Bohnert joined UIUC in August 2001 as a faculty member in two departments: Plant Biology and Crop Sciences. He was hired through the Faculty Excellence Program, sponsored by the Office of the Provost "to hire individuals who have an outstanding record of accomplishment and will be able to provide scholarly leadership from the outset of their appointments here" (Provost Communication 4).

The research in the Bohnert group is centered on plants, their reactions to stressful environments, and then trying to genetically engineer stress tolerance or resistance. "The term plant stress may need some explanation to make sure no Freudian complexity is meant: plants – most of which are stuck in a place for life – are stressed by light and darkness, by bugs, molds, car exhaust, trampling, wind, too much or too little ion nutrients, by high and low and freezing temperatures or by too much or too little water. The fact that plants exist is, one might say, a major achievement of evolution," says Bohnert.



Figure 1: Ice Plant, a plant that can tolerate high concentrations of salt. Often used as a model organism for salt stress.

The group's research uses models, species that can tolerate salt stress, such as seawater irrigation in a plant called "ice plant" (figure 1), or extreme drought, exemplified by resurrection plants, to find mechanisms that protect these plants. Bohnert explains: "From mechanisms found in models, we go to a crop plant such as rice, related to corn, sorghum, wheat, barley, because many of the genes in rice have been completely sequenced. The lowly ice plant, for example, develops special cells in the

leaf surface into which salt is deposited at extremely high concentrations – getting the bad stuff out of the way (figure 2). We begin to know the genes that are responsible. Without attempting to recreate these salt storage cells in a crop plant, it helps knowing the genes that are involved in getting salt (sodium) out of harms way in the ice plant. We can look for these genes in rice or corn, to find what is missing in the not-so-tolerant crop species. In fact, it seems that practically all genes necessary are present in corn, but corn is essentially incapable in activating them in a way that would improve tolerance when a stress comes along. One



Hans Bohnert

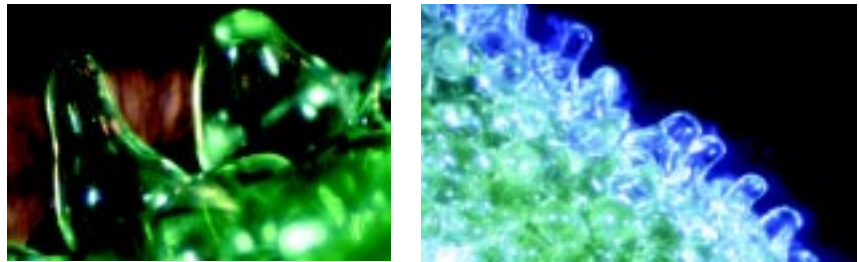


Figure 2 and 3: The ice plant has special cells in the leaf surface into which salt is deposited at extremely high concentrations – getting the bad stuff out of the way.

might use a computer analogy – the hardware exists but the software is outdated, or missing, or not properly installed. In principle, one can now begin to correct such a "deficiency" that is explained by the life style of corn or rice which evolved in the absence of stress."

Using different model strategies, drought is another topic, which has an even greater impact on crop yield. Does that mean we will be able to grow crops in the sea or in a true dry desert? This seems unlikely, even impossible and probably undesirable. Plants need water, and high salt in the water makes it energetically difficult to get to the water. What may be possible is that one could engineer moderate stress tolerance, for example in a way that drought lets an engineered soybean continue to grow for 20 days without water while the original line might stop growing after 10 days.

For Bohnert one major reason, apart from climate, for the move to UIUC is the prospect of collaborating with the resident faculty in a stimulating university environment. A second important incentive, reflected by the choice of departments, is a growing interest in research that integrates different disciplines, and a vision of how the results of such work might be applied in the real world.

*H. Bohnert
Edited by B. Whitmarsh*



Paul Hergenrother

Organic Molecules: Tools to Modulate Biochemical Pathways

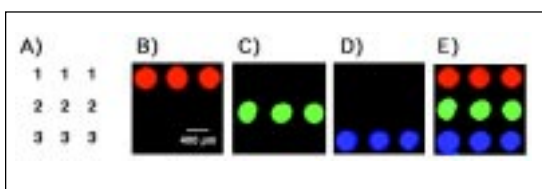
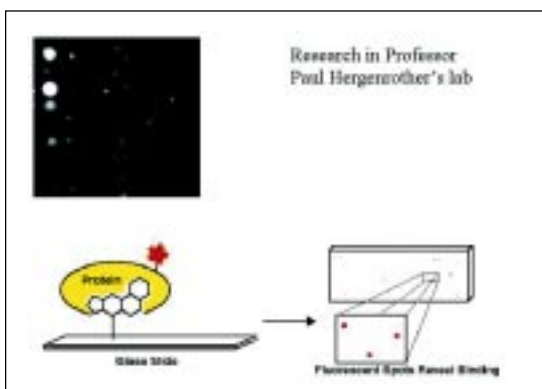
Paul Hergenrother recently joined the Department of Chemistry after completing his Ph.D. at the University of Austin, Texas in 1999 and a post-doctoral position at Harvard University, sponsored by the American Cancer Society. Asked what brought him to Illinois, Hergenrother replies: "I accepted the offer, because the University of Illinois Chemistry Department is well-known for its excellent faculty and great graduate students. After living in Boston, the Champaign-Urbana area seems like the right place for my family to settle down."

Trained in both, chemical and biochemical methods, Hergenrother takes a fresh approach to identifying protein-ligand interactions and biochemical pathways. By combining traditional methods of synthetic organic chemistry and biochemistry with modern techniques such as combinatorial chemistry and high throughput screening, Hergenrother

creates a library of small molecules that bind specifically to individual proteins, covalently attaches them to glass slides, creating *small molecule microarrays*. He probes the arrays with a fluorescently labeled protein to identify stable ligand-protein complexes; for example, he may be looking for a compound that binds to a specific isozyme within a family of enzymes. He then uses these organic compounds *in vivo* in an attempt to block a specific enzyme within a biochemical pathway to elucidate the overall mechanism. Hergenrother is applying this technique to study the apoptosis pathway, or programmed cell death, which is critical in both, the development and maintenance of higher organisms. Modulations of this pathway are associated with a variety of deleterious conditions, from cancer to Alzheimer's disease. Ultimately he is searching for compounds that modulate a biochemical pathway in a beneficial manner and could be used as drugs.

Hergenrother's research also focuses on the discovery of new antibiotics. Multi-drug resistant bacteria commonly infect hospital patients and in some cases have leapt to the larger community. These bacterial foes promise to become a significant threat to human health unless new tactics are devised to combat them. Hergenrother's lab is seeking to create antibiotics with a novel mode of action, compounds that would be effective against multi-drug resistant bacteria. This investigation involves the synthesis of natural product derivatives, and the subsequent antibiotic evaluation of the synthetic compounds.

A third area of research is the creation of new biocatalysts for organic synthesis. The ability of enzymes to catalyze complicated reactions in a chemo- and stereoselective manner under mild reaction conditions is unsurpassed. These traits make the use of enzymes in synthetic organic chemistry very attractive. Unfortunately, enzymes that catalyze many of the reactions of importance to synthetic chemists do not exist in nature. Hergenrother currently is attempting to evolve known enzymes, such as aldolases, into an enzyme that performs the Mannich reaction, a reaction that both forms a carbon-carbon bond and sets new stereocenters. In the process, it is hoped that general tactics for enzyme engineering will be developed.



Small molecule microarray to detect stable protein-ligand complexes. Small organic molecules are printed on a microarray and probed with a fluorescently labeled protein. If the protein binds to one of the compounds by forming a stable protein-ligand complex, the protein attaches to the spot on the slide. Subsequent chemical analysis reveals the structure of the organic compound that binds to the protein.

How Does Chromatin Structure Regulate Gene Expression?

Peter Jones arrived in November in Urbana-Champaign to start his career as Assistant Professor of Cell and Structural Biology. He was hired through the Post Genomic Initiative: Proteomics: Molecular Function in Biology and Macromolecular Assemblies and Machines as Determinants of Cell Function. Jones' research fits well within this programmatic area. His lab investigates how chromatin structure, which is the natural form of eukaryotic DNA intimately complexed with proteins, is utilized in repression of gene expression. Using conventional biochemistry he purifies chromatin remodeling machines, large protein complexes that contain chromatin modifying enzymatic activities, termed co-repressor complexes (Figure 1). Using these purified complexes he is able to 1) identify novel components of the chromatin regulatory network and 2) determine what is required to establish and maintain a repressive chromatin state. The system for these studies is the early development of the African clawed frog, *Xenopus laevis*.

X. laevis develops from a single cell embryo to around the 4000-cell stage in the absence of any zygotic transcription. Thus, *X. laevis* oocytes and eggs must contain large maternal stores of proteins required for replication, cell division, and DNA packaging. "We use this wealth of soluble endogenous protein for our biochemical purifications. Thus, we are able to use protein complexes in their native form as opposed to epitope tagging and over-expression often used in other systems," explains Jones when asked why he chose *X. laevis* as his model organism. "In addition, *X. laevis* has a well-studied external development from embryo to adult that is readily visualized on the live specimen under the microscope. Coupled with recent advances in *Xenopus* transgenesis techniques, this makes for an excellent system to study early events in development," Jones points out.

Increasing evidence indicates that mutations affecting chromatin remodeling play important roles in a variety of human disorders including Rett Syndrome, ICF Syndrome, and numerous cancers. Rett Syndrome is the most common form of mental retardation in females and recently has been shown to be due to mutations in the gene MeCP2. Previously, Jones has shown that MeCP2 functions as a transcriptional repressor through the recruitment of a chromatin remodeling co-repressor complex to regions of methylated DNA (Figure 1). It appears that the Rett phenotype is due to a lack of necessary repression resulting from the failure of MeCP2 to target the chromatin remodeling machine. Understanding the nature of the repressive environment as well as characterization of the remodeling machine will give more insight into the disease and may provide targets for therapy.

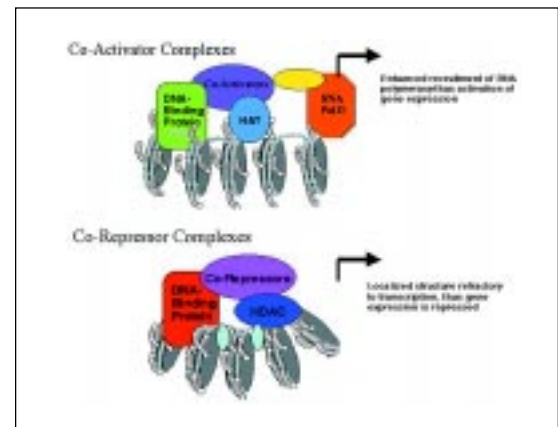
When asked what attracted him to join this campus, Jones answers: "I chose to pursue my career at the University of Illinois in large part because of the scientific infrastructure. This includes excellent colleagues with diverse areas of expertise as well as on campus facilities such as the Biotechnology Center with its Immunological Resource Center, and the Protein Sciences Facility. I never dreamed I would move back to the Midwest, but after ten years in Atlanta and Washington DC, Urbana is a welcome change of pace."

P. Jones

Edited by B. Whitmarsh



Peter Jones



Biotechnology Center at the University of Illinois at Urbana-Champaign

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Chris Schoenherr

Genomic Imprinting: How does it work?

This question is on the mind of Chris Schoenherr who recently assumed a position as Assistant Professor in the Department of Cell and Structural Biology. Schoenherr was hired as part of the Post Genomic Initiative that provides state funds for new faculty hires in four areas of Biology and Microtechnology. Schoenherr's biggest draw to come to this university is the opportunity to collaborate with at least four other faculty members who are working on chromatin structure and gene silencing. "I felt right at home when I joined this university. Both the intellectual community and the supportive environment makes this a great place to pursue my goals," says Schoenherr.

Trained as a molecular biologist, Schoenherr works on the mechanism of imprinting of two genes—insulin-like growth factor II (Igf2) and H19.

Igf2 and H19 are about 100kb apart and have almost identical expression patterns. Igf2 is paternally expressed and is a key growth factor in determining the overall size of fetuses. It is also important in cancer biology as the loss of imprinting and overexpression seen in many tumors promotes cell proliferation. H19 is maternally expressed but its function is much more enigmatic. It is a highly expressed, untranslated RNA that has no known biochemical activity. In addition, H19 deletion mice show no obvious phenotype.

Most genes are transcribed from both parental alleles.

Genes subject to genomic imprinting, however, are transcribed from only one copy; the other is completely repressed. Unlike the random repression seen in X chromosome inactivation, the repressed or *imprinted* allele depends on the parent of origin. Thus, there are genes in which the maternally inherited copy is silent and for others, the paternal copy is silenced. After an intensive search

of the human and mouse genomes, about 50 imprinted genes have been found to date.

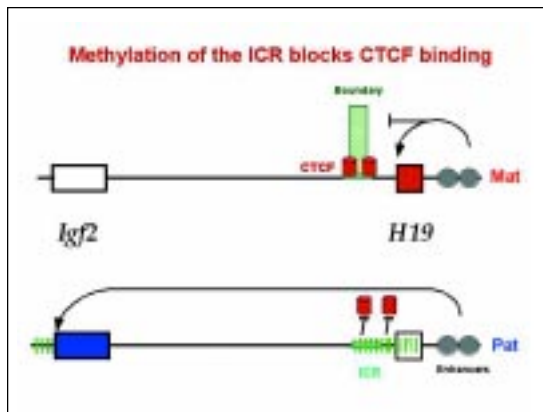
So, how does the cell distinguish between the two copies of a gene and how does it use that information to repress one copy? Differences in DNA methylation between the maternal and paternal gene are an important part of the answer. These methylation differences begin in the gametes and are maintained throughout development. Although methylation is often associated with gene silencing, its role in imprinting is more complicated, as it can both activate and repress imprinted genes.

As expected from their shared expression pattern, Igf2 and H19 share multiple tissue specific enhancers that reside downstream of H19. The imprinting of the two genes is also determined by a shared DNA element, known as an *imprinting control region*, or ICR. It is completely methylated on the paternal chromosome and completely unmethylated on the maternal. The ICR acts as switch. When methylated, it turns paternal H19 off and allows paternal Igf2 to be transcribed and when unmethylated on the maternal chromosome it does the opposite, silencing Igf2 and allowing H19 transcription.

While in Shirley Tilghman's lab at Princeton, Schoenherr determined that the maternal ICR repressed the Igf2 gene 100kb away by functioning as a chromatin boundary. Chromatin boundaries are thought to act like a fence that can keep enhancer activity restricted to a defined chromosomal domain. Schoenherr also found that the boundary activity required the zinc finger protein CTCF. CTCF binds to multiple sites in the unmethylated ICR and by an unknown mechanism prevents enhancers from activating Igf2 transcription. CTCF, however, cannot bind the methylated paternal ICR, which allows the enhancers to activate Igf2.

"My future work will focus on how chromatin structure regulates gene expression. There is still much to be learned about the interplay between chromatin structure, methylation, and regulatory elements, such as enhancers and boundaries," says Schoenherr.

C. Schoenherr
Edited by B. Whitmarsh



This figure shows the present model for the reciprocal imprinting of Igf2 and H19. On the maternal chromosome, CTCF (red cylinder) binds to the ICR (turquoise) and blocks the enhancers (gray circles) from activating Igf2 while allowing activation of H19. On the paternal chromosome, the ICR is methylated (green bars) which prevents CTCF from binding and enables the enhancers to activate transcription of Igf2. H19, however, is repressed by methylation of its promoter.

Facility Updates



W.M. Keck Center for Comparative and Functional Genomics

Sharon Bachman, Laboratory Supervisor at the High-Throughput Sequencing and Genotyping Facility



The Biotechnology Center promoted Ms. Sharon Bachman to Laboratory Supervisor to take on the responsibilities of High-throughput Sequencing and also oversee the DNA Core facility. Bachman joined the W.M. Keck Center in August of 1999 and first worked as research specialist in DNA core sequencing, which serves over 400 users campus-wide. About six months later she was promoted to senior research specialist and became the team leader of the DNA sequencing core operation, a position that, besides well-developed technical skills, requires excellent organizational, managerial and communication skills. Bachman proved to be a very effective and responsible team leader. This summer, when the situation arose that the lab needed a team leader for high-throughput sequencing, Bachman agreed to take over this service. Although similar in nature to the DNA core, high-throughput sequencing is carried out in microtiter plates and is conducted semi-automatically, using robotic equipment for several steps of the procedures, and capillary DNA sequencers for sequencing. Only an efficiently set-up pipeline allows the facility to run at or close to the capacity of the instrumentation involved. When Bachman took on these new responsibilities, she greatly improved general operating procedures and team management, ensuring that the service runs smoothly and efficiently. The Keck Center staff is happy to have such a dedicated and exceptional team leader.

Last summer, **Thomas Dekoj, Chris Wright and Adrienne Kurtz** joined the High-throughput lab. While Tom Dekoj is work-

ing on two large-scale BAC DNA preparation and BAC DNA sequencing projects, Chris Wright is part of the high-throughput team and primarily involved in DNA isolation and sequencing set-up. Adrienne Kurtz started out as research specialist in the DNA sequencing core team, but soon after, because of her well-developed skills and strong dedication, was promoted to senior research specialist taking on the team leader position for this service.

The Functional Genomics Lab added **Denizhan Akan** to its staff last fall. Dennis will be in charge of establishing and running the Affymetrix Gene Chip system by performing labeling, hybridizations, staining and chip analysis. **Al Bari**, also working in the Functional Genomics Lab, was promoted to senior research specialist. The Bioinformatics lab also added three new staff members to their lab, **Richard LeDuc, Dr. Yong Liu** and **Jennifer Edwards**. LeDuc is primarily in charge of software development, including creating a P450 information web site. Liu works on microarray databases and data mining, as well as in high performance computing. Jennifer Edwards focuses on DNA sequence analysis, including sequence assembly and oligonucleotide design.

The Administrative Office

The Administrative Office is welcoming **Randy Musselman**, who as an accountant, is responsible for purchasing, billings and account balances.



Flow Cytometry Facility

Ben Montez was promoted to senior research specialist to take over additional responsibilities and assist Barbara Pilas in managing the day-to-day operations of the Flow Cytometry Facility. Having worked for over 5 years at the flow lab, Montez is very experienced and familiar with many different types of assays. He also has an in-depth technical understanding of the instrumentation, which is a great asset to the facility.

Peter Schweitzer, continued from page 1

In this capacity he supervised a staff of 14 scientists and technicians, dedicated to genetic analysis of over 500 transgenic and knock-out mice strains. Using microsatellite markers for speed congenic breeding projects, and biochemical and molecular assays to detect genetic contamination and/or mutations, this laboratory is in charge of ensuring genetic purity of the Jackson Laboratory mice.

Schweitzer's multidisciplinary background and extensive training in genetics and genomics research will create opportunities for new initiatives and collaborations with our faculty. Says Schweitzer: "I'm looking forward to the opportunity to apply the tools of genetics and genomics that I've learned in rodent systems to the new and ongoing challenging projects involving different species here at the Biotechnology Center."



Liping Wang



Liping Wang, Laboratory Supervisor of the Immunological Resources Center

The Biotechnology Center is pleased to announce that Liping Wang has accepted the position of Laboratory Supervisor for the Immunological Resources Center. Ms. Wang, with more than 10 years of experience in biotechnology research, is ideally suited to take on the leadership of the center.

Ms. Wang received a master's degree in biology from Western Carolina University, North Carolina in 1989. From 1989 - 1990 she worked at the Laboratory of Radiobiology and Environmental Health at the University of California, San Francisco as an assistant research biochemist, participating in point mutation analysis of the *ras* oncogene in mouse skin tumor. She also constructed human cDNA libraries.

From 1991 until the end of 1993 she worked as research specialist in the Department of Chemical Therapeutics for Chiron Corp. in Emeryville, California, where she was primarily in charge of construction and verification of phage peptide libraries, including panning against antibodies and receptors. She then moved within the same company to the Department of Virology where she primarily conducted structural and serological studies of hepatitis G and C viruses. After moving to Urbana-Champaign in February of 1997 she worked as research specialist in the Department of Cell and Structural Biology before joining the Immunological Resources Center in March of 1999. Please join the Biotechnology Center in welcoming Ms. Wang to her new position.



Barbara Pilas



Barbara Pilas, Laboratory Coordinator for Flow Cytometry Facility

Barbara Pilas recently assumed the position of Laboratory Coordinator for the Flow Cytometry facility, bringing six years of flow cytometry experience to the lab. She comes well prepared, with a strong background in biophysical and biochemical methodology, which she acquired through her graduate training at Jagiellonian University in Krakow, Poland. Because her graduate research required sophisticated instrumentation that was unavailable in Poland at the time, she spent almost two years in the United States, working as graduate research assistant at several universities, including Johns Hopkins University and the University of Illinois. After returning to Poland and

completing her Ph.D., she assumed a postdoctoral position at Argonne National Laboratory. Since 1991, she has been living with her family in Champaign-Urbana, where she joined the Flow Cytometry facility in 1995. Pilas combines cell culture methodology with an in-depth knowledge of spectroscopic methods, such as fluorescence spectroscopy, electron spin resonance, optical spectroscopy, flash photolysis and others. She has introduced several new applications to the Flow Cytometry facility, such as apoptosis, multicolor immunophenotyping, fluorescence resonance energy transfer, environmental microbiology, intracellular enzyme kinetics and others.



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