



LIFE SCIENCES DIVISION E-NEWSLETTER

November/December, 2009

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DOE scientific focus area notes

Low Dose Radiation Research

Life Sciences Division Hosts DOE Program Manager: Low Dose SFA Research Presented

DOE Program Manager Noelle Metting visited the DOE Low Dose Scientific Focus Area (SFA) of the Life Sciences Division on November 30 – December 3, 2009. The agenda of the 3-day visit, hosted by SFA leader **Joe Gray**, was focused on scientific progress presentations by the SFA members on the first two days and it was concluded with tours of Berkeley Lab's Joint Genome Institute, Molecular Foundry, the Joint BioEnergy Institute, Advanced Light source and Berkeley West Biocenter Labs (Potter Street).

Joe Gray introduced the SFA research program that addresses aspects of low dose radiobiology that may inform regulatory decision making for exposure to low dose and low dose rate radiation. Specifically, the SFA focuses on systems biology approaches to the assessment of (a) how interactions between cells at risk of developing into cancer cells (target cells) and surrounding cells not at risk of developing into cancer (non target cells) influence cancer incidence in target cells, (b) whether and how biological systems adapt to ionizing radiation so that response to a later dose is conditioned by the action of the first exposure, and (c) influence of the epigenome on cancer incidence. Several members of the SFA presented recent progress in these areas and they presented on the development of new technologies to support low dose radiobiology.

Target and nontarget cells. **Mina Bissell** provided an overview of the project investigating the targeted and non-targeted effects of ionizing radiation (IR) on the mammary gland. In collaboration with **Jian-Hua Mao** and **Eleanor Blakely**, the chimeric mouse model - developed by Mary Helen Barcellos-Hoff while she was a member of the Life Sciences Division - is being utilized to examine the genetic components, the cytokine response, as well as the microenvironmental contributions that may lead to mammary tumor formation following exposure to low levels of IR. In addition to the extensive in vivo studies coordinated by Mao and Blakely, three-dimensional culture assays, developed in the Bissell lab are being used to investigate the hidden properties that would be difficult to see in vivo and are basically masked in two dimensional-cell culture. Exciting but as yet preliminary data comparing the two species used in these studies (Balb/c and Spretus) showed that other than size there were no discernable differences observed between the whole mounts of the untreated mammary glands. However, interestingly, the two species have very dramatic differences in their branching pattern in the three-dimensional assay. Together, the in vivo studies and the three-dimensional culture assays provide complementary modes of investigation to unravel mechanisms leading to the differences in mammary tumors induced by low dose radiation.

Adaptive response. **Andrew Wyrobek**, **Priscilla Cooper**, **Debo Das**, and **Judith Campisi** reviewed recent progress on understanding the molecular mechanisms by which cells respond to low dose radiation, illustrating the advantages of the model systems, biotechnologies and bioinformatic tools developed at Berkeley Lab. Evidence was presented that low dose response mechanisms depend on the genetic background of the individual, vary across tissues, cell types and tissue microenvironment, and cannot be predicted from high dose responses. Also, a novel parallelogram strategy was introduced for integrating

mechanistic and dose response information from various animal and human cell culture models to predict low dose human cancer risks.

Exposure to low dose ionizing radiation can also alter responses to subsequent exposures to cancer causing agents, a phenomenon known as the adaptive response. This response is inducible and can protect a broad range of biological endpoints, including senescence (permanent cellular dormancy), chromosome damage, developmental defects, and excess cancers. The mechanisms of protection are complex and may vary by endpoint, involving inducible DNA repair and various cellular damage response pathways. Interestingly, individuals and cell types appear to differ in their ability to mount an adaptive response, but it is not yet known how this may affect an individual's health. The goals of this research are to understand the molecular mechanisms of the low dose and adaptive responses within complex tissue environments, and how genetic differences in low dose damage response may predict susceptibilities to breast cancer, lymphoma, and other radiation-sensitive cancers.

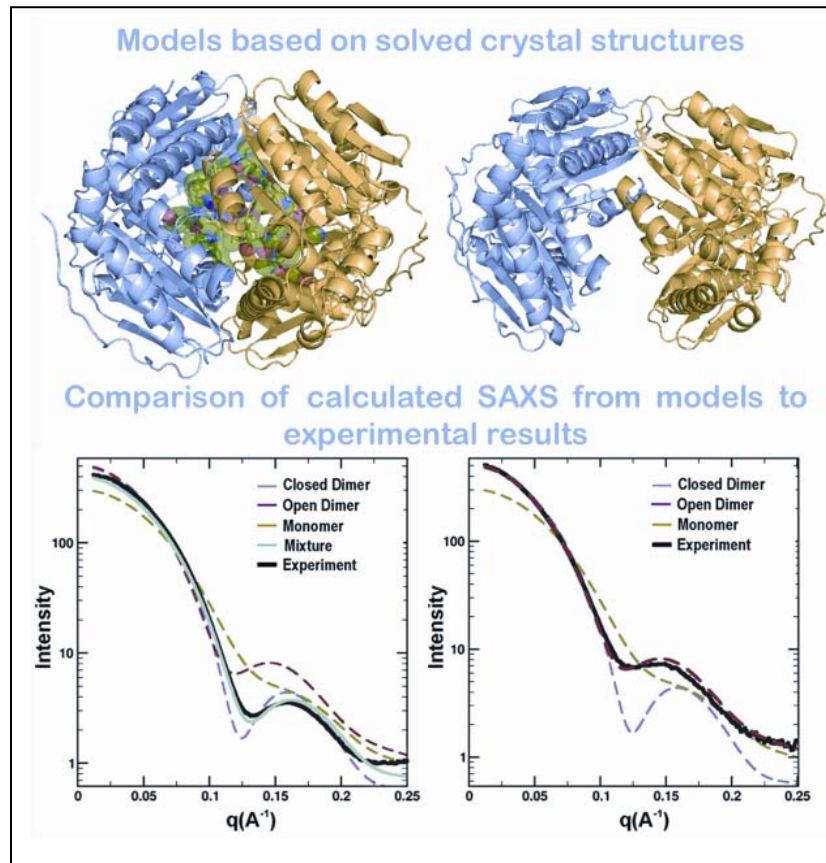
Epigenomics. **Kohwi-Shigematsu, Hunter Richards, Sylvain Costes, Gary Karpen and Paul Yaswen** described progress on elucidating the effect of x-ray irradiation on changes in chromatin structure. Using massively parallel sequencing, they detected rapid changes in chromatin structure marked by specific histone modifications throughout the genome of immortalized human breast epithelial cells after low dose radiation. They also observed global changes in chromatin packaging throughout the genome in irradiated drosophila - especially in heterochromatic regions known to be required for chromosome inheritance and genome stability. The changes in chromatin structure and histone marks induced by x-ray irradiation may poise cells to acquire new phenotypes, such as cancer progression or positive adaptation to low levels of irradiation.

Technological and infrastructure advances. Several technological advances were described including microenvironment microarrays to interrogate the role of the microenvironment of adaptive response, by **Mark LaBarge**; computational strategies for analysis of low dose and adaptive responses, by **Debo Das**; protein and mRNA imaging using mass spectrometry, by **Frank Chen**; metabolome imaging using nanostructure initiated mass spectrometry, by **Trent Northen**; high resolution anatomic 3D imaging, by **Al Thompson and Matt Francis**; new approaches to quantitative protein analysis, by **Demetris Iacovides**; and Bioinformatics and integrative analysis by **Braham Parvin**. In addition, **Francisco Marchetti** described new developments in the animal care facility.

The SFA's research is supported by several "work for others" (non-DOE) projects such as the SBDR (DNA repair machines) program project, the Integrative Cancer Biology Program, the Physical Sciences Oncology Center, The Cancer Genome Atlas Program, the Bay Area Breast Cancer Specialized Program on Research Excellence (SPORE), and the Stand Up to Cancer Program. The research is supported by several technologies and supporting facilities such as the animal facility, Mass Spec imaging, RPPA arrays and Firefly, Bioinformatics and integrative analysis, and anatomic imaging.

Gray, et al., 12/09

Novel Insights into Peptidases Published in *Structure*



The structural and conformational changes of a ubiquitous class of enzymes called metalloendopeptidases determined at SIBYLS, the Berkeley Lab X-ray beamline "Structurally-Integrated Biology for Life Sciences", is shown in the figure on the left. This class of enzymes recognizes specific proteins, encapsulates them and rather than degrading the entire protein cleaves off short targeting sequences. The processing is crucial for function of the target protein in chloroplasts or mitochondria. These "processing peptidases" are present in most organisms. Combining crystallography and SAXS the conformational mechanism has been elucidated. The metalloendopeptidase from the prokaryotic organism *Bacillus halodurans* used in this study

belongs to the M16B family of peptidases. Models of the dimeric protein from crystal structures for various conformations are shown in ribbons. Upon recognition and ingestion of a specific target protein (surface purple/green) the metalloendopeptidase closes around the protein via a conformational hinge motion. Comparison of these results to other available structural data on peptidases provides novel insights into the evolution of the entire superfamily of peptidases. The study is published in the November issue of *Structure*:

Aleshin AE, Gramatikova S, **Hura GL**, Bobkov A, Strongin AY, Stec B, **Tainer JA**, Liddington RC, Smith JW. Crystal and solution structures of a prokaryotic M16B peptidase: an open and shut case. *Structure*, 2009 Nov 11;17(11):1465-75. [PubMed - in process] PMID: 19913481

Greg Hura, 11/09

Radiochemistry & Instrumentation

Collaboration with Earth Sciences Division

Members of the DOE-OBER Scientific Focus Area (SFA) entitled “Radiotracer Imaging Technologies for Plant, Microbial, and Environmental Systems” have established a collaboration with members of the Earth Sciences Division at Berkeley Lab. The objective is to use nuclear medical imaging techniques to study how contaminants, especially heavy metals and radioactive ions, are transported through soils and groundwater aquifers. Contaminants are frequently observed to migrate further than conventional theory predicts. Their propagation is studied experimentally using “soil columns”—tubes (with dimensions ranging from about a centimeter to a meter) packed with soils having a variety of chemical and biological properties through which water-bearing contaminants are flowed. Currently, tedious chemical assays are used to determine the distribution of the contaminant in these columns.

Bill Moses explains that they “propose radiotracer imaging (positron tomography and single-photon imaging) as a more effective technique, as it is more sensitive and allows direct measurement of the contaminants in permeable materials without physically removing and measuring numerous samples. Positron- and gamma-emitting isotopes with suitable half-lives (days or longer) exist for many of the important contaminants (Cr, Cs, Tc, U, Sr, I, Se, As, etc.). We have performed several pilot studies in which we use SPECT imaging to study transport of Tc-99m in soil, sand, clay, dirt and gravel to model Tc-99 contamination around former nuclear facilities such as the Hanford reactors in Washington. We are currently developing and validating methods for imaging Tc-99m flow, exclusion fraction, and hydrodynamic dispersion in soil columns containing FeO₂ and Fe₂O₃ coated sand and bacterial compositions”.

Bill Moses, 11/09

Optimizing Biofuels Production

Members of the above are also developing tools to optimize biofuels production. **Bill Moses** reports: “There is a critical need to maximize the latent energy content of the compounds that plants produce and to maximize the efficiency of their conversion to biofuels. One of the keys to this development is to further our understanding of both the biochemical pathways involved in producing biofuel “raw materials” (such as cellulose, starch, terpenoids and lipids) and the genes that control these pathways. One vital tool for this enterprise is the use of radiolabeled metabolic intermediates to determine the dynamics of crucial control points along the pathways. We propose to further develop the use of short-lived, cyclotron-produced, positron-emitting isotopes (C-11, N-13 and F-18) to elucidate these processes. This involves radiochemistry (to synthesize the appropriate radiolabeled compounds as well as methods for introducing them into the biological system), instrumentation (to develop a radio-HPLC [high-pressure liquid chromatography] device that has two orders of magnitude higher sensitivity than existing devices), data analysis (kinetic modeling is necessary to extract the rates at which the various biological processes are occurring), and modeling (to use these data to determine the biochemical pathways). This strategy will be applied to both terrestrial plant systems and microalgae. To this end, we have developed a prototype HPLC instrument that can accurately measure the concentrations of radiolabeled compounds that are two orders of magnitude smaller than that possible with existing techniques.

A provisional patent for this instrument has been received, and we are working to use it to perform experiments on carbon utilization in algae”.

Bill Moses, 11/09

Scientific & divisional news

Gray Presents Keynote Address in Beijing

At the invitation of Anna Barker, Deputy Director National Cancer Institute (NCI) of the National Institutes of Health, **Joe Gray** presented a keynote address entitled “Cancer Genomics: State of the Science and Future Directions” at a joint meeting of the NCI and the Chinese Academy of Medical Sciences. The title of the meeting was "Enabling a Future of Personalized Cancer Medicine: Leveraging 30 years of China –U.S. Scientific Progress. The high level meeting, held November 9-10, 2009 in Beijing, China, was planned to celebrate the 30 year history of cooperation in cancer research. The meeting highlighted past successes and focused on opportunities for development of personalized cancer medicine in the U.S. and China – with an emphasis on future collaboration.

CG, 11/09

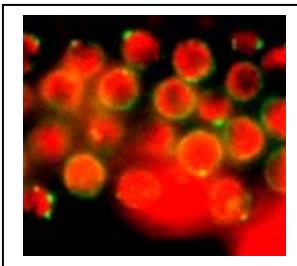
Bissell’s Impact on Breast Cancer Research

The National Breast Cancer Awareness Month organization just celebrated its 25th anniversary. Since that campaign began, the conversation about breast cancer has significantly changed — moving beyond genetics to include the cellular microenvironment and other factors, with profound implications for breast cancer awareness and therapies. It is widely recognized that the research of **Mina Bissell**, a Berkeley Lab Distinguished Scientist, has been a primary reason behind this change in the breast cancer conversation. More> <http://newscenter.lbl.gov/feature-stories/2009/11/10/breast-cancer-conversation-change/>

Today at Berkeley Lab, 11/11/09



Chromosomes Dance, Pair Up on Nuclear Membrane



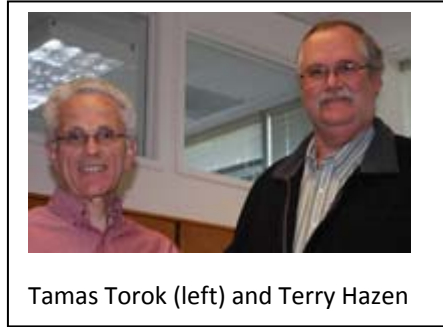
Meiosis — the pairing and recombination of chromosomes, followed by segregation of half to each egg or sperm cell — is a major crossroads in all organisms reproducing sexually. Yet how the cell precisely choreographs these chromosomal interactions is a long-standing question. New findings by UC Berkeley and Life Sciences scientist **Abby Dernburg** show that the cell's cytoskeleton, which moves things around in the cell, plays a critical role, essentially reaching into the nucleus to bring chromosome pairs together in preparation for recombination and segregation. More>

http://www.berkeley.edu/news/media/releases/2009/11/12_meiosis.shtml

Today at Berkeley Lab, 11/17/09

New Biosafety Committee Chair Appointed

The Earth Sciences Division's Terry Hazen has been appointed the Chair of the Institutional Biosafety Committee (IBC). The IBC provides institutional assurance of safety by reviewing Berkeley Lab policies and projects involving research with biological materials that may pose safety, health, or environmental risks. Hazen brings to the IBC extensive experience in microbiology and biotechnology, and a strong desire to promote research within a safe work environment. He is a fellow of the American Academy of Microbiologists, the American Society for Microbiologists, and the Society for Industrial Microbiology. He replaces Life Sciences Division's **Tamas Torok**, who held the IBC chair for 10 years. More > <http://www.lbl.gov/ehs/biosafety/bioSafetyCommittee.pdf>
Today at Berkeley Lab, 11/19/09



Tamas Torok (left) and Terry Hazen

Kronenberg to Serve on German Advisory Committee

Amy Kronenberg has been nominated by the Scientific Council of GSI to be a member of the Biophysics and Radiobiology Program Advisory Committee for the GSI Helmholtz Centre for Heavy Ion Research. The committee's task is to meet (in Germany) to review experimental proposals in the fields of biophysics, radiobiology and ion-therapy-relevant developments to recommend them for granting to the Scientific Director of GSI. Kronenberg has expertise in heavy ion biology and biophysics, with particular emphases in apoptotic regulation, DNA repair and mutagenesis.

From the GSI website: "GSI operates a large, in many aspects worldwide unique accelerator facility for heavy-ion beams. Researchers from around the world use the facility for experiments that help point the way to new and fascinating discoveries in basic research. In addition, the scientists use their findings to continually develop new applications. The research program at GSI covers a broad range of activities extending from nuclear and atomic physics to plasma and materials research to biophysics and cancer therapy. Probably the best-known results are the discovery of six new chemical elements and the development of a new type of tumor therapy using ion beams". More > http://www.gsi.de/portrait/ueberblick_e.html
CG, 11/09

Gray to Join AIMBE College of Fellows

Joe Gray has been elected to the College of Fellows of the American Institute for Medical Biological Engineering (AIMBE). Gray was recognized for "developing chromosome sorting, bi-variate cell cycle analysis, fluorescence in situ hybridization, competitive genomic hybridization, cancer specific markers." He was selected for "his significant contributions to the field" and will join an exemplary group of over 900 engineers and scientists.

Located in Washington D.C., AIMBE is the leading advocacy group for medical and biological engineering and is comprised of some of the most important leaders in science and engineering, the top 2% of medical and biological engineers. AIMBE (www.aimbe.org) was founded in 1991 to establish a clear and comprehensive identity for the field of medical and biological engineering – which is the bridge between the principles of engineering science and practice, and the problems and issues of biological and medical

science and practice. It has since earned a reputation as a prestigious public policy leader on issues impacting the medical and biological community. AIMBE is regarded by key legislators as the preeminent voice on the subject. More >

https://www.netforumondemand.com/eWeb/DynamicPage.aspx?Site=AIMBE&WebCode=ArticleDetail&faq_key=c4bb6c8f-1a68-4568-9343-d0f4762d0853

CG, Today at Berkeley Lab, 12/8/09

Budinger Appointed to National Academies Panel on Physics

Thomas Budinger has been appointed to the National Academies Panel on Physics. The Panel on Physics



Thomas Budinger

is part of the National Research Council, Division on Engineering and Physical Sciences, Laboratory Assessments Board. This panel assesses the scientific and technical work performed by the National Institute of Standards and Technology (NIST). It is one of nine panels that assess NIST and is specific for the NIST Physics Laboratory.

Founded in 1901, NIST is a non-regulatory federal agency within the U.S. Department of Commerce. NIST's mission is to promote United States innovation and industrial competitiveness by advancing measurement science, standards, and technology in ways that enhance economic security and improve our quality of life. The Physics Laboratory is one of the major operating units of

NIST. Its mission is to support United States industry by providing measurement services and research for electronic, optical, and radiation technologies.

The appointment is in addition to Budinger's current responsibilities as Home Secretary for the National Academy of Engineering and participant on the Board of the National Research Council.

Thomas Budinger, 11/09

Life Sciences Undergraduate Interns Present their Work

The Center for Science and Engineering Education (CSEE) hosted a poster session on December 16, 2009. Featuring the work of five fall college undergraduates, the event highlighted the breadth of research conducted at the Laboratory through the experience of the students who have participated in CSEE Science Undergraduate Laboratory Internship (SULI) fall program. Four of the participating undergraduates interned in the Life Sciences Division: Sean Christensen, Michael Lysonski and Marcin Zemla (mentored by **Manfred Auer**), and Jenny Hung (mentored by **Joe Gray** and **James Korkola**).



Christensen, who presented his work on "Ultrastructural Analysis of Extracellular Filaments Produced by the Bacterium *Shewanella Oneidensis* MR-1," recognizes the value of his internship, commenting: "rarely do students grasp the independence necessary for progressive research when they're still learning in the classroom. Manfred Auer offered myself and other interns the opportunity to participate in cutting edge research while having well-informed colleagues at our side when questions surfaced.

Along with presenting at the poster session, I was given the opportunity to speak at our lab meetings and discuss my research at length, allowing my colleagues to ask me questions and delve deeper into my project. My experience was unforgettable and helped me understand that being a researcher isn't just following instructions, it is a synthesis of science, philosophy, communication and determination".

Auer's main motivation for hosting and mentoring interns, he says, is "to give back to the community, knowing that I am here in this place only because of the mentoring I received. The reward is the impact one has on the interns' life and careers, and to see them becoming excited about a career in research. This way, hopefully, values of scientific integrity, intellectual curiosity and the love for discovery are passed on to a next generation of scientific leaders."

CG, Today at Berkeley Lab, 12/16/09

Life Sciences Featured in "Science and Health" Overview

The Year of Science 2009, an initiative of the Coalition on the Public Understanding of Science (COPUS), concluded 2009 with the December theme "Science and Health." As a participating member of this coalition, Berkeley Lab presented a brief overview entitled "Cancer research at Berkeley Lab: the intersection of science and health" on their Year of Science 2009 web page, featuring research in the Life Sciences Division of **Mina Bissell**, **Judith Campisi**, **Joe Gray** and others. Visit here to see a brief snapshot of where we're going, and where we've been: <http://www.lbl.gov/Publications/YOS/Dec/CG, 12/09>



Awards

Kohwi-Shigematsu Receives NIH Roadmap Grant

Life Sciences Division's scientist Terumi **Kohwi-Shigematsu** has received an NIH grant for studying "Determinants for Genome-Wide Epigenomics in Metastatic Breast Cancer". Breast cancer is the most common cancer in women in the United States, causing ~40,000 deaths each year. Mounting evidence suggest that cancer is not necessarily a result of accumulated genetic mutations, but may also involve alterations in the epigenome, the modifications on the DNA or the proteins that package the DNA. In fact, recent evidence suggests that these modifications are different between metastatic and non-metastatic breast cancer. The funded study will determine what epigenomic changes underlie metastatic breast cancer and the mechanisms by which these changes are established.

The grant is one of 22 grants funded by the National Institutes of Health on genome-wide studies of how epigenetic changes - chemical modifications to genes that result from diet, aging, stress, or environmental exposures - define and contribute to specific human diseases and biological processes. The awards build on the important work undertaken as part of the NIH Roadmap for Medical Research's Epigenomics Program. The



Terumi Kohwi-Shigematsu

Program was designed to characterize epigenetic modifications and to correlate the presence or absence of specific modifications with disease status.

CG, 11/09

New Funding for Protein Radiation Biodosimetry and Technology from HHS

In December 2009, a team from Berkeley Lab and Stanford University was awarded a multi-center research contract from the Biomedical Advanced Research and Development Authority (BARDA) in the U.S. Department of Health and Human Services to develop panels of blood protein markers and nano-devices for high-throughput radiation biodosimetry. The risk of a terrorist attack with radiological or nuclear devices is existent, and current technologies are largely inadequate for mass exposure scenarios. Life Sciences Division's bioscientists lead by **Andrew Wyrobek** and **Francesco Marchetti** teamed with the engineering group from Stanford University, lead by Shan Wang, to organize a research consortium including seven other academic and industry centers.

The goal of this consortium is to produce a novel protein chip platform based on a small set of established protein radiation biomarkers to rapidly assess the dose range of radiation exposure of a large number of individuals in a short period of time. At the heart of this technology are magneto-nano protein chips functionalized with radiation-sensitive protein biomarkers that the Berkeley Lab team will validate using complementary models of irradiated cells from rodents, humans, and non-human primates. This research will establish the sensitivity and specificity of the protein panels, characterize responses and inter-individual variations, and assess the effects of partial exposures, combined injury, as well as gender, age and other population factors. The technology platform to be developed under this project is expected to have broad applications for assessing exposures, tissue damage and health consequences in persons exposed to ionizing radiation from various environmental, medical and energy technologies, including low-dose exposures of interest to the U.S. Department of Energy.

Andrew Wyrobek, 12/09

Recent publications

Li C, Knierim B, Manisseri C, Arora R, Scheller HV, **Auer M**, Vogel KP, Simmons BA, Singh S. Comparison of dilute acid and ionic liquid pretreatment of switchgrass: Biomass recalcitrance, delignification and enzymatic saccharification. *Bioresource Technology*, 2009 Nov 28. [Epub ahead of print]
PMID: 19945861

The efficiency of two biomass pretreatment technologies, dilute acid hydrolysis and dissolution in an ionic liquid, are compared in terms of delignification, saccharification efficiency and saccharide yields with switchgrass serving as a model bioenergy crop. When subject to ionic liquid pretreatment (dissolution and precipitation of cellulose by anti-solvent) switchgrass exhibited reduced cellulose crystallinity, increased surface area, and decreased lignin content compared to dilute acid pretreatment. Pretreated material was characterized by powder X-ray diffraction, scanning electron microscopy, Fourier transform infrared spectroscopy, Raman spectroscopy and chemistry methods. Ionic liquid pretreatment enabled a significant enhancement in the rate of enzyme hydrolysis of the cellulose component of switchgrass, with a rate increase of 16.7-fold, and a glucan yield of 96.0% obtained in 24h. These results indicate that ionic

liquid pretreatment may offer unique advantages when compared to the dilute acid pretreatment process for switchgrass. However, the cost of the ionic liquid process must also be taken into consideration.

Su X, Kells AP, Huang EJ, Lee HS, Hadaczek P, **Beyer J, Bringas J, Pivrotto P**, Penticuff J, **Eberling J**, Federoff HJ, Forsayeth J, Bankiewicz KS. Safety evaluation of AAV2-GDNF gene transfer into the dopaminergic nigrostriatal pathway in aged and Parkinsonian Rhesus Monkeys. *Human Gene Therapy*, 2009 Nov 12. [Epub ahead of print] PMID: 19671001

We evaluated neuropathological findings in two studies of AAV2-GDNF efficacy and safety in naive aged (>20 years) or MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-lesioned rhesus macaques. In the first study, a total of 17 animals received one of two doses of AAV2-GDNF into either putamen or substantia nigra (SN). To control for surgical variables, all animals received identical putaminal and nigral infusions in which phosphate-buffered saline was substituted for vector as appropriate. All 17 aged monkeys were studied for 6 months before necropsy. In a separate study, 11 MPTP-lesioned rhesus macaques with extensive lesions in the right SN and mild lesions in the left SN received bilateral infusions of AAV2-GDNF (9.9×10^{11} vector genomes) or PBS into the putamen and were then studied for up to 14 months. In the current analysis, we addressed safety issues regarding AAV2-GDNF administration. An extensive series of assessments of in-life behavioral and clinical parameters was conducted. No overt histopathology or immune responses were detected in any experimental monkey. However, the delivery of AAV2-GDNF to the SN of aged monkeys caused a marked and significant loss of body weight (-19.4%). No weight loss was observed in the MPTP-lesioned monkeys despite bilateral axonal transport of glial cell line-derived neurotrophic factor (GDNF) to the SN from the putamen. These findings indicate that putaminal administration of AAV2-GDNF by convection-enhanced delivery shows therapeutic promise without any apparent side effects. Importantly, nigral administration of AAV2-GDNF caused significant weight loss that raises substantial concern for clinical application of this approach.

Hiraoka Y, **Dernburg AF**. The SUN rises on meiotic chromosome dynamics. *Developmental Cell*, 2009 Nov;17(5):598-605. PMID: 19922865 [Review]

Recent studies in diverse eukaryotes have implicated a family of nuclear envelope proteins containing SUN domains as key components of meiotic nuclear organization and chromosome dynamics. In many cases, these transmembrane proteins are also known to contribute to centrosome or spindle pole body function in mitotically dividing cells. During meiotic prophase, the apparent role of these SUN-domain proteins, together with their partner KASH-domain proteins, is to connect chromosomes through the intact nuclear envelope to force-generating mechanisms in the cytoplasm.

Ho MC, Johnsen H, Goetz SE, Schiller BJ, Bae E, Tran DA, Shur AS, Allen JM, Rau C, Bender W, Fisher WW, **Celniker SE**, Drewell RA. Functional evolution of cis-regulatory modules at a homeotic gene in *Drosophila*. *PLoS Genetics*, 2009 Nov;5(11):e1000709. PMID: 19893611

It is a long-held belief in evolutionary biology that the rate of molecular evolution for a given DNA sequence is inversely related to the level of functional constraint. This belief holds true for the protein-coding homeotic (Hox) genes originally discovered in *Drosophila melanogaster*. Expression of the Hox genes in *Drosophila* embryos is essential for body patterning and is controlled by an extensive array of cis-regulatory modules (CRMs). How the regulatory modules functionally evolve in different species is not clear. A comparison of the CRMs for the Abdominal-B gene from different *Drosophila* species reveals relatively low levels of overall sequence conservation. However, embryonic enhancer CRMs from other *Drosophila* species direct transgenic reporter gene expression in the same spatial and temporal patterns during development as their *D. melanogaster* orthologs. Bioinformatic analysis reveals the presence of short conserved sequences within defined CRMs, representing gap and pair-rule transcription factor

binding sites. One predicted binding site for the gap transcription factor KRUPPEL in the IAB5 CRM was found to be altered in Superabdominal (Sab) mutations. In Sab mutant flies, the third abdominal segment is transformed into a copy of the fifth abdominal segment. A model for KRUPPEL-mediated repression at this binding site is presented. These findings challenge our current understanding of the relationship between sequence evolution at the molecular level and functional activity of a CRM. While the overall sequence conservation at *Drosophila* CRMs is not distinctive from neighboring genomic regions, functionally critical transcription factor binding sites within embryonic enhancer CRMs are highly conserved. These results have implications for understanding mechanisms of gene expression during embryonic development, enhancer function, and the molecular evolution of eukaryotic regulatory modules.

Choong WS. The timing resolution of scintillation-detector systems: Monte Carlo analysis. *Physics in Medicine and Biology*, 2009 Nov 7;54(21):6495-513. PMID: 19820267

We use Monte Carlo analysis to model the physical processes (crystal geometry, crystal surface finish, scintillator rise time, scintillator decay time, photoelectron yield, PMT transit time spread, PMT single-electron response, amplifier response, and time pick-off method) that can contribute to the timing resolution of scintillation-detector systems. The best timing resolution is achieved with the first photoelectron timing, which is limited by the photoelectron statistics independent of the properties of the photodetector. One of the important and interesting results is that the calculated timing resolution of a leading edge discriminator gives better timing performance than a constant fraction discriminator and can potentially approach the first photoelectron timing. Experimental measurements provide reasonably good agreement with the calculated timing resolution. The Monte Carlo analysis developed in this work will allow us to optimize the scintillation-detector systems for timing and to understand the physical factors limiting their performance.

Turker MS, Connolly L, Dan C, Lasarev M, **Gauny S, Kwoh E, Kronenberg A.** Comparison of autosomal mutations in mouse kidney epithelial cells exposed to iron ions in situ or in culture. *Radiation Research*, 2009 Nov;172(5):558-66. PMID: 19883223

Exposure to accelerated iron ions represents a significant health risk in the deep space environment because it induces mutations that can cause cancer. A mutation assay was used to determine the full spectrum of autosomal mutations induced by exposure to 2 Gy of 1 GeV/nucleon iron ions in intact kidney epithelium, and the results were compared with mutations induced in cells of a kidney epithelial cell line exposed in vitro. A molecular analysis for loss of heterozygosity (LOH) for polymorphic loci on chromosome 8, which harbors *Aprt*, demonstrated iron-ion induction of mitotic recombination, interstitial deletion, and discontinuous LOH events. Iron-ion-induced deletions were detected more readily with the in vitro assay, whereas discontinuous LOH was detected more readily in the intact kidney. The specific induction of discontinuous LOH in vivo suggests that this mutation pattern may serve as an indicator of genomic instability. Interestingly, the frequency of small intragenic events increased as a function of time after exposure, suggesting non-targeted effects. In total, the results demonstrate that 1 GeV/nucleon iron ions can elicit a variety of autosomal mutations and that the cellular microenvironment and the sampling time after exposure can influence the distribution of these mutations in epithelial cell populations.

Gundiah G, Hanrahan S, Hollander F, and Bourret-Courchesne E. Europium-doped barium bromide iodide. *Acta Crystallographica Section E: Structure Reports Online*, E65, i76-i77 (November 2009) <doi:10.1107/S1600536809041105>.

The structure of Eu-activated barium bromide iodide (BaBrI:Eu) was determined using single-crystal X-ray diffraction. This material is a new scintillator discovered at LBNL that has excellent gamma-ray detection

properties. Single crystals were grown by the melt-based Bridgman technique. The compound has a PbCl_2 structure, similar to the related compositions SrBrI and EuBrI . The Eu atom was found to replace Ba in the lattice and is surrounded by nine anions.

Rodriguez B, Yang Y, Guliaev AB, Chenna A, **Hang B**. Benzene-derived N(2)-(4-hydroxyphenyl)-deoxyguanosine adduct: UvrABC incision and its conformation in DNA. *Toxicology Letters*, 2009 Dec 16. [Epub ahead of print] PMID: 20006688

Benzene, a ubiquitous human carcinogen, forms DNA adducts through its metabolites such as p-benzoquinone (p-BQ) and hydroquinone (HQ). N(2)-(4-Hydroxyphenyl)-2'-deoxyguanosine (N(2)-4-HOPh-dG) is the principal adduct identified in vivo by (32)P-postlabeling in cells or animals treated with p-BQ or HQ. To study its effect on repair specificity and replication fidelity, we recently synthesized defined oligonucleotides containing a site-specific adduct using phosphoramidite chemistry. We here report the repair of this adduct by *Escherichia coli* UvrABC complex, which performs the initial damage recognition and incision steps in the nucleotide excision repair (NER) pathway. We first showed that the p-BQ-treated plasmid was efficiently cleaved by the complex, indicating the formation of DNA lesions that are substrates for NER. Using a 40-mer substrate, we found that UvrABC incises the DNA strand containing N(2)-4-HOPh-dG in a dose- and time-dependent manner. The specificity of such repair was also compared with that of DNA glycosylases and damage-specific endonucleases of *E. coli*, both of which were found to have no detectable activity toward N(2)-4-HOPh-dG. To understand why this adduct is specifically recognized and processed by UvrABC, molecular modeling studies were performed. Analysis of molecular dynamics trajectories showed that stable G:C-like hydrogen bonding patterns of all three Watson-Crick hydrogen bonds are present within the N(2)-4-HOPh-G:C base pair, with the hydroxyphenyl ring at an almost planar position. In addition, N(2)-4-HOPh-dG has a tendency to form more stable stacking interactions than a normal G in B-type DNA. These conformational properties may be critical in differential recognition of this adduct by specific repair enzymes.

Aleshin AE, Gramatikova S, **Hura GL**, Bobkov A, Strongin AY, Stec B, **Tainer JA**, Liddington RC, Smith JW. Crystal and solution structures of a prokaryotic M16B peptidase: an open and shut case. *Structure*, 2009 Nov 11;17(11):1465-75. PMID: 19913481

See also GTL-Genomics Highlight, page 4.

The M16 family of zinc peptidases comprises a pair of homologous domains that form two halves of a "clam-shell" surrounding the active site. The M16A and M16C subfamilies form one class ("peptidasomes"): they degrade 30-70 residue peptides, and adopt both open and closed conformations. The eukaryotic M16B subfamily forms a second class ("processing proteases"): they adopt a single partly-open conformation that enables them to cleave signal sequences from larger proteins. Here, we report the solution and crystal structures of a prokaryotic M16B peptidase, and demonstrate that it has features of both classes: thus, it forms stable "open" homodimers in solution that resemble the processing proteases; but the clam-shell closes upon binding substrate, a feature of the M16A/C peptidasomes. Moreover, clam-shell closure is required for proteolytic activity. We predict that other prokaryotic M16B family members will form dimeric peptidasomes, and propose a model for the evolution of the M16 family.

Schuff N, Matsumoto S, Kmiecik J, Studholme C, Du A, Ezekiel F, Miller BL, Kramer JH, **Jagust WJ**, Chui HC, Weiner MW. Cerebral blood flow in ischemic vascular dementia and Alzheimer's disease, measured

by arterial spin-labeling magnetic resonance imaging. *Alzheimer's & Dementia : the Journal of the Alzheimer's Association*, 2009 Nov;5(6):454-62. PMID: 19896584

BACKGROUND: Our objectives were to compare the effects of subcortical ischemic vascular dementia (SIVD) and Alzheimer's disease (AD) on cerebral blood flow (CBF), and then to analyze the relationship between CBF and subcortical vascular disease, measured as volume of white-matter lesions (WMLs). **METHODS:** Eight mildly demented patients with SIVD (mean +/- SD; aged 77 +/- 8 years; Mini-Mental State Examination score 26 +/- 3 years) and 14 patients with AD were compared with 18 cognitively normal elderly subjects. All subjects had CBF measured using arterial spin-labeling magnetic resonance imaging, and brain volumes were assessed using structural magnetic resonance imaging. **RESULTS:** AD and SIVD showed marked CBF reductions in the frontal (P = 0.001) and parietal (P = 0.001) cortices. In SIVD, increased subcortical WMLs were associated with reduced CBF in the frontal cortex (P = 0.04), in addition to cortical atrophy (frontal, P = 0.05; parietal, P = 0.03). **CONCLUSIONS:** Subcortical vascular disease is associated with reduced CBF in the cortex, irrespective of brain atrophy.

Kronenberg A, Gauny S, Kwoh E, Connolly L, Dan C, Lasarev M, Turker MS. Comparative analysis of cell killing and autosomal mutation in mouse kidney epithelium exposed to 1 GeV/nucleon iron ions in vitro or in situ. *Radiation Research*, 2009 Nov;172(5):550-7. PMID: 19883222

Astronauts receive exposures to high-energy heavy ions from galactic cosmic radiation. Although high-energy heavy ions are mutagenic and carcinogenic, their mutagenic potency in epithelial cells, where most human cancers develop, is poorly understood. Mutations are a critical component of human cancer, and mutations involving autosomal loci predominate. This study addresses the cytotoxic and mutagenic effects of 1 GeV/nucleon iron ions in mouse kidney epithelium. Mutant fractions were measured for an endogenous autosomal locus (Aprt) that detects all types of mutagenic events contributing to human cancer. Results for kidneys irradiated in situ are compared with results for kidney cells from the same strain exposed in vitro. The results demonstrate dose-dependent cell killing in vitro and for cells explanted 3-4 months postirradiation in situ, but in situ exposures were less likely to result in cell death than in vitro exposures. Prolonged incubation in situ (8-9 months) further attenuated cell killing at lower doses. Iron ions were mutagenic to cells in vitro and for irradiated kidneys. No sparing was seen for mutant frequency with a long incubation period in situ. In addition, the degree of mutation induction (relative increase over background) was similar for cells exposed in vitro or in situ. We speculate that the latent effects of iron-ion exposure contribute to the maintenance of an elevated mutation burden in an epithelial tissue.

Zhang X, Sebastiani P, Liu G, Schembri F, Zhang X, Dumas YM, Langer EM, Alekseyev Y, O'Connor GT, Brooks DR, **Lenburg ME**, Spira A. Similarities and differences between smoking-related gene expression in nasal and bronchial epithelium. *Physiological Genomics*, 2009 Dec 1. [Epub ahead of print] PMID: 19952278

Previous studies have shown that physiological responses to cigarette smoke can be detected via bronchial airway epithelium gene-expression profiling, and that heterogeneity in this gene-expression response to smoking is associated with lung cancer. In this study, we sought to determine the similarity of the effects of tobacco-smoke throughout the respiratory tract by determining patterns of smoking-related gene expression in paired nasal and bronchial epithelial brushings collected from 14 healthy nonsmokers and 13 healthy current smokers. Using whole-genome expression arrays, we identified 119 genes whose expression was affected by smoking similarly in both bronchial and nasal epithelium, including genes related to detoxification, oxidative stress, and wound healing. While the vast majority of smoking-related gene-expression changes occur in both bronchial and nasal epithelium, we also identified 27 genes whose expression was affected by smoking more dramatically in bronchial epithelium than nasal epithelium. Both common and site-specific smoking-related gene-expression profiles were validated using

independent microarray datasets. Differential expression of select genes was also confirmed by RT-PCR. That smoking induces largely similar gene-expression changes in both nasal and bronchial epithelium suggests that the consequences of cigarette smoke exposure can be measured in tissues throughout the respiratory tract. Our findings suggest that nasal epithelial gene expression may serve as a relatively non-invasive surrogate to measure physiological responses to cigarette smoke and/or other inhaled exposures in large-scale epidemiological studies.

Steiling K, **Lenburg ME**, Spira A. Airway gene expression in chronic obstructive pulmonary disease. *Proceedings of the American Thoracic Society*, 2009 Dec;6(8):697-700. PMID: 20008878

Although cigarette smoking is the major cause of chronic obstructive pulmonary disease (COPD), only a subset of smokers develops this disease. There is significant clinical, radiographic, and pathologic heterogeneity within smokers who develop COPD that likely reflects multiple molecular mechanisms of disease. It is possible that variations in the individual response to cigarette smoking form the basis for the distinct clinical and molecular phenotypes and variable natural history associated with COPD. Using the biologic premise of a molecular field of airway injury created by cigarette smoking, this response to tobacco exposure can be measured by molecular profiling of the airway epithelium. Noninvasive study of this field effect by profiling airway gene expression in patients with COPD holds important implications for our understanding of disease heterogeneity, early disease detection, and identification of novel disease-modifying therapies.

Blanc L, **Liu J**, Vidal M, **Chasis JA**, An X, Mohandas N. The water channel aquaporin-1 partitions into exosomes during reticulocyte maturation: implication for the regulation of cell volume. *Blood*, 2009 Oct 29;114(18):3928-34. PMID: 19724054

Aquaporin-1 (AQP-1), the universal water channel, is responsible for rapid response of cell volume to changes in plasma tonicity. In the membrane of the red cell the concentration of the protein is tightly controlled. Here, we show that AQP-1 is partially lost during in vitro maturation of mouse reticulocytes and that it is associated with exosomes, released throughout this process. AQP-1 in young reticulocytes localizes to the plasma membrane and also in endosomal compartments and exosomes, formed both in vitro and in vivo. During maturation a part of the total pool of AQP-1 is differentially sorted and released via the exosomal pathway. A proteasome inhibitor, MG132, suppresses secretion of AQP-1, implying that ubiquitination is a sorting signal for its release. We further show that modulation of medium tonicity in vitro regulates the secretion of AQP-1, thus showing that extracellular osmotic conditions can drive sorting of selected proteins by the exosomal pathway. These results lead us to suggest that AQP-1 sorting into exosomes may be the mechanism by which the reticulocyte adapts to environmental changes during its maturation.

Nam JM, Chung Y, **Hsu HC**, **Park CC**. beta1 integrin targeting to enhance radiation therapy. *International Journal of Radiation Biology*, 2009 Nov;85(11):923-8. PMID: 19895268

PURPOSE: Cell adhesion to extracellular matrix (ECM) proteins is mediated by the integrin family and has been known to modify radiation sensitivity and resistance in several cell types, including cancer cells. In particular, beta1 integrin signaling has been implicated in the progression and metastasis of various cancers and has been shown to facilitate resistance to radiation therapy. **CONCLUSION:** In this mini-review, we provide a brief overview of integrin targeting in radiation therapy. We specifically focus on the updated findings of beta1 integrin-mediated signaling pathways after exposure to ionising radiation (IR) using in vitro and in vivo experimental models, which could represent promising therapeutic targets for breast cancer.

Nogales E, Ramey VH. Structure-function insights into the yeast Dam1 kinetochore complex. *Journal of Cell Science*, 2009 Nov 1;122(Pt 21):3831-6. PMID: 19889968

Faithful segregation of genetic material during cell division requires the dynamic but robust attachment of chromosomes to spindle microtubules during all stages of mitosis. This regulated attachment occurs at kinetochores, which are complex protein organelles that are essential for cell survival and genome integrity. In budding yeast, in which a single microtubule attaches per kinetochore, a heterodecamer known as the Dam1 complex (or DASH complex) is required for proper chromosome segregation. Recent years have seen a burst of structural and biophysical data concerning this interesting complex, which has caught the attention of the mitosis research field. In vitro, the Dam1 complex interacts directly with tubulin and self-assembles into ring structures around the microtubule surface. The ring is capable of tracking with depolymerizing ends, and a model has been proposed whereby the circular geometry of the oligomeric Dam1 complex allows it to couple the depolymerization of microtubules to processive chromosome movement in the absence of any additional energy source. Although it is attractive and simple, several important aspects of this model remain controversial. Additionally, the generality of the Dam1 mechanism has been questioned owing to the fact that there are no obvious Dam1 homologs beyond fungi. In this Commentary, we discuss recent structure-function studies of this intriguing complex.

Hammel M, Yu Y, Mahaney BL, Cai B, Ye R, Phipps BM, **Rambo RP, Hura GL**, Pelikan M, So S, Abolfath RM, Chen DJ, Lees-Miller SP, **Tainer JA.** KU and DNA-Dependant Protein Kinase (DNA-PK) dynamic conformations and assembly regulate DNA binding and the initial nonhomologous end joining complex. *The Journal of Biological Chemistry*, 2009 Nov 5. [Epub ahead of print] PMID: 19893054

DNA double-strand break (DSB) repair by non-homologous end joining (NHEJ) is initiated by DSB detection by Ku70/80 (Ku) and DNA-dependent protein kinase catalytic subunit (DNA-PKcs) recruitment, which promotes pathway progression through poorly defined mechanisms. Here, Ku and DNA-PKcs solution structures alone and in complex with DNA, defined by X-ray scattering (SAXS), reveal major structural reorganizations that choreograph NHEJ initiation. The Ku80 C-terminal region forms a flexible arm that extends from the DNA-binding core to recruit and retain DNA-PKcs at DSBs. Furthermore, Ku and DNA promoted assembly of a DNA-PKcs dimer facilitates trans-autophosphorylation at the DSB. The resulting site-specific autophosphorylation induces a large conformational change that opens DNA-PKcs and promotes its release from DNA ends. These results show how protein and DNA interactions initiate large Ku and DNA-PKcs rearrangements to control DNA-PK biological functions as a macromolecular machine orchestrating assembly and disassembly of the initial NHEJ complex on DNA.

Sato A, Isaac B, Phillips CM, Rillo R, Carlton PM, Wynne DJ, Kasad RA, Dernburg AF. Cytoskeletal forces span the nuclear envelope to coordinate meiotic chromosome pairing and synapsis. *Cell*, 2009 Nov 11. [Epub ahead of print] PMID: 19913287

During meiosis, each chromosome must pair with its unique homologous partner, a process that usually culminates with the formation of the synaptonemal complex (SC). In the nematode *Caenorhabditis elegans*, special regions on each chromosome known as pairing centers are essential for both homologous pairing and synapsis. We report that during early meiosis, pairing centers establish transient connections to the cytoplasmic microtubule network. These connections through the intact nuclear envelope require the SUN/KASH domain protein pair SUN-1 and ZYG-12. Disruption of microtubules inhibits chromosome pairing, indicating that these connections promote interhomolog interactions. Dynein activity is essential to license formation of the SC once pairing has been accomplished, most likely by overcoming a barrier imposed by the chromosome-nuclear envelope connection. Our findings thus provide insight into how homolog pairing is accomplished in meiosis and into the mechanisms regulating synapsis so that it occurs selectively between homologs.

Schild D, Wiese C. Overexpression of RAD51 suppresses recombination defects: a possible mechanism to reverse genomic instability. *Nucleic Acids Research*, 2009 Nov 26. [Epub ahead of print] PMID: 19942681

RAD51, a key protein in the homologous recombinational DNA repair (HRR) pathway, is the major strand-transferase required for mitotic recombination. An important early step in HRR is the formation of single-stranded DNA (ss-DNA) coated by RPA (a ss-DNA-binding protein). Displacement of RPA by RAD51 is highly regulated and facilitated by a number of different proteins known as the 'recombination mediators'. To assist these recombination mediators, a second group of proteins also is required and we are defining these proteins here as 'recombination co-mediators'. Defects in either recombination mediators or co-mediators, including BRCA1 and BRCA2, lead to impaired HRR that can genetically be complemented for (i.e. suppressed) by overexpression of RAD51. Defects in HRR have long been known to contribute to genomic instability leading to tumor development. Since genomic instability also slows cell growth, precancerous cells presumably require genomic re-stabilization to gain a growth advantage. RAD51 is overexpressed in many tumors, and therefore, we hypothesize that the complementing ability of elevated levels of RAD51 in tumors with initial HRR defects limits genomic instability during carcinogenic progression. Of particular interest, this model may also help explain the high frequency of TP53 mutations in human cancers, since wild-type p53 represses RAD51 expression.

Fang Q, Kanugula S, Tubbs JL, **Tainer JA**, Pegg AE. Repair of O4-alkylthymine by O6-alkylguanine-DNA alkyltransferases. *The Journal of Biological Chemistry*, 2009 Dec 21. [Epub ahead of print] PMID: 20026607

O6-Alkylguanine-DNA alkyltransferase (AGT) plays a major role in repair of the cytotoxic and mutagenic lesion O6-methylguanine (m6G) in DNA. Unlike the *Escherichia coli* alkyltransferase Ogt that also repairs O4-methylthymine (m4T) efficiently, the human AGT (hAGT) acts poorly on m4T. Here we made several hAGT mutants in which residues near the cysteine acceptor site were replaced by corresponding residues from Ogt to investigate the basis for the efficiency of hAGT in repair of m4T. Construct hAGT-03 (where hAGT sequence V149CSSGAVGN157- was replaced with the corresponding Ogt I143GRNGTMTG151-), exhibited enhanced m4T repair activity in vitro compared to hAGT. Three AGT proteins (hAGT, hAGT-03 and Ogt) exhibited similar protection from killing by N-methyl-N'-nitro-N-nitrosoguanidine and caused a reduction in m6G-induced G:C to A:T mutations in both nucleotide excision repair (NER) proficient and deficient *E. coli* strains that lack endogenous AGTs. hAGT-03 resembled Ogt in totally reducing the m4T-induced T:A to C:G mutations in NER proficient and deficient strains. Surprisingly, wild-type hAGT expression caused a significant but incomplete decrease in NER deficient strains but a slight increase in T:A to C:G mutation frequency in NER proficient strains. The T:A to C:G mutations due to O4-alkylthymine formed by ethylating and propylating agents were also efficiently reduced by either hAGT-03 or Ogt while hAGT had little effect irrespective of NER status. These results show that specific alterations in the hAGT active site facilitate efficient recognition and repair of O4-alkylthymines and reveal damage-dependent interactions of base and nucleotide excision repair.

Savarese F, Dávila A, Nechanitzky R, De La Rosa-Velazquez I, Pereira CF, Engelke R, **Takahashi K**, Jenuwein T, **Kohwi-Shigematsu T**, Fisher AG, Grosschedl R. Satb1 and Satb2 regulate embryonic stem cell differentiation and Nanog expression. *Genes & Development*, 2009 Nov 15;23(22):2625-38. PMID: 19933152

Satb1 and the closely related Satb2 proteins regulate gene expression and higher-order chromatin structure of multigene clusters in vivo. In examining the role of Satb proteins in murine embryonic stem (ES) cells, we find that Satb1(-/-) cells display an impaired differentiation potential and augmented

expression of the pluripotency determinants Nanog, Klf4, and Tbx3. Metastable states of self-renewal and differentiation competence have been attributed to heterogeneity of ES cells in the expression of Nanog. Satb1(-/-) cultures have a higher proportion of Nanog(high) cells, and an increased potential to reprogram human B lymphocytes in cell fusion experiments. Moreover, Satb1-deficient ES cells show an increased expression of Satb2, and we find that forced Satb2 expression in wild-type ES cells antagonizes differentiation-associated silencing of Nanog and enhances the induction of NANOG in cell fusions with human B lymphocytes. An antagonistic function of Satb1 and Satb2 is also supported by the almost normal differentiation potential of Satb1(-/-)Satb2(-/-) ES cells. Taken together with the finding that both Satb1 and Satb2 bind the Nanog locus in vivo, our data suggest that the balance of Satb1 and Satb2 contributes to the plasticity of Nanog expression and ES cell pluripotency.

Thrash JC, Pollock J, **Torok T**, Coates JD. Description of the novel perchlorate-reducing bacteria *Dechlorobacter hydrogenophilus* gen. nov., sp. nov. and *Propionivibrio militaris*, sp. nov. *Applied Microbiology and Biotechnology*, 2009 Nov 18. [Epub ahead of print] PMID: 19921177

Novel dissimilatory perchlorate-reducing bacteria (DPRB) were isolated from enrichments conducted under conditions different from those of all previously described DPRB. Strain LT-1(T) was enriched using medium buffered at pH 6.6 with 2-(N-morpholino)ethanesulfonic acid (MES) and had only 95% 16S rRNA gene identity with its closest relative, *Azonexus caeni*. Strain MP(T) was enriched in the cathodic chamber of a perchlorate-reducing bioelectrical reactor (BER) and together with an additional strain, CR (99% 16S rRNA gene identity), had 97% 16S rRNA gene identity with *Propionivibrio limicola*. The use of perchlorate and other electron acceptors distinguished strains MP(T) and CR from *P. limicola* physiologically. Strain LT-1(T) had differences in electron donor utilization and optimum growth temperatures from *A. caeni*. Strains LT-1(T) and MP(T) are the first DPRB to be described in the Betaproteobacteria outside of the *Dechloromonas* and *Azospira* genera. On the basis of phylogenetic and physiological features, strain LT-1(T) represents a novel genus in the Rhodocyclaceae; strain MP(T) represents a novel species within the genus *Propionivibrio*. The names *Dechlorobacter hydrogenophilus* gen. nov., sp. nov. and *Propionivibrio militaris* sp. nov. are proposed.

Wang HW, Noland C, Siridechadilok B, Taylor DW, Ma E, Felderer K, Doudna JA, **Nogales E**. Structural insights into RNA processing by the human RISC-loading complex. *Nature Structural & Molecular Biology*, 2009 Nov;16(11):1148-53. PMID: 19820710

Targeted gene silencing by RNA interference (RNAi) requires loading of a short guide RNA (small interfering RNA (siRNA) or microRNA (miRNA)) onto an Argonaute protein to form the functional center of an RNA-induced silencing complex (RISC). In humans, Argonaute2 (AGO2) assembles with the guide RNA-generating enzyme Dicer and the RNA-binding protein TRBP to form a RISC-loading complex (RLC), which is necessary for efficient transfer of nascent siRNAs and miRNAs from Dicer to AGO2. Here, using single-particle EM analysis, we show that human Dicer has an L-shaped structure. The RLC Dicer's N-terminal DExH/D domain, located in a short 'base branch', interacts with TRBP, whereas its C-terminal catalytic domains in the main body are proximal to AGO2. A model generated by docking the available atomic structures of Dicer and Argonaute homologs into the RLC reconstruction suggests a mechanism for siRNA transfer from Dicer to AGO2.

Wu DJ, **Wang NJ**, Driscoll J, Dorrani N, Liu D, Sigman M, Schanen NC. Autistic disorder associated with a paternally derived unbalanced translocation leading to duplication of chromosome 15pter-q13.2: a case report. *Molecular Cytogenetics*, 2009 Dec 18;2(1):27. [Epub ahead of print] PMID: 20021661

ABSTRACT: Autism spectrum disorders have been associated with maternally derived duplications that involve the imprinted region on the proximal long arm of chromosome 15. Here

we describe a boy with a chromosome 15 duplication arising from a 3:1 segregation error of a paternally derived translocation between chromosome 15q13.2 and chromosome 9q34.12, which led to trisomy of chromosome 15pter-q13.2 and 9q34.12-qter. Using array comparative genome hybridization, we localized the breakpoints on both chromosomes and sequence homology suggests that the translocation arose from non-allelic homologous recombination involving the low copy repeats on chromosome 15. The child manifests many characteristics of the maternally-derived duplication chromosome 15 phenotype including developmental delays with cognitive impairment, autism, hypotonia and facial dysmorphisms with nominal overlap of the most general symptoms found in duplications of chromosome 9q34. This case suggests that biallelically expressed genes on proximal 15q contribute to the idic(15) autism phenotype.

Washington NL, Haendel MA, Mungall CJ, Ashburner M, Westerfield M, Lewis SE. Linking human diseases to animal models using ontology-based phenotype annotation. *PLoS Biology*, 2009 Nov;7(11):e1000247. PMID: 19956802

Scientists and clinicians who study genetic alterations and disease have traditionally described phenotypes in natural language. The considerable variation in these free-text descriptions has posed a hindrance to the important task of identifying candidate genes and models for human diseases and indicates the need for a computationally tractable method to mine data resources for mutant phenotypes. In this study, we tested the hypothesis that ontological annotation of disease phenotypes will facilitate the discovery of new genotype-phenotype relationships within and across species. To describe phenotypes using ontologies, we used an Entity-Quality (EQ) methodology, wherein the affected entity (E) and how it is affected (Q) are recorded using terms from a variety of ontologies. Using this EQ method, we annotated the phenotypes of 11 gene-linked human diseases described in Online Mendelian Inheritance in Man (OMIM). These human annotations were loaded into our Ontology-Based Database (OBD) along with other ontology-based phenotype descriptions of mutants from various model organism databases. Phenotypes recorded with this EQ method can be computationally compared based on the hierarchy of terms in the ontologies and the frequency of annotation. We utilized four similarity metrics to compare phenotypes and developed an ontology of homologous and analogous anatomical structures to compare phenotypes between species. Using these tools, we demonstrate that we can identify, through the similarity of the recorded phenotypes, other alleles of the same gene, other members of a signaling pathway, and orthologous genes and pathway members across species. We conclude that EQ-based annotation of phenotypes, in conjunction with a cross-species ontology, and a variety of similarity metrics can identify biologically meaningful similarities between genes by comparing phenotypes alone. This annotation and search method provides a novel and efficient means to identify gene candidates and animal models of human disease, which may shorten the lengthy path to identification and understanding of the genetic basis of human disease.

Kwan J, Baumgartner A, Lu CM, Wang M, Weier JF, Zitzelsberger HF, Weier HU. BAC-FISH assays delineate complex chromosomal rearrangements in a case of post-Chernobyl childhood thyroid cancer. *Folia histochemica et cytobiologica / Polish Academy of Sciences, Polish Histochemical and Cytochemical Society*, 2009;47(2):135-42. PMID: 19995698

Structural chromosome aberrations are known hallmarks of many solid tumors. In the papillary form of thyroid cancer (PTC), for example, activation of the receptor tyrosine kinase (RTK) genes, RET and neurotrophic tyrosine kinase receptor type I (NTRK1) by intra- and interchromosomal rearrangements has been suggested as a cause of the disease. However, many phenotypically similar tumors do not carry an activated RET or NTRK-1 gene or express abnormal ret or NTRK-1 transcripts. Thus, we hypothesize that other cellular RTK-type genes are aberrantly expressed in these tumors. Using fluorescence in situ

hybridization-based methods, we are studying karyotype changes in a relatively rare subgroup of PTCs, i.e., tumors that arose in children following the 1986 nuclear accident in Chernobyl, Ukraine. Here, we report our technical developments and progress in deciphering complex chromosome aberrations in case S48TK, an aggressively growing PTC cell line, which shows an unusual high number of unbalanced translocations.