GENETIC MATERIAL

Center for Genetic Medicine | *Northwestern University*

Spring 2007

Featured in the film are (from top) Allison Kessler, John Kessler, Carrie Kaufman, Vicki Mattiace, and Laurie Zoloth.

Silverstein Lecture Series Presents New Documentary Film on Stem Cell Research

The Center for Genetic Medicine Silverstein Lecture Series is proud to sponsor the Chicago-area premieres of *Terra Incognita: The Promise and Peril of Stem Cell Research*, a feature-length documentary film directed by Maria Finitzo and released by Kartemquin Films, producer of the acclaimed 1994 documentary *Hoop Dreams*. Screenings of the film will take place on May 10 in Thorne Auditorium on the Chicago campus and on May 16 in Ryan Family Auditorium on the Evanston campus.

Terra Incognita offers a close-up look at two years in the life and laboratory of stem cell expert John Kessler, Ken and Ruth Davee Professor in Stem Cell Biology, chair of neurology, and director of the Feinberg Clinical Neuroscience Research Institute and the Feinberg School of Medicine Stem Cell Institute at Northwestern. In 2001, Kessler's 15-year-old daughter, Allison, fell while skiing, leaving her paralyzed below the waist. In response to her accident, Kessler redirected the focus of his research lab to using stem cells to repair damage to the spinal cord and brain. In documenting Kessler's personal and professional quest to advance stem cell medicine, the film traces the painstaking work of Kessler's graduate students Vibhu Sahni and Vicki Mattiace and reveals the myriad, at times unexpected, ways in which spinal cord injury alters lives. This point is dramatically reinforced in the story of another young woman featured — Carrie Kaufman, the daughter of Kessler's Feinberg School colleague Dale M. Kaufman — whose paralyzing injury in a diving accident coincided with the making of the film.

Terra Incognita director Maria Finitzo is based in Evanston and has been making documentary films for more than two decades. Earlier she directed another Kartemquin Films release, *5 Girls*, which explored the lives of five young women as they dealt with the complexities of adolescence. It won the Council on Foundations' Henry Hampton Award for Excellence, the Silver Award in the Chicago Film and Television Competition, and an Outstanding Achievement Award from Parent's Guide to Children's Media.

Finitzo will be at both the Chicago and Evanston screenings to answer questions. Joining her will be John Kessler and another participant in the film, Laurie Zoloth. A widely respected expert on ethics, religion and science, and social policy, Zoloth directs the Center for Bioethics at the Feinberg School and is a founding board member of the International Society for Stem Cell Research. She is a member of the faculty in both the Feinberg School, where she is Science and Society Professor of Medical Ethics and Humanities, and the Judd A. and Marjorie Weinberg College of Arts and Sciences, where she is professor of religion.

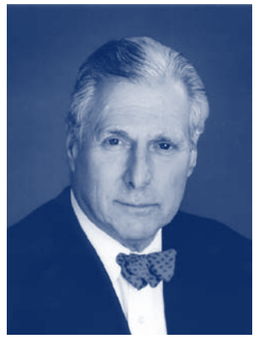
Funding for the Silverstein Lecture Series is provided by the Herman M. and Bea L. Silverstein Medical Research Fund for Genetic Medicine. Events in the series are free, open to the public, and designed to provide forums where leading experts discuss the importance of developments in genetics research in terms understandable to nonscientists.

Chicago-area premieres of *Terra Incognita: The Promise and Peril of Stem Cell Research*:

May 10, 7 p.m.
Thorne Auditorium
375 East Chicago Avenue
Chicago

May 16, 7 p.m.
Ryan Family Auditorium
2145 Sheridan Road
Evanston

CGM Acknowledges a Remarkable Dean



Lewis Landsberg

The Center for Genetic Medicine has garnered strong support from Lewis Landsberg, dean of the Feinberg School of Medicine and vice president for medical affairs at Northwestern. As he plans to step down as dean next June to embark on projects of his own, the CGM thanks Dean Landsberg for making the center part of his legacy.

The CGM was founded in September 2000, shortly after Landsberg became dean, and is one of four research centers and institutes created during his eight-year tenure. Thanks to his dedication to building the Feinberg School's research infrastructure and faculty, the number of CGM faculty members in genetics research grew from 73 in 2001 to 130 in 2007, and the majority also hold positions at the medical school. Six recent Feinberg School faculty recruits share open laboratory space with four established labs based in the Robert H. Lurie Medical Research Center that opened two years ago. As a result, CGM members from seven different departments are able to share resources and equipment across disciplinary boundaries in conducting their genetics research.

Landsberg has enthusiastically supported NUgene, the center's DNA-banking project, since its inception. The CGM and Landsberg both foresee NUgene's sample collection growing into a valuable resource that will have a broad impact on future genetics research. The dean's office has also provided generous support to establish CGM core facilities that offer genomic and transgenic and targeted-mutagenesis services to researchers on both campuses.

Landsberg's dedication to genetics education contributed to the rapid growth of the Graduate Program in Genetic Counseling. Since he entrusted administration of the program to the CGM in September 2002, enrollment has risen, fellowship opportunities have expanded, and its directors are nationally known leading experts in the field.

Landsberg's fundraising efforts and work to strengthen relationships between the Feinberg School and Northwestern's hospital partners have resulted in seed funding and support for endowments that will help sustain the CGM, its research infrastructure, academic programs, and public outreach initiatives.

Many thanks go to Dean Landsberg for helping launch and develop the CGM, which offers its best wishes as he brings another new center to life at the Feinberg School.

Dean Landsberg Reflects on His Legacy and Future

What are your proudest accomplishments as dean?
Four things stand out for me: first and foremost are the people I have recruited and appointed, because getting good leadership for the school is the most important thing a dean can do; second, the Robert H. Lurie Medical Research Center, which provides much-needed laboratory and education space; third, the development of a new and critically important relationship with Northwestern Memorial Hospital that promises to immensely benefit the school and the hospital; and finally, the relocation of Children's Memorial Hospital to the medical school campus, which will provide a range of new opportunities in research and clinical care.

What are your plans for the near future and the years ahead?
After taking a few months off for planning and reflection on Cape Cod and the Cote d'Azur, I will return to the faculty. I plan to launch a center for the study and management of obesity with [Feinberg faculty colleagues] Jim Young, Bob Kushner, Linda Van Horn, and Kathy Kristoffel. Its focus will be on children and minorities, emphasizing primary obesity prevention, as well as on research into fundamental causes. As genetics obviously plays a very important role in the disease, collaboration with the CGM should be very fruitful, and NUgene provides a great opportunity. I also hope to resume teaching activities with residents and students.

Incoming Feinberg Dean Sees CGM Focusing on Innovative Genetics Research Areas

J. Larry Jameson

On July 1 the Feinberg School will welcome J. Larry Jameson as dean and vice president for medical affairs. Jameson is the Irving S. Cutter Professor and chair of the Department of Medicine as well as an active member of the Center for Genetic Medicine who has served on the center's executive committee for the past six years. His laboratory specializes in molecular endocrinology research and is on the CGM floor in the Robert H. Lurie Medical Research Center.

In a recent interview, Jameson was asked about the future of genetic medicine.

Genetics research is a broad, dynamic field. How can the CGM best advance its development?
The CGM should identify a unifying theme and focus on new areas in the field. We can strive to be better at new things instead of competing in traditional areas others have already claimed.

How do today's genetics research trends differ from earlier ones?
Historically, themes in genetics have been mostly technical in nature — sequencing, cloning, and other technical approaches. In contrast, contemporary themes are in the areas of complex genetics, bioinformatics, genetic epidemiology, and translational research.

CGM has developed the NUgene DNA-banking project and core facilities to promote genetics research at Northwestern. How can these research resources be improved?
Our faculty are interested in NUgene, but the sample size is still small. NUgene has 6,000 samples now, but we need 60,000 to make the collection really valuable. Genotyping the existing samples would significantly lower barriers to use, but genotyping is costly and would require external resources. The Genomics Core and the Transgenic and Targeted Mutagenesis Laboratory (TTML) facilities offer important services, but we need to look for ways to improve efficiency, offer competitive pricing, and get user feedback on a regular basis. The TTML should expand technology to efficiently handle and process frozen embryos as opposed to live animals; this service exists only in partial form today. The Genomics Core should prepare for a shift from DNA analysis to protein analysis. The costs of storing and handling sera and other protein-containing samples will be much higher and more technically challenging than for DNA, since these samples do not withstand repeated freezing and thawing.

How can the CGM promote academic education and public outreach programs?
Both academic and public education programs are very important, and coordinating such programs is one of the center's strengths. Tightly focused academic programs such as minisymposia are well suited for busy faculty members. In the public sphere, I would like to see the CGM develop programs for practicing physicians, maybe create something that would send them away with a "toolbox" of genetics-related advice. Such a toolbox does not currently exist, but the Graduate Program in Genetic Counseling is well positioned to help create something like this.

GPGC News

The Graduate Program in Genetic Counseling (GPGC) annual colloquium and graduation took place on March 9 and 10. This year's Beth Fine Kaplan Lecture was presented by Amy Cronister. A regional manager for genetic services at Genzyme Genetics since 1996, Cronister is responsible for developing and implementing genetic counseling services and programs in the southern and northeastern United States. She received her MS in human genetics from Sarah Lawrence College, and throughout her career has focused her work on Fragile X Syndrome, coauthoring more than a dozen articles and book chapters on the subject. Most recently, she served as a consultant on a Centers for Disease Control project titled "Fragile X Syndrome Cascade Testing and Genetic Counseling Protocols." The Beth Fine Kaplan Lecture was presented as part of the Grand Round Series of the Department of Obstetrics and Gynecology.

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The GPGC was granted full accreditation for the next eight years by the American Board of Genetic Counselors.

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In January GPGC associate director Cathy Wicklund began her term as president of the National Society of Genetic Counselors.

CGM Faculty Profile: Michael Kennedy

Last November the Center for Genetic Medicine welcomed Michael Kennedy as director of educational and research programs. Since then the center has begun to capitalize on Kennedy's long-standing interests in promoting scientific literacy, teaching and mentoring students, and facilitating biomedical research.

Michael Kennedy

A chief outreach objective for Kennedy is forming relationships with community-based partners — especially those in minority communities — that will inform and support the development of the CGM's public educational programming. For example, in collaboration with Kelly Ormond, director of the Graduate Program in Genetic Counseling, and Cathy Wicklund, GPGC associate director, Kennedy is initiating a dialogue with community leaders who can both raise awareness of genetics-related issues among their constituents and bring their constituents' interests and concerns to the CGM's attention. The outcome will be to hold seminars that address those concerns.

Another project is the Mouse Genetic Technologies Working Group, which Kennedy is undertaking in collaboration with Tom Bozza, assistant professor of neurobiology and physiology, and Raj Awatramani, assistant professor of neurology. The working group's goals are to provide a forum for transgenic mouse users to share ideas, experimental approaches, and resources; to enable labs developing transgenic mouse models to connect with the broader community; and to foster interdepartmental collaborations.

Kennedy is also developing a centralized web-based e-magazine that will increase the impact and visibility of Northwestern's life sciences–based public outreach efforts. The web site, known as Science in Society, will serve as a platform for cross-promoting events and disseminating articles by University faculty as well as a repository for video archives and other educational content from past events. The Office of the Vice President for Research provided funding for the first phase of the project, and Kennedy plans to launch the site in late spring or early summer.

In addition, Kennedy is working with colleagues on the CGM's strategic planning process, participating in an upcoming grant proposal to the National Human Genome Research Institute for NUgene, and directing event planning and outreach for the Silverstein and Scott Lecture Series.

Kennedy received his PhD in biochemistry in 1997 from Mayo Graduate School of Medicine, where he studied macrophage-mediated inflammation in the lung and investigated the structure, function, and properties of calcineurin, a key signaling enzyme with roles in metabolism and immune-response mediation. Later, as a postdoctoral fellow in Robert MacDonald's lab in the Department of Biochemistry, Molecular Biology, and Cell Biology at Northwestern, he worked on a project using novel cationic lipids to deliver DNA into cells. For eight years before joining the CGM, Kennedy was associate chair for administration in the Department of Neurobiology and Physiology in Weinberg College at Northwestern. He oversaw all aspects of department administration, directed the master's program, and taught an undergraduate course on reproductive genetics.

Investigator Studies Motives of Selected NUgene Participants

What motivates individuals of the Jewish faith to participate in genetics research?

This question has been the subject of study for Carly Siskind, an investigator at Northwestern University and a graduate of its master's program in genetic counseling.

"Historically, members of the Ashkenazi Jewish community have been willing to participate in genetic research on disorders known to be prevalent in the Jewish population," says Siskind, "but little is known about whether the community similarly supports nontherapeutic research that does not directly benefit the participant."

Carly Siskind

For her research, Siskind interviewed individuals who both identified themselves as related to the Ashkenazi Jews — the Jews of Eastern and Central Europe whose descendents make up the majority of today's U.S. Jewish population — and are enrolled in the NUgene Project, an ongoing long-term study initiated by Northwestern's Center for Genetic Medicine. Unique in Chicago and one of only a few like it in the nation, NUgene allows patients of Northwestern-affiliated hospitals and clinics to voluntarily donate a sample of their genetic information (DNA) accompanied by their personal health history to be stored for research purposes. While they themselves receive no direct benefit, participants help enable NUgene Project researchers to identify the genetic causes of common diseases and to devise new treatments and cures.

"This study and others showed culture has an influence on decision making, and researchers should be aware of and sensitive to the proper courtesies and the particular environment from which they are recruiting."

Carly Siskind

To date, of NUgene's approximately 6,000 participants, about 11 percent are of Ashkenazi Jewish descent. Those who took part in Siskind's study had to have identified themselves as Ashkenazi Jewish descendents as well as agreed to be contacted for future studies. Siskind's aim was to find out to what extent, if any, these NUgene participants are motivated by Jewish values and/or religiosity — devoutness or piety — to participate in nontherapeutic genetic research. She conducted 10-minute interviews with 54 subjects who answered questions about their religiosity, demographic traits, and motives for participating in NUgene.

In her analysis of the interviews, Siskind showed that while there was no association between degree of religiosity and willingness to participate in nontherapeutic research, there were common motivating factors among people of Ashkenazi Jewish descent. Some of these factors, such as "helping others," have been reported as motives by other populations who have taken part in nontherapeutic research. But Siskind also identified factors for the Ashkenazi Jewish population that were previously unreported by other groups, such as "personal values" and the incorporation of Jewish values in a person's life.

Siddique Lab Conducts Whole Genome–Association Study for ALS

Half of all people diagnosed with amyotrophic lateral sclerosis (ALS), the degenerative disorder also known as Lou Gehrig's disease, become paralyzed and die within three to five years. Roughly 90 percent of the time, these are cases of sporadic ALS (SALS), in which only one member of a family is affected. A complex disorder of motor neurons, SALS is thought to be produced by genes interacting with each other or with environmental factors, or possibly by environmental factors acting alone. Understanding its causes is especially challenging because ALS is relatively rare, its course relatively short, and testing its genetics requires large sample sizes.

Fifteen years ago CGM member and neurology professor Teepu Siddique led pioneering studies that identified SOD1 and ALSIN, the two known genes implicated in familial ALS. Today the Siddique lab's large DNA and cell-line repository of samples from SALS patients, their relatives, and unaffected controls is making it possible to explore the genetics of SALS.

A whole genome–association study (WGAS) for SALS is now under way in the Siddique lab, initially using 1,000 samples, with a similar number to be used to replicate and validate findings. Because a WGAS allows comprehensive coverage of the human genome using a dense-marker panel, it can efficiently identify susceptibility genes for common disorders and, it is hoped, for rarer, multifactorial disorders like SALS. The lab is using the Illumina Beadstation 500GX system and is screening 500 cases and 500 age- and gender-matched controls with the Infinium II BeadChip (300K SNPs) in the lab. Through in-house collaboration the lab has access to a second Illumina Beadstation in the Genomics Core Facility and has obtained the DNA of 30 controls from the NUgene repository.

Genomics Core Facility Consolidates Resources

Genomics Core technicians (from left) Mary Majewski, Vickie Thomas, Vivi Frangidakis, Jenny Che, and Hannah Hwang

Genetic Material reported last fall that the CGM's microarray, genotyping, and sequencing activities would be merged to form a Genomics Core Facility. With the relocation of the sequencing lab from the Health Sciences Building in January, all operations have been consolidated on the ninth floor of the Montgomery Ward Building. Credit for the success of the transition goes to Genomics Core Facility director Nadereh Jafari, program assistant Anne Hubbard, and technicians Jenny Che, Vivi Frangidakis, Hannah Hwang, Mary Majewski, and Vickie Thomas.

For more information on services provided by the Genomics Core Facility, visit www.cgm.northwestern.edu/genomics.htm.

CGM Faculty Profile: Markus Bredel

Markus Bredel joined the Feinberg School of Medicine in January as assistant professor in the Department of Neurological Surgery and director of the Northwestern Brain Tumor Institute Basic Science Program. He is a member of the Center for Genetic Medicine and the Cancer Genes and Molecular Targeting Program of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University.

Markus Bredel

Bredel's laboratory, based in the CGM at the Robert H. Lurie Medical Research Center, uses functional and computational genomics tools to discern the architecture and laws of complex networks of molecules and cellular components. In particular, his research focuses on the molecular mechanisms of the evolution of gliomas — tumors developing from supporting tissue, or glia, in the brain — and their resistance to existing therapies. His approach has succeeded in prioritizing hub genes whose role may be to determine network behavior and in identifying lead molecules that may factor in the susceptibility of gliomas to therapy.

Bredel has discovered that the TNFAIP3 gene is a molecular determinant of glioblastomas' resistance to alkylating drug therapies. This gene is part of a complex network of endogenous modulators that act upon nuclear factor-κB, a eukaryotic transcription regulator that promotes cell survival. Bredel has identified several networking endogenous modulators of nuclear factor-κB activation that affect cell resistance and patient outcomes in cases of high-grade gliomas.

Bredel was first in using cDNA microarray-based comparative genomic hybridization to map genome-wide alterations in gene dosage in gliomas. He has shown that such high-resolution mapping can precisely localize and size tumor regions where gene-dosage change recurs. This research has identified novel common minimally deleted regions — areas where the instance of missing chromosomal material or DNA is minor — that involve genes assumed to be tumor suppressors, such as the TOPORS gene on the long arm of chromosome 9.

Another area of Bredel's research focuses on nonrandom chromosomal abnormalities in gliomas. Because somatic evolution naturally selects cells that are adept at surviving, such genetic coincidence might affect function, presumably giving an advantage to glioma cells. By linking network modeling to the known functional interactions of orthologous mammalian genes, Bredel has described a nonrandom genetic landscape that, through its facilitation of gene interactions, promotes gliomagenesis.

As reported in *Cell* last February, Bredel is part of a team of researchers that has pinpointed CHD5 as a novel tumor suppressor on the long arm of chromosome 1 (1p36). He has led efforts to translate the findings in mice to human tumors and has shown this gene to be altered in about 20 percent of human gliomas.

Bredel has received prestigious awards for his research, including the Merit Award of the American Society of Clinical Oncology, the Preuss Research Award of the American Association of Neurological Surgeons and the Congress of Neurological Surgeons, and the Rhone-Poulenc-Rorer Young Investigator Award of the American Association for Cancer Research. Bredel is on the editorial board of *The Lancet Oncology* and is the scientific and translational chair of the CNS Tumors Subcommittee of the Scientific Program Committee of the American Society of Clinical Oncology.

Bredel received his MD and PhD summa cum laude from the University of Vienna and the Free University of Berlin in 2000 and 2001, respectively. He was a research fellow at the University of Pittsburgh Cancer Institute Brain Tumor Center and received training in neurosurgery at the University of Freiburg. Before joining the Feinberg faculty, Bredel had been at Stanford University since 2003, most recently as visiting assistant professor in the Department of Neurosurgery and the Division of Oncology.

CGM Travel Fellowship Profile: Laura De Laporte

Laura De Laporte

Laura De Laporte came to the United States four years ago, after obtaining her master's degree from the University of Ghent in her native Belgium. Now a third-year graduate student in Lonnie D. Shea's lab in the Department of Chemical and Biological Engineering at Northwestern, she focuses on building gene-therapy approaches to tissue-engineering applications.

De Laporte works with DNA-loaded multiple-channel bridges that she and her coworkers designed for use in treating spinal cord injury — a condition affecting approximately 2 million people worldwide for which there are few therapies. Various factors inhibit the regeneration of spinal cord cells: the necessary growth factors are lacking, certain molecules inhibit cell regeneration, and scar tissue forms. Genetics-based therapies could provide a tool to cause inductive proteins to be expressed or to block the expression of inhibitory factors.

Along with Northwestern colleagues Yang Yang and Marina L. Zelivyanskaya, and assisted by their collaborator, Aileen J. Anderson of the University of California, Irvine, De Laporte started an in vivo rat hemisection model to assess the functionality of implanted multiple-channel bridges. The bridges can function as a drug-delivery device that simultaneously provides a substrate with linear channels to guide nerve cell regrowth and extension. According to De Laporte, the rats with spinal cord injury improved after the bridges were implanted in the absence of gene delivery. The DNA-loaded bridges have induced local transgene expression in the spinal cord up to two weeks. Combining the bridges with local, sustained delivery of tissue-inductive factors could further improve behavioral recovery. Bridges loaded with plasmids encoding for these factors could provide a powerful tool for spinal cord regeneration.

Travel Fellowship Winners Present Research at National and International Meetings

CGM Travel Fellowships of $500 each assist graduate students and postdoctoral fellows in presenting their work at national or international meetings. Recipients may also take part in the Robert H. Lurie Comprehensive Cancer Center's Annual Scientific Poster Session at the end of the academic year. The next application deadline will be July 6. Information on the fellowships can be found at www.cgm.northwestern/travel_app.htm.

Following are six recent fellowship recipients:

Souvik Bhattacharjee, "Pathogenic Protein Secretion in Malaria-Infected Erythrocyte Red Cells," to be presented at the Gordon Research Conference, May 20–25, Aussois, France. Adviser/mentor: Kasturi Haldar, professor of pathology, Feinberg School of Medicine.

Laura De Laporte, "DNA-Loaded Multiple-Channel Bridges for Spinal Cord Regeneration," presented at the American Institute of Chemical Engineers annual meeting, November 12-17, 2006, San Francisco. Adviser/mentor: Lonnie D. Shea, associate professor of chemical and biological engineering, Robert R. McCormick School of Engineering and Applied Science.

Iiro T. Helenius, "Modeling Hypercapnia-Induced Injury in Drosophila Melanogaster: Insights into the Physiological and Molecular Effects of CO_2," presented at the 47th annual Drosophila Research Conference, March 7–11, Philadelphia. Adviser/mentor: Greg Beitel, assistant professor of biochemistry, molecular biology, and cell biology, Judd A. and Marjorie Weinberg College of Arts and Sciences.

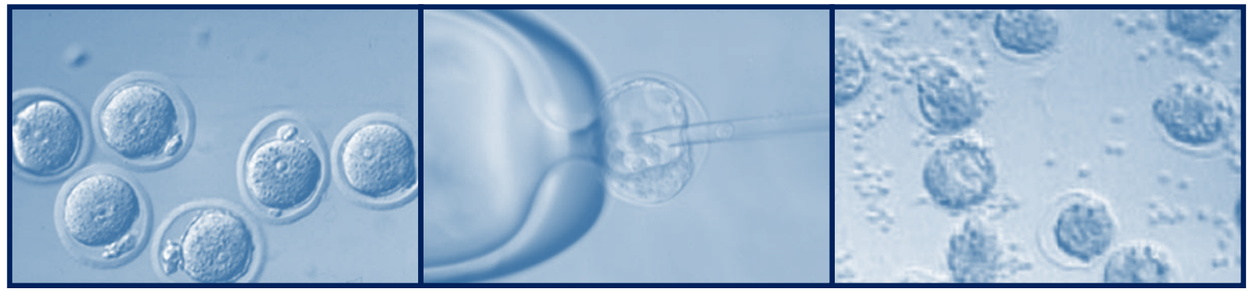
Robert P. Johnson, "Genetic Analysis of *C. elegans* Dystroglycan DGN-1," presented at the American Society for Matrix Biology biennial meeting, November 1–4, 2006, Nashville. Adviser/mentor: James Kramer, professor of cell and molecular biology, Feinberg School.

Jonathan Preall, "Biochemical Analysis of RNAi," presented at the Keystone Symposia Conference on "MicroRNAs and siRNAs: Biological Functions and Mechanisms," sponsored by Novartis Institutes for BioMedical Research, January 27–February 2, Keystone, Colorado. Adviser/mentor: Erik Sontheimer, associate professor of biochemistry, molecular biology, and cell biology, Weinberg College.

Carly Siskind, "Two Families with a Deletion/Duplication of the X Chromosome," presented at the 2007 American College of Medical Genetics annual meeting, March 21-25, Nashville. Adviser/mentor: Rebecca Anderson, Graduate Program in Genetic Counseling, Feinberg School, and certified genetic counselor, Department of Human Genetics, University of Chicago.

TTML Expands Services for Genetics Researchers

The CGM's Transgenic and Targeted Mutagenesis Laboratory (TTML) has the specialized equipment and staff necessary to offer mouse-based genomic services to all Northwestern University investigators. Services provided by the TTML are described below.



Left to right: Two pronuclei can be seen in single-cell fertilized eggs under microscope prior to microinjection; ES cells deposited into blastocoel cavity; collapsed blastocysts following ES cell injection

Generation of transgenic mice

Transgenic systems serve as gain-of-function models that can be used to study the effects of altering gene expression in a particular tissue and time during development. Traditional transgenic constructs are plasmid based and up to 25Kb in size. The TTML has been able to generate mice from much larger transgenes derived from bacterial artificial chromosomes (BAC), which can be as large as 250Kb. TTML technicians microinject transgenic DNA constructs into the pronucleus of a single-cell embryo, then surgically transplant the microinjected embryos into surrogate mothers. The injected DNA randomly integrates into the genome of some of the injected embryos. The investigator receives tissue samples from the resulting offspring to determine which mice inherited the microinjected transgene.

Targeted mutagenesis or gene targeting

Gene targeting can be used to introduce site-specific mutations into particular mouse genes to more faithfully mimic human disease. Key to this process is the ability to create stem cells from early preimplantation embryos that can be maintained and manipulated in vitro. The TTML maintains and routinely uses pluripotential HM1 (129/Ola) embryonic stem (ES) cells for gene-targeting projects. The investigator designs a targeting vector that contains regions of identity (homology) on both sides of the endogenous gene being targeted (mutated). High-quality targeting vector DNA is provided to the TTML technician, who introduces the targeting vector into ES cells through electroporation. Electroporated DNA will pair through the regions of identity with the endogenous gene being targeted. The endogenous gene will then be replaced with the targeting vector sequences carrying the mutation by a process called homologous recombination. Electroporated ES cells are grown under drug selection, and the TTML technician picks from the surviving colonies those that appear undifferentiated (still pluripotent). The investigator genotypes these colonies to determine which clones have incorporated the DNA by homologous recombination. Clones that have incorporated the DNA by homologous recombination (targeted ES cells) are expanded and microinjected into blastocysts to create chimeric mice.

ES cell microinjection into blastocysts

Mice carrying a site-specific mutation are produced by TTML staff microinjecting targeted ES cells into the blastocyst cavity of an embryo 3½ days old. ES cells are derived from cells within the blastocyst's intercell mass (ICM) — cells that give rise to all the tissues of the embryo. Following injection the cavity collapses, forcing the injected ES cells into the ICM. Reexpanded blastocysts are then surgically transplanted into female recipient mice. Among the resulting litters are colorful chimeric pups with tissues, including germ tissue, made of cells from both the targeted ES cells and the host blastocyst. The degree to which the targeted ES cells populate the ICM during injection is shown by the coat color of the chimera: all skin and hair derived from the host blastocyst will be black, whereas skin and hair coming from the white/agouti ES cell will be white, tan, golden, or agouti. Chimeric mice are given to the investigator for breeding to identify ES cell–derived offspring that are heterozygous for the targeted mutation.

Rederivation

Rederivation eliminates pathogens such as pinworm, parvovirus, and mouse hepatitis virus from infected mouse lines, allowing investigators to import established mouse lines with known pathogens or unknown health status from anywhere in the world. From infected mice TTML research staff collects preimplantation embryos, which are relatively resistant to such pathogenic microorganisms, washes the embryos through several droplets of embryo culture media, and then surgically transfers the embryos into specific pathogen-free pseudopregnant females. Resulting mice can then be transferred to the new barrier-level vivarium in the Lurie Research Center.

Cryopreservation and cryorecovery

Cryopreservation affordably and safely allows the stable long-term storage of frozen embryos or sperm at extremely low temperatures. Frozen preimplantation embryos can later be thawed and surgically transferred into surrogate females to recover the line. Investigators supply the mice, typically males, for cryopreservation. Early-stage preimplantation embryos are then collected by TTML staff, frozen, and stored in liquid-nitrogen freezer tanks. "Speed-cryogenic" methods can be used for mouse strains, such as C57BL/6, that are amenable to in vitro fertilization (IVF). In this method hundreds of eggs are fertilized via IVF using only one or two males. Fertilized embryos are cultured overnight, and those that have continued to develop are frozen.

For access to TTML technologies or advice on vector and experimental design, animal protocol issues, grant proposals, and screening analysis, investigators may contact core operations director Lynn Doglio at l-doglio@northwestern.edu.

CGM Research Profile: Warren Tourtellotte

Warren Tourtellotte, assistant professor of pathology at the Feinberg School, is the scientific director of the Transgenic and Targeted Mutagenesis Laboratory (TTML). He draws on his own research experience to advise other investigators who are developing transgenic/knockout mouse models.

Tourtellotte began designing gene-targeting vectors and working with embryonic stem (ES) cells in 1992, while a postdoctoral fellow at Washington University. Now focusing on the early-growth response (Egr) family of transcriptional regulators, Tourtellotte uses transgenic and knockout/knockin mouse models to understand the role of Egr-mediated transcriptional regulation in growth and differentiation processes, including those of the sympathetic nervous system and muscle stretch receptors.

By transiently expressing Egr3 in all skeletal muscle fibers during their development, investigators in Tourtellotte's lab showed that Egr3 dictates the fate of early muscle cells and their differentiation into stretch receptors. Tourtellotte has since developed a conditional knockin model to regulate where and when Egr3 expression occurs. In this model, homologous recombination is used to integrate Egr3 into the ROSA26 locus, an easily targeted locus that confers ubiquitous expression of the selected transgene. To restrict Egr3 expression, a transcriptional stop sequence is inserted upstream of Egr3 in the Rosa targeted locus. Using homologous recombination in *E. coli* (recombineering) technologies, Tourtellotte is able to clone recombination elements, called LoxP sites, on both sides of the transcription termination sequences. When Cre recombinase is present, these elements pair, and all DNA lying between the two LoxP sites is excised. Egr3 can then be conditionally expressed in specific tissues or cells by mating Rosa26-Egr3 knockin mice with transgenic mice that express Cre recombinase in the right tissue or cell at the right time and point. Tourtellotte has been able to study the role of Egr3 in skeletal muscle fibers and Egr transcription activity in sympathetic neurons by mating these mice to Mlc1f-cre (to activate Egr3 expression specifically in skeletal muscle) and dbH-Cre-IRES-taulacz transgenic mice (to activate Egr3 in sympathetic neurons).

Tourtellotte is now creating a conditional Egr3 knockout mouse line as part of a targeting scheme that uses the two different but functionally similar Cre- and Flp-recombinase systems. By incorporating both Cre- and Flp-recombinase elements (LoxP and Frt, respectively) into the targeting vector, the ES cell–selection cassette, which controls the initial selection of targeted clones in vitro but can also lead to incorrect expression of the targeted gene in vivo, can be excised in the targeted ES cells before they are injected into the blastocyst. The targeted Egr3 gene will be expressed normally in chimeric mice and their offspring but can be functionally deleted in specified tissue (i.e., myotubes or specific subpopulations of neurons) in offspring that express Cre recombinase in particular cells when they are mated to particular Cre recombinase–expressing transgenic mice.

Honors and Awards for CGM Members


Serdar Bulun


Kathleen Green


Amy Paller


Steven Wolinsky


Teresa Woodruff

Serdar E. Bulun, chief of reproductive biology research in the Department of Obstetrics and Gynecology at the Feinberg School, was elected to the American Gynecological and Obstetrical Society (AGOS) in December 2006. The AGOS is made up of physician scientists attaining national prominence in scholarship in the discipline of obstetrics, gynecology, and women's health. Only 6 to 10 new members are elected each year, Bulun was also named the George H. Gardner Professor of Clinical Gynecology at Northwestern. Bulun's lab researches estrogen biosynthesis and metabolism, in particular aromatase expression, in hormone-dependent human diseases such as breast cancer, endometriosis, and uterine fibroids.

Barbara Burton, professor of pediatrics at the Feinberg School and a clinical geneticist at Children's Memorial Hospital, was elected president of the Society for Inherited Metabolic Disorders (SIMD) and will serve a two-year term in 2007–08. The SIMD was founded to develop a group of metabolic experts who could advise the government on using medical products to treat inborn errors of metabolism; to establish a connected group of centers for the diagnosis and treatment of patients with metabolic diseases; and to foster research in this area. Burton's special interests are in the Marfan syndrome, Ehlers-Danlos syndrome, phenylketonuria, and other metabolic disorders and neurogenetic and ophthalmic genetic disorders.

Kathleen J. Green, Joseph L. Mayberry Sr. Professor in the Department of Pathology at the Feinberg School, has been appointed to the advisory council of the National Institute of Arthritis Musculoskeletal and Skin Disease (NIAMS). The mission of the NIAMS is to support research into the causes, treatment, and prevention of arthritis and musculoskeletal and skin diseases, the training of basic and clinical scientists to carry out this research, and the dissemination of information on research progress in these diseases. Green's research is focused on the assembly and regulation of an intercellular junction called the desmosome, the most prominent adhesive structure in complex epithelial tissues such as the epidermis. Her work was influential in the discovery of the plakin family of genes and facilitated the identification of heart and skin diseases resulting from mutations in desmoplakin.

J. Larry Jameson, Irving S. Cutter Professor of Medicine and incoming dean of the Feinberg School, became a member of the Institute of Medicine (IOM). The IOM was established to honor professional achievement in the health sciences and to serve as a national resource for independent analysis and recommendations on issues related to medicine, biomedical sciences, and health. Jameson's research is internationally recognized and has defined the genetic basis of more than a dozen endocrine disorders. His laboratory studies fundamental mechanisms that control the transcription of endocrine genes. He also focuses on bridging laboratory studies with clinical endocrinology using recombinant-DNA methods to investigate the pathophysiology of endocrine disorders.

Amy Paller, Walter J. Hamlin Professor and chair of dermatology and professor of pediatrics at the Feinberg School, becomes president of the Society for Investigative Dermatology (SID) in May. The SID advances and promotes the sciences relevant to skin health and disease through education, advocacy, and scholarly exchange of scientific information. Seminal studies in Paller's laboratory on the role of gangliosides in epithelial cell function have provided new insights into how glycosphingolipids modulate signaling at membrane raft regions. An active clinical trials investigator, Paller is currently researching new therapies for atopic dermatitis, pediatric psoriasis, epidermolysis bullosa, and keratinizing genetic disorders.

Patricia Spear, John Evans Professor of Microbiology-Immunology at the Feinberg School, was elected to the governing board of the American Academy of Microbiology (AAM) in 2006. The academy is the only group of its kind devoted entirely to microbiologists and the science of microbiology, and Spear has been a fellow since 1999. Fellowship nominations are reviewed annually, and successful candidates must be approved by both the Committee on Election to Fellowship and the Board of Governors. Spear's research investigates herpes simplex virus, the cause of various forms of disease from lesions on the lips, eyes, or genitalia to encephalitis or disseminated disease.

Steven M. Wolinsky, Samuel J. Sackett Professor of Medicine and chief of the Division of Infectious Diseases in the Department of Medicine at the Feinberg School, was awarded a fellowship of the American Academy of Microbiology, the privileged leadership group within the American Society for Microbiology. Fellows are chosen through a highly selective peer-reviewed process based on their records of scientific achievement and original contributions to the advancement of microbiology. Wolinsky's research has provided a fundamental understanding of the factors that affect HIV infection and the rate of disease progression — knowledge that is critical to establishing vaccine and treatment strategies for the disease.

Teresa K. Woodruff, professor of obstetrics and gynecology and of endocrinology at the Feinberg School and professor of biochemistry, molecular biology, and cell biology in the Judd A. and Marjorie Weinberg College of Arts and Sciences, has been appointed the Thomas J. Watkins Memorial Professor in Obstetrics and Gynecology. Woodruff has also been appointed director of the Feinberg School's new Institute for Women's Health Research and is chief of the new Division of Fertility Preservation. Woodruff's research focuses on ovarian biology, interdisciplinary approaches to problems in reproductive science, and the translation of her work to humans. Her work addresses methods to preserve fertility for young women and girls with a cancer diagnosis and the role of two peptide hormones, inhibin and activin, in regulating the reproductive axis.

CGM Faculty Profile: Douglas W. Losordo

Douglas W. Losordo, Eileen M. Foell Professor of Heart Research, joined the Feinberg School of Medicine in December 2006 as the director of the Feinberg Cardiovascular Research Institute of Northwestern University and the Program in Cardiovascular Regenerative Medicine in the Bluhm Cardiovascular Institute. He is also professor of medicine and a member of the Center for Genetic Medicine.

Before coming to Northwestern, Losordo held positions at St. Elizabeth's Medical Center and Tufts University in Boston, where he was professor of medicine and chief of cardiovascular research. Fifteen members of Losordo's laboratory and clinical research team moved with him from Boston to Chicago.


Douglas Losordo

Losordo is an internationally recognized cardiologist and physician-scientist. His research in stem cell biology, tissue repair and regeneration, and therapeutic angiogenesis (new blood vessel growth) focuses on developing therapies for cardiovascular diseases. In 1998 he published a report on the first successful application of gene therapy for coronary heart disease.

With funding from the National Institutes of Health, Losordo heads a team of physician-scientists that study adult stem cell and gene therapies for heart and vascular diseases. Recently the team completed the first approved U.S. trial of adult stem cell therapy for heart disease. In clinical trials of the fairly noninvasive procedure, stem cells extracted from a patient's own bone marrow were injected back into the blood supply. The team's key finding was that adult human bone marrow contains cells that can differentiate into heart muscle cells and cells that form new blood vessels.

Losordo received his medical degree from the University of Vermont and is board certified in internal medicine, cardiovascular medicine, and interventional cardiology. He is a fellow of the American College of Cardiology, the American Heart Association, the American Association for the Advancement of Science, the American College of Physicians, the American College of Chest Physicians, and the Society for Cardiac Interventions and Angiography.

Events

The CGM Silverstein Lecture Series presents the Chicago-area premieres of the documentary film *Terra Incognita: The Promise and Peril of Stem Cell Research*.

May 10, 7 p.m.
Thorne Auditorium
375 East Chicago Avenue
Chicago

May 16, 7 p.m.
Ryan Family Auditorium
2145 Sheridan Road
Evanston

Questions and answers with Northwestern Feinberg School stem cell specialist John Kessler, bioethicist and Northwestern professor Laurie Zoloth, and filmmaker Maria Finitzo of Kartemquin Films will follow the screenings, which are free and open to the public. *(See article on page 1 and www.cgm.northwestern.edu.)*

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