



LIFE SCIENCES DIVISION E-NEWSLETTER

January, 2009

In this issue:

- **DOE Scientific Focus Area Notes**
 - **Low Dose Radiation Research - International Meeting on Carcinogenesis and Low Dose Radiation Exposure** 2
 - **GTL-Genomics - Parvin's Work Presented** 3
 - **Nuclear Medicine - Funding for New Research Plans; Crystal Growth Facility** 3

- **Scientific & Division News**
 - **Cover Article: MEK Inhibitors Hold Promise for Breast Cancer Treatment** 4
 - **Cover Article: Chromosomal Mosaicism in Mouse Two-cell Embryos** 4
 - **Gazing Deeper into Cancer: TCGA Study Analyzed** 5
 - **Scientists Link Technologies to Locate Stem Cell Reservoirs in Tissue** 5
 - **Johansen Outgoing Staff Advisor to the Regents** 6

- **Awards**
 - **Torok to Receive Mentor Award** 6

- **Recent Publications** 6

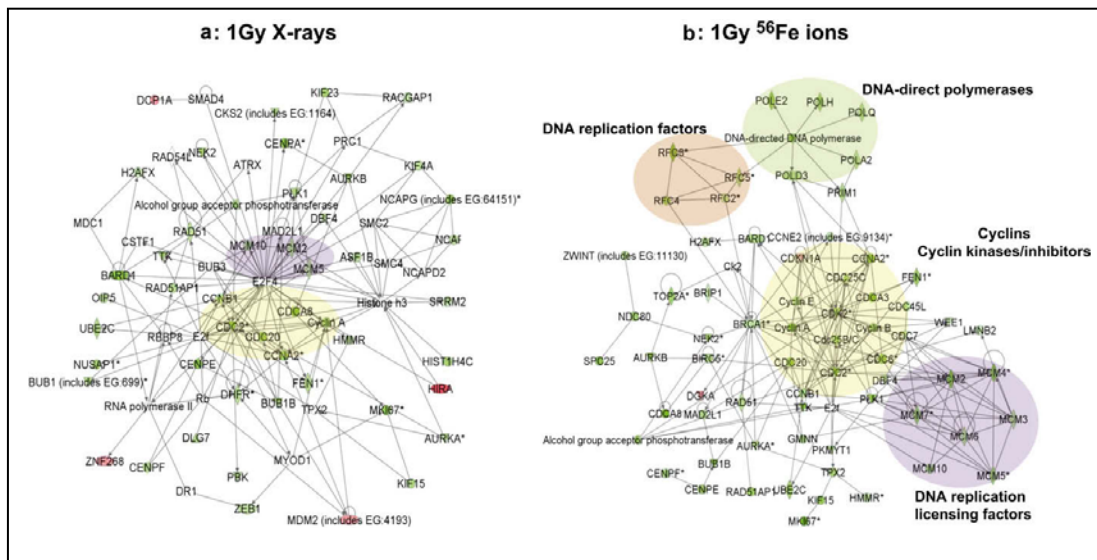
DOE scientific focus area notes

Low Dose Radiation Research

Differential Effects of X-Rays and High-energy 56Fe Ions on Human Mesenchymal Stem Cells

A study of Life Sciences **Doajing Wang, Sanchita Bhattacharya, Andrew Wyrobek, and Bjorn Rydberg** shows both phenotypically and mechanistically, the differential effects of X-rays and high-energy 56Fe ions on hMSC. These results will assist the Department of Energy with risk estimations for low-dose radiation, and the National Aeronautics and Space Administration with HZE risk estimations and countermeasures for space exploration. Their work, supported by the DOE Low Dose program jointly with NASA, was published in the January issue of the *International Journal of Radiation Oncology, Biology, Physics*.

From the authors: Stem cells hold great potential for regenerative medicine, but they have also been implicated in cancer and aging. How different kinds of ionizing radiation affect stem cell biology remains unexplored. This study was designed to compare the biological effects of X-rays and of high-linear energy transfer (LET) 56Fe ions on human mesenchymal stem cells (hMSC). Methods and Materials: A multi-functional comparison was carried out to investigate the differential effects of X-rays and 56Fe ions on hMSC. The end points included modulation of key markers such as p53, cell cycle progression, osteogenic differentiation, and pathway and networks through transcriptomic profiling and bioinformatics analysis. Results: X-rays and 56Fe ions differentially inhibited the cell cycle progression of hMSC in a p53-dependent manner without impairing their in vitro osteogenic differentiation process. Pathway and network analyses revealed that cytoskeleton and receptor signaling were uniquely enriched for low-dose (0.1 Gy) X-rays. In contrast, DNA/RNA metabolism and cell cycle regulation were enriched for high-dose (1 Gy) X-rays and 56Fe ions, with more significant effects from 56Fe ions. Specifically, DNA replication, DNA strand elongation, and DNA binding/transferase activity were perturbed more severely by 1 Gy 56Fe ions than by 1 Gy X-rays, consistent with the significant G2/M arrest for the former while not for the latter. Conclusions: 56Fe ions exert more significant effects on hMSC than X-rays. Since hMSC are the progenitors of osteoblasts in vivo, this study provides new mechanistic understandings of the relative health risks associated with low- and high-dose X-rays and high-LET space radiation.



Kurpinski K, Jang DJ, Bhattacharya S, Rydberg B, Chu J, So J, Wyrobek A, Li S, Wang D. Differential effects of x-rays and high-energy (56)Fe Ions on human mesenchymal stem cells. *International Journal of Radiation Oncology, Biology, Physics*, 2008 Dec 18. [Epub ahead of print]PMID: 19101095

Daojing Wang, 1/09

GTL-Genomics

Parvin's Work Presented

Life Sciences Bahram Parvin gave an invited talk on "High content screening of multicellular systems" at the High Content Analysis Conference, which was sponsored by the Cambridge Health Institute, in San Francisco, CA. Current high content screening systems are limited to 2D cell culture models, which may not faithfully represent in vivo models. Parvin's group has developed image analysis technologies to quantify the structure and organization of 3D cell culture models at multiple endpoints through a multivariate representation so that (i) these endpoints can be compared with the classical monolayer system, and (ii) subpopulations with distinct morphological signatures can be discovered. Subsequently, computed phenotypic endpoints or subpopulations can be associated with molecular features for generating new hypotheses. These can be used, for example, to identify sub-populations of cells that are particularly sensitive or resistant to drugs under test in order to reach a better understanding of conditions where specific drugs will be effective.

Bahram Parvin, 1/09

Nuclear Medicine

Funding for New Research Plans

The Department of Energy Office of Biological and Environmental Research (DOE BER) held workshops in November 2008 and January 2009, focusing on how the radiotracers and dynamical imaging systems developed by the Department of Energy for Nuclear Medical Imaging can benefit the study of plant physiology for the production of biofuels, and the transport of contaminants in the environment. Members of the Life Sciences Division Department of Radiotracer Development and Imaging Technology participated in these workshops and developed new research plans that were outlined in a brief proposal that was submitted to BER. The plans' primary goal is to improve the technical capability of researchers at Berkeley Lab and nearby University of California campuses who are working in the areas of biofuels and environmental remediation. The proposal was accepted, resulting in 2009 funding and an invitation to develop a full proposal for future years.

Stephen Derenzo, 1/09

Crystal Growth Facility Now Operational

The Berkeley Lab crystal growth facility has just been made operational with support from Berkeley Lab, the DOE Office of Non-Proliferation, the DOE Office of Science, and the DHS Domestic Nuclear Detection Office. The first crystals to be grown include new superconductors and new scintillator radiation detectors.

Stephen Derenzo, 1/09

Scientific & division news

Cover Article: MEK Inhibitors Hold Promise for Breast Cancer Treatment

MEK enzyme mutations are players in cancer cell proliferation and metastasis; drugs that inhibit MEK are a promising treatment for cancers including breast cancer. A team led by Michael Korn of UC San Francisco and including **Debopriya Das, Laura Heiser, Sanchita Bhattacharya, Nora Bayani, Nicholas Wang, Richard Neve, Yinghui Guan, Zhi Hu, Heidi Feiler, Philippe Gascard, Bahram Parvin, Paul Spellman, Andrew Wyrobek, Mina Bissell, Wen-Lin Kuo, and Joe Gray** of Berkeley Lab's Life Sciences Division have found that basal-type breast cancer cells are particularly susceptible to some MEK inhibitors. Their results, the cover story of the January 15 *Cancer Research*, suggest how clinical trials of these drugs can benefit from better patient selection and a rational combination of therapies. More>

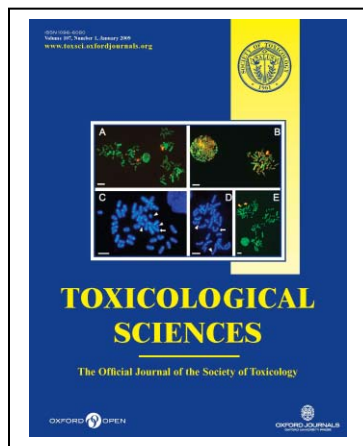
http://www.lbl.gov/publicinfo/newscenter/tab/2009/january/01-16-09/Korn_Can_Res_2009.pdf



Mirzoeva OK, Das D, Heiser LM, Bhattacharya S, Siwak D, Gendelman R, Bayani N, Wang NJ, Neve RM, Guan Y, Hu Z, Knight Z, Feiler HS, Gascard P, Parvin B, Spellman PT, Shokat KM, Wyrobek AJ, Bissell MJ, McCormick F, Kuo WL, Mills GB, Gray JW, Korn WM. Basal subtype and MAPK/ERK kinase (MEK)-phosphoinositide 3-kinase feedback signaling determine susceptibility of breast cancer cells to MEK inhibition. *Cancer Research*, 2009 Jan 15;69(2):565-72. PMID: 19147570
Today at Berkeley Lab, 1/16/09

Cover Article: Chromosomal Mosaicism in Mouse Two-cell Embryos after Paternal Exposure to Acrylamide

In the cover article of the January *Toxicological Sciences*, Life Sciences **Francesco Marchetti, Andrew Wyrobek**, et al. summarize their findings on the high incidence of chromosomal mosaicism observed in two-cell mouse embryos after paternal treatment with acrylamide.



From the authors: Chromosomal mosaicism in human preimplantation embryos is a common cause of spontaneous abortions, however, our knowledge of its etiology is limited. We used multicolor fluorescence in situ hybridization painting to investigate whether paternally transmitted chromosomal aberrations result in mosaicism in mouse two-cell embryos. Paternal exposure to acrylamide, an important industrial chemical also found in tobacco smoke and generated during the cooking process of starchy foods, produced significant increases in chromosomally defective two-cell embryos, however, the effects were transient primarily affecting the postmeiotic stages of spermatogenesis. Comparisons with our previous study of zygotes demonstrated similar frequencies of chromosomally abnormal zygotes and two-cell embryos

suggesting that there was no apparent selection against numerical or structural chromosomal aberrations. However, the majority of affected two-cell embryos were mosaics showing different chromosomal abnormalities in the two blastomeric metaphases. Analyses of chromosomal aberrations in zygotes and two-cell embryos showed a tendency for loss of acentric fragments during the first mitotic division of embryogenesis, whereas both dicentrics and translocations apparently underwent proper segregation. These results suggest that embryonic development can proceed up to the end of the second cell cycle of development in the presence of abnormal paternal chromosomes and that even dicentrics can persist through cell division. The high incidence of chromosomally mosaic two-cell embryos suggests that the first mitotic division of embryogenesis is prone to missegregation errors and that paternally transmitted chromosomal abnormalities increase the risk of missegregation leading to embryonic mosaicism.

Marchetti F, Bishop J, Lowe X, Wyrobek AJ. Chromosomal mosaicism in mouse two-cell embryos after paternal exposure to acrylamide. *Toxicological Sciences: an official journal of the Society of Toxicology*, 2009 Jan;107(1):194-205. Epub 2008 Oct 16. PMID: 18930949
CG, 1/09

Gazing Deeper into Cancer: TCGA Study Analyzed

The January 8 issue of *SciBX*, a publishing collaboration between BioCentury Publications, Inc. and Nature Publishing Group, included the article "Gazing Deeper into Cancer" on three important recent genomic studies that reveal "potential new drug targets and provide a next step toward personalized, whole genome-based cancer diagnostics. Genome sequencing could provide more robust data than existing cancer tests, which sample only a handful of the genes that could be mutated". One of the studies analyzed in this article is the glioblastoma multiforme study report of the NIH/NCI-NHGRI consortium Cancer Genome Atlas Research Network (TCGA) in which the Berkeley Cancer Genome Center, led by **Paul Spellman** and **Joe Gray**, played a major role. Joe Gray, one of the authors of "The Cancer Genome Atlas (TCGA) Research Network" study published in the October 2008 issue of *Nature*, was interviewed for and quoted in the *SciBX* article, available for *Nature* subscribers at:

<http://www.nature.com/scibx/journal/v2/n1/full/scibx.2009.3.html>

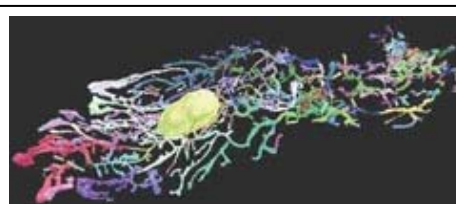
CG, 1/09

Scientists Link Technologies to Locate Stem Cell Reservoirs in Tissue

[*Chemistry World*] During pregnancy the mammary gland goes through changes which suggest the tissue contains a reservoir of undifferentiated cells — cells that can convert into more specialized types. The researchers — led by Berkeley Lab life scientist **Mary Helen Barcellos-Hoff** — built a computational microscopy platform that will allow them to understand how cells are affected by different microenvironments within the same tissue.

More>http://www.rsc.org/Publishing/Journals/cb/Volume/2009/2/searching_for_stem_cells.asp

Today at Berkeley Lab, 1/12/09



Label retaining cells form small, non-random clusters in large mammary ducts

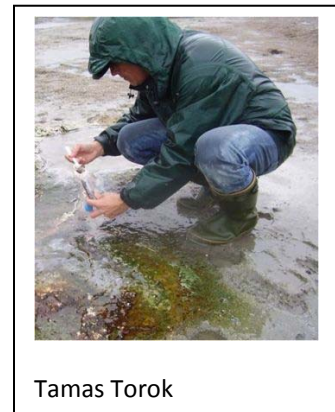
Johansen Outgoing Staff Advisor to the Regents

Berkeley Lab staff have been invited to apply for the position of Staff Advisor Designate to The UC Regents. The outgoing Staff Advisor is **Bill Johansen**, Sr. Business Manager of the Life Sciences Division, whose one year term will end on June 30, 2009. The position helps foster two-way communication between the Regents and UC staff. Staff Advisors serve as non-voting advisors to designated Regents' committees and participate in committee and board meetings throughout their term of service.
Today at Berkeley Lab, CG, 1/16/09

Awards

Torok to Receive Mentor Award from Biotech Partners

Life Sciences **Tamas Torok** will be awarded a Biotech Partners Proven Power Award next month for his mentor work. The award, which he will receive at the Biotech Partners 15th Anniversary Benefit Gala, is given to "dedicated scientists who by sharing their wisdom and passion bring to life the dream of a biotech career." The gala will take place in the Exploratorium in San Francisco on February 19, 2009. Biotech Partners' mission is to prepare high school and community college students - particularly female and minority youths from low-income or other disadvantaged families - for careers in biotechnology. The program combines a specialized biotech curriculum with paid internships and comprehensive student support. Torok has been working with Biotech Partners (formerly Berkeley Biotechnology Education, Inc.) for 13 years.
CG, 1/09



Recent publications (selected)

Shawkey MD, Saranathan V, Pálsdóttir H, Crum J, Ellisman MH, **Auer M**, Prum RO. Electron tomography, three-dimensional Fourier analysis and colour prediction of a three-dimensional amorphous biophotonic nanostructure. *Journal of the Royal Society, Interface*, 2009 Jan 20. [Epub ahead of print] PMID: 19158016

Organismal colour can be created by selective absorption of light by pigments or light scattering by photonic nanostructures. Photonic nanostructures may vary in refractive index over one, two or three dimensions and may be periodic over large spatial scales or amorphous with short-range order. Theoretical optical analysis of three-dimensional amorphous nanostructures has been challenging because these structures are difficult to describe accurately from conventional two-dimensional electron microscopy alone. Intermediate voltage electron microscopy (IVEM) with tomographic reconstruction adds three-dimensional data by using a high-power electron beam to penetrate and image sections of material sufficiently thick to contain a significant portion of the structure. Here, we use IVEM tomography to characterize a non-iridescent, three-dimensional biophotonic nanostructure: the spongy medullary layer from eastern bluebird *Sialia sialis* feather barbs. Tomography and three-dimensional Fourier analysis

reveal that it is an amorphous, interconnected bicontinuous matrix that is appropriately ordered at local spatial scales in all three dimensions to coherently scatter light. The predicted reflectance spectra from the three-dimensional Fourier analysis are more precise than those predicted by previous two-dimensional Fourier analysis of transmission electron microscopy sections. These results highlight the usefulness, and obstacles, of tomography in the description and analysis of three-dimensional photonic structures.

Kurpinski K, Jang DJ, **Bhattacharya S**, **Rydberg B**, Chu J, So J, **Wyrobek A**, Li S, **Wang D**. Differential effects of x-rays and high-energy (56)Fe Ions on human mesenchymal stem cells. *International Journal of Radiation Oncology, Biology, Physics*, 2008 Dec 18. [Epub ahead of print] PMID: 19101095

(See Scientific News, page 2)

Rønnov-Jessen L, **Bissell MJ**. Breast cancer by proxy: can the microenvironment be both the cause and consequence? *Trends in Molecular Medicine*, 2009 Jan;15(1):5-13. Epub 2008 Dec 16. PMID: 19091631

Breast cancer is one of the most clear-cut examples of a solid tumor in which systemic cues play a decisive part in its development. The breast tissue is constantly subjected to changes in hormone levels and modifications in the microenvironment. This scenario is even more striking during tumor development because of the dramatic loss or aberration of basement membrane (BM) and myoepithelial cells and the gain of peritumoral myofibroblasts. We suggest that the microenvironment, defined here as all components of the mammary gland other than luminal and/or tumor epithelial cells, might be instrumental in maintaining organ integrity and in promoting, and at times even initiating, breast cancer development. As such, the tumor microenvironment and its constituents, alone or in combination, might serve as promising targets for therapy.

Chen CS, Nelson CM, Khauv D, Bennett S, Radisky ES, Hirai Y, **Bissell MJ**, **Radisky DC**. Homology with vesicle fusion mediator syntaxin-1a predicts determinants of epimorphin/syntaxin-2 function in mammary epithelial morphogenesis. *The Journal of Biological Chemistry*, 2009 Jan 12. [Epub ahead of print] PMID: 19129200

We have shown that branching morphogenesis of mammary ductal structures requires the action of the morphogen epimorphin/syntaxin-2. Epimorphin, originally identified as an extracellular molecule, is identical to syntaxin-2, an intracellular molecule that is a member of the extensively investigated syntaxin family of proteins that mediate vesicle trafficking. We show here that although epimorphin/syntaxin-2 is highly homologous to syntaxin-1a, only epimorphin/syntaxin-2 can stimulate mammary branching morphogenesis. We construct a homology model of epimorphin/syntaxin-2 based on the published structure of syntaxin-1a, and we use this model to identify the structural motif responsible for the morphogenic activity. We identify four residues located within the cleft between helices B and C that differ between syntaxin-1a and epimorphin/syntaxin-2; through site-directed mutagenesis of these four amino acids, we confer the properties of epimorphin for cell adhesion, gene activation, and branching morphogenesis onto the inactive syntaxin-1a template. These results provide a dramatic demonstration of the use of structural information about one molecule to define a functional motif of a second molecule that is related at the sequence level but highly divergent functionally.

Xu R, **Boudreau A**, **Bissell MJ**. Tissue architecture and function: dynamic reciprocity via extra- and intra-cellular matrices. *Cancer Metastasis Reviews*, 2009 Jan 23. [Epub ahead of print] PMID: 19160017

Mammary gland development, functional differentiation, and homeostasis are orchestrated and sustained by a balance of biochemical and biophysical cues from the organ's microenvironment. The three-dimensional microenvironment of the mammary gland, predominantly 'encoded' by a collaboration between the extracellular matrix (ECM), hormones, and growth factors, sends signals from ECM receptors through the cytoskeletal intracellular matrix to nuclear and chromatin structures resulting in gene expression; the ECM in turn is regulated and remodeled by signals from the nucleus. In this chapter, we discuss how coordinated ECM deposition and remodeling is necessary for mammary gland development, how the ECM provides structural and biochemical cues necessary for tissue-specific function, and the role of the cytoskeleton in mediating the extra-to intracellular dialogue occurring between the nucleus and the microenvironment. When operating normally, the cytoskeletal-mediated dynamic and reciprocal integration of tissue architecture and function directs mammary gland development, tissue polarity, and ultimately, tissue-specific gene expression. Cancer occurs when these dynamic interactions go awry for an extended time.

Braskie MN, Wilcox CE, Landau SM, O'Neil JP, Baker SL, Madison CM, Kluth JT, Jagust WJ. Relationship of striatal dopamine synthesis capacity to age and cognition. *Journal of Neuroscience*, 2008 Dec 24;28(52):14320-8. PMID: 19109513

Past research has demonstrated that performance on frontal lobe-dependent tasks is associated with dopamine system integrity and that various dopamine system deficits occur with aging. The positron emission tomography (PET) radiotracer 6-[[18F]fluoro-L-m-tyrosine (FMT) is a substrate of the dopamine-synthesizing enzyme, aromatic amino acid decarboxylase (AADC). Studies using 6-[[18F]fluorodopa (FDOPA) (another AADC substrate) to measure how striatal PET signal and age relate have had inconsistent outcomes. The varying results occur in part from tracer processing that renders FDOPA signal subject to aspects of postrelease metabolism, which may themselves change with aging. In contrast, FMT remains a purer measure of AADC function. We used partial volume-corrected FMT PET scans to measure age-related striatal dopamine synthesis capacity in 21 older (mean, 66.9) and 16 younger (mean, 22.8) healthy adults. We also investigated how striatal FMT signal related to a cognitive measure of frontal lobe function. Older adults showed significantly greater striatal FMT signal than younger adults. Within the older group, FMT signal in dorsal caudate (DCA) and dorsal putamen was greater with age, suggesting compensation for deficits elsewhere in the dopamine system. In younger adults, FMT signal in DCA was lower with age, likely related to ongoing developmental processes. Younger adults who performed worse on tests of frontal lobe function showed greater FMT signal in right DCA, independent of age effects. Our data suggest that higher striatal FMT signal represents nonoptimal dopamine processing. They further support a relationship between striatal dopamine processing and frontal lobe cognitive function.

Lüke I, **Butland G**, Moore K, Buchanan G, Lyall V, Fairhurst SA, Greenblatt JF, Emili A, Palmer T, Sargent F. Biosynthesis of the respiratory formate dehydrogenases from *Escherichia coli*: characterization of the FdhE protein. *Archives of Microbiology*, 2008 Dec;190(6):685-96. Epub 2008 Aug 21. PMID: 18716757

Escherichia coli can perform two modes of formate metabolism. Under respiratory conditions, two periplasmically-located formate dehydrogenase isoenzymes couple formate oxidation to the generation of a transmembrane electrochemical gradient; and under fermentative conditions a third cytoplasmic isoenzyme is involved in the disproportionation of formate to CO₂ and H₂. The respiratory formate dehydrogenases are redox enzymes that comprise three subunits: a molybdenum cofactor- and FeS cluster-containing catalytic subunit; an electron-transferring ferredoxin; and a membrane-integral cytochrome b. The catalytic subunit and its ferredoxin partner are targeted to the periplasm as a complex by the twin-arginine transport (Tat) pathway. Biosynthesis of these enzymes is under control of an accessory protein termed FdhE. In this study, it is shown that *E. coli* FdhE interacts with the catalytic subunits of the respiratory formate dehydrogenases. Purification of recombinant FdhE demonstrates the

protein is an iron-binding rubredoxin that can adopt monomeric and homodimeric forms. Bacterial two-hybrid analysis suggests the homodimer form of FdhE is stabilized by anaerobiosis. Site-directed mutagenesis shows that conserved cysteine motifs are essential for the physiological activity of the FdhE protein and are also involved in iron ligation.

Zhou L, Gao C, Zhu D, Xu W, **Chen FF**, Palkar A, Echegoyen L, Kong ES. Facile functionalization of multilayer fullerenes (Carbon Nano-Onions) by nitrene chemistry and "grafting from" strategy. *Chemistry*, 2009;15(6):1389-96. PMID: 19115308

Facile functionalization of multilayer fullerenes (carbon nano-onions, CNOs) was carried out by [2+1] cycloaddition of nitrenes. The products were further derivatized by using the "grafting from" strategy of in situ ring-opening polymerization (ROP) and atom transfer radical polymerization (ATRP). Using one-step nitrene chemistry with high-energy reagents, such as azidoethanol and azidoethyl 2-bromo-2-methyl propanoate, in N-methyl-2-pyrrolidone at 160 degrees C for 16 h, hydroxyl and bromide functionalities were introduced onto the surfaces of CNOs. These hydroxyl CNOs (CNO-OH) and bromic CNOs (CNO-Br) were extensively characterized by various techniques such as thermal gravimetric analysis (TGA), transmission electron microscopy (TEM), Raman spectroscopy and X-ray photo electron spectroscopy (XPS). TGA measurements indicated that the surface hydroxyl and bromide group density reached 1.49 and 0.49 mmol g⁻¹, respectively. The as-functionalized CNOs showed much better solubility in solvents than pristine CNOs. The CNO-OH were also observed to fluoresce at $\lambda=453$ nm in water. The CNO-OH and CNO-Br can be conveniently utilized as macroinitiators to conduct surface-initiated in-situ polymerizations. Poly(epsilon-caprolactone) (PCL, 45wt %) and polystyrene (PS, 60 wt%) were then grafted from surfaces of CNOs through the ROP of epsilon-caprolactone with the macroinitiator CNO-OH and the ATRP of styrene with the macroinitiator CNO-Br, respectively. The structures and morphology of the resulting products were characterized by ¹H NMR, scanning electron microscopy (SEM), TEM, and atomic force microscopy (AFM). The polymer functionalized CNOs have good solubility/dispersibility in common organic solvents. The facile and scalable functionalization approaches can pave the way for the comprehensive investigation of chemistry of CNOs and fabrication of novel CNO-based nanomaterials and nanodevices.

Mirzoeva OK, **Das D**, **Heiser LM**, **Bhattacharya S**, Siwak D, Gendelman R, **Bayani N**, **Wang NJ**, Neve RM, Guan Y, **Hu Z**, Knight Z, **Feiler HS**, Gascard P, **Parvin B**, **Spellman PT**, Shokat KM, **Wyrobek AJ**, **Bissell MJ**, McCormick F, **Kuo WL**, Mills GB, **Gray JW**, Korn WM. Basal subtype and MAPK/ERK kinase (MEK)-phosphoinositide 3-kinase feedback signaling determine susceptibility of breast cancer cells to MEK inhibition. *Cancer Research*, 2009 Jan 15;69(2):565-72. PMID: 19147570

(See Scientific News, page 4)

Kotwaliwale CV, **Dernburg AF**. Old nuclei spring new leaks. *Cell*, 2009 Jan 23;136(2):211-2. PMID: 19167324

The nuclear pore complex (NPC) regulates the bidirectional movement of cell components across the nuclear envelope. In this issue, D'Angelo et al. (2009) demonstrate that the NPC loses essential protein subunits as cells age, resulting in increased nuclear permeability and potentially contributing to organismal aging.

Danev R, **Glaeser RM**, Nagayama K. Practical factors affecting the performance of a thin-film phase plate for transmission electron microscopy. *Ultramicroscopy*, 2008 Dec 11. [Epub ahead of print] PMID: 19157711

A number of practical issues must be addressed when using thin carbon films as quarter-wave plates for Zernike phase-contrast electron microscopy. We describe, for example, how we meet the more stringent requirements that must be satisfied for beam alignment in this imaging mode. In addition we address the concern that one might have regarding the loss of some of the scattered electrons as they pass through such a phase plate. We show that two easily measured parameters, (1) the low-resolution image contrast produced in cryo-EM images of tobacco mosaic virus particles and (2) the fall-off of the envelope function at high resolution, can be used to quantitatively compare the data quality for Zernike phase-contrast images and for defocused bright-field images. We describe how we prepare carbon-film phase plates that are initially free of charging or other effects that degrade image quality. We emphasize, however, that even though the buildup of hydrocarbon contamination can be avoided by heating the phase plates during use, their performance nevertheless deteriorates over the time scale of days to weeks, thus requiring their frequent replacement in order to maintain optimal performance.

Huang Q, You J, Zeng GL, **Gullberg GT**. Reconstruction from uniformly attenuated SPECT projection data using the DBH method. *IEEE transactions on Medical Imaging*, 2009 Jan;28(1):17-29. PMID: 19116185

An algorithm was developed for the 2-D reconstruction of truncated and nontruncated uniformly attenuated data acquired from single photon emission computed tomography (SPECT). The algorithm is able to reconstruct data from half-scan (180 (degrees)) and short-scan (180 (degrees) +fan angle) acquisitions for parallel- and fan-beam geometries, respectively, as well as data from full-scan (360 (degrees)) acquisitions. The algorithm is a derivative, backprojection, and Hilbert transform (DBH) method, which involves the backprojection of differentiated projection data followed by an inversion of the finite weighted Hilbert transform. The kernel of the inverse weighted Hilbert transform is solved numerically using matrix inversion. Numerical simulations confirm that the DBH method provides accurate reconstructions from half-scan and short-scan data, even when there is truncation. However, as the attenuation increases, finer data sampling is required.

Shatsky M, **Hall RJ**, Brenner SE, **Glaeser RM**. A method for the alignment of heterogeneous macromolecules from electron microscopy. *Journal of Structural Biology*, 2008 Dec 30. [Epub ahead of print] PMID: 19166941

We propose a feature-based image alignment method for single-particle electron microscopy that is able to accommodate various similarity scoring functions while efficiently sampling the two-dimensional transformational space. We use this image alignment method to evaluate the performance of a scoring function that is based on the Mutual Information (MI) of two images rather than one that is based on the cross-correlation function. We show that alignment using MI for the scoring function has far less model-dependent bias than is found with cross-correlation based alignment. We also demonstrate that MI improves the alignment of some types of heterogeneous data, provided that the signal-to-noise ratio is relatively high. These results indicate, therefore, that use of MI as the scoring function is well suited for the alignment of class-averages computed from single-particle images. Our method is tested on data from three model structures and one real dataset.

Deheuninck J, **Luo K**. Ski and SnoN, potent negative regulators of TGF-beta signaling. *Cell Research*, 2009 Jan;19(1):47-57. PMID: 19114989 (Review Article)

Ski and the closely related SnoN were discovered as oncogenes by their ability to transform chicken embryo fibroblasts upon overexpression. While elevated expressions of Ski and SnoN have also been reported in many human cancer cells and tissues, consistent with their pro-oncogenic activity, emerging evidence also suggests a potential anti-oncogenic activity for both. In addition, Ski and SnoN have been implicated in regulation of cell differentiation, especially in the muscle and neuronal lineages. Multiple cellular partners of Ski and SnoN have been identified in an effort to understand the molecular mechanisms underlying the complex roles of Ski and SnoN. In this review, we summarize recent findings on the biological functions of Ski and SnoN, their mechanisms of action and how their levels of expression are regulated.

Xu R, Nelson CM, **Muschler JL**, **Veiseh M**, Vonderhaar BK, **Bissell MJ**. Sustained activation of STAT5 is essential for chromatin remodeling and maintenance of mammary-specific function. *The Journal of Cell Biology*, 2009 Jan 12;184(1):57-66. PMID: 19139262

Epithelial cells, once dissociated and placed in two-dimensional (2D) cultures, rapidly lose tissue-specific functions. We showed previously that in addition to prolactin, signaling by laminin-111 was necessary to restore functional differentiation of mammary epithelia. Here, we elucidate two additional aspects of laminin-111 action. We show that in 2D cultures, the prolactin receptor is basolaterally localized and physically segregated from its apically placed ligand. Detachment of the cells exposes the receptor to ligation by prolactin leading to signal transducers and activators of transcription protein 5 (STAT5) activation, but only transiently and not sufficiently for induction of milk protein expression. We show that laminin-111 reorganizes mammary cells into polarized acini, allowing both the exposure of the prolactin receptor and sustained activation of STAT5. The use of constitutively active STAT5 constructs showed that the latter is necessary and sufficient for chromatin reorganization and beta-casein transcription. These results underscore the crucial role of continuous laminin signaling and polarized tissue architecture in maintenance of transcription factor activation, chromatin organization, and tissue-specific gene expression.

Coppé JP, **Patil CK**, **Rodier F**, Sun Y, Muñoz DP, Goldstein J, Nelson PS, **Desprez PY**, **Campisi J**. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biology*, 2008 Dec 2;6(12):2853-68. PMID: 19053174

Cellular senescence suppresses cancer by arresting cell proliferation, essentially permanently, in response to oncogenic stimuli, including genotoxic stress. We modified the use of antibody arrays to provide a quantitative assessment of factors secreted by senescent cells. We show that human cells induced to senesce by genotoxic stress secrete myriad factors associated with inflammation and malignancy. This senescence-associated secretory phenotype (SASP) developed slowly over several days and only after DNA damage of sufficient magnitude to induce senescence. Remarkably similar SASPs developed in normal fibroblasts, normal epithelial cells, and epithelial tumor cells after genotoxic stress in culture, and in epithelial tumor cells in vivo after treatment of prostate cancer patients with DNA-damaging chemotherapy. In cultured premalignant epithelial cells, SASPs induced an epithelial-mesenchyme transition and invasiveness, hallmarks of malignancy, by a paracrine mechanism that depended largely on the SASP factors interleukin (IL)-6 and IL-8. Strikingly, two manipulations markedly amplified, and accelerated development of, the SASPs: oncogenic RAS expression, which causes genotoxic stress and senescence in normal cells, and functional loss of the p53 tumor suppressor protein. Both loss of p53 and gain of oncogenic RAS also exacerbated the promalignant paracrine activities of the SASPs. Our findings define a central feature of genotoxic stress-induced senescence. Moreover, they suggest a cell-nonautonomous mechanism by which p53 can restrain, and oncogenic RAS can promote, the development of age-related cancer by altering the tissue microenvironment.

Palsdottir H, **Remis JP**, Schaudinn C, O'Toole E, Lux R, Shi W, McDonald KL, Costerton JW, **Auer M**. 3D macromolecular organization of cryofixed *Myxococcus xanthus* biofilms as revealed by EM tomography. *Journal of Bacteriology*, 2009 Jan 23. [Epub ahead of print] PMID: 19168614

Despite the fact that most bacteria grow in biofilms in natural and pathogenic ecosystems, very little is known about the ultrastructure of their component cells or about the details of their community architecture. We used high-pressure freezing and freeze substitution to minimize the artifacts of chemical fixation, sample aggregation, and sample extraction. As a further innovation we have, for the first time in biofilm research, used electron tomography and 3D visualization to better resolve the macromolecular 3D ultrastructure of a biofilm. This combination of superb specimen preparation, and greatly improved resolution in z-axis, has opened a window in studies of *Myxococcus xanthus* cell ultrastructure and biofilm community architecture. New structural information is presented on the chromatin body, cytoplasmic organization, membrane apposition between adjacent cells, and the structure and distribution of pili and vesicles in the biofilm matrix.

Durinck S, Bullard J, **Spellman PT**, Dudoit S. GenomeGraphs: integrated genomic data visualization with R. *BMC Bioinformatics*, 2009 Jan 6;10:2. PMID: 19123956

BACKGROUND: Biological studies involve a growing number of distinct high-throughput experiments to characterize samples of interest. There is a lack of methods to visualize these different genomic datasets in a versatile manner. In addition, genomic data analysis requires integrated visualization of experimental data along with constantly changing genomic annotation and statistical analyses. **RESULTS:** We developed GenomeGraphs, as an add-on software package for the statistical programming environment R, to facilitate integrated visualization of genomic datasets. GenomeGraphs uses the biomaRt package to perform on-line annotation queries to Ensembl and translates these to gene/transcript structures in viewports of the grid graphics package. This allows genomic annotation to be plotted together with experimental data. GenomeGraphs can also be used to plot custom annotation tracks in combination with different experimental data types together in one plot using the same genomic coordinate system. **CONCLUSION:** GenomeGraphs is a flexible and extensible software package which can be used to visualize a multitude of genomic datasets within the statistical programming environment R.

Min X, Akella R, He H, Humphreys JM, **Tsutakawa SE**, Lee SJ, **Tainer JA**, Cobb MH, Goldsmith EJ. The structure of the MAP2K MEK6 reveals an autoinhibitory dimer. *Structure*, 2009 Jan;17(1):96-104. PMID: 19141286

MAP2Ks are dual-specificity protein kinases functioning at the center of three-tiered MAP kinase modules. The structure of the kinase domain of the MAP2K MEK6 with phosphorylation site mimetic aspartic acid mutations (MEK6/DeltaN/DD) has been solved at 2.3 Å resolution. The structure reveals an autoinhibited elongated ellipsoidal dimer. The enzyme adopts an inactive conformation, based upon structural queues, despite the phosphomimetic mutations. Gel filtration and small-angle X-ray scattering analysis confirm that the crystallographically observed ellipsoidal dimer is a feature of MEK6/DeltaN/DD and full-length unphosphorylated wild-type MEK6 in solution. The interface includes the phosphate binding ribbon of each subunit, part of the activation loop, and a rare "arginine stack" between symmetry-related arginine residues in the N-terminal lobe. The autoinhibited structure likely confers specificity on active MAP2Ks. The dimer may also serve the function in unphosphorylated MEK6 of preventing activation loop phosphorylation by inappropriate kinases.

Popova EY, Krauss SW, Short SA, Lee G, **Villalobos J**, Etzell J, Koury MJ, Ney PA, **Chasis JA**, Grigoryev SA. Chromatin condensation in terminally differentiating mouse erythroblasts does not involve special

architectural proteins but depends on histone deacetylation. *Chromosome Research*, 2009 Jan 27. [Epub ahead of print] PMID: 19172406

Terminal erythroid differentiation in vertebrates is characterized by progressive heterochromatin formation and chromatin condensation and, in mammals, culminates in nuclear extrusion. To date, although mechanisms regulating avian erythroid chromatin condensation have been identified, little is known regarding this process during mammalian erythropoiesis. To elucidate the molecular basis for mammalian erythroblast chromatin condensation, we used Friend virus-infected murine spleen erythroblasts that undergo terminal differentiation in vitro. Chromatin isolated from early and late-stage erythroblasts had similar levels of linker and core histones, only a slight difference in nucleosome repeats, and no significant accumulation of known developmentally regulated architectural chromatin proteins. However, histone H3(K9) dimethylation markedly increased while histone H4(K12) acetylation dramatically decreased and became segregated from the histone methylation as chromatin condensed. One histone deacetylase, HDAC5, was significantly upregulated during the terminal stages of Friend virus-infected erythroblast differentiation. Treatment with histone deacetylase inhibitor, trichostatin A, blocked both chromatin condensation and nuclear extrusion. Based on our data, we propose a model for a unique mechanism in which extensive histone deacetylation at pericentromeric heterochromatin mediates heterochromatin condensation in vertebrate erythroblasts that would otherwise be mediated by developmentally-regulated architectural proteins in nucleated blood cells.

Hodgson JG, Yeh RF, Ray A, **Wang NJ**, Smirnov I, Yu M, Hariono S, Silber J, **Feiler HS**, **Gray JW**, **Spellman PT**, Vandenberg SR, Berger MS, James CD. Comparative analyses of gene copy number and mRNA expression in GBM tumors and GBM xenografts. *Neuro-Oncology*, 2009 Jan 12. [Epub ahead of print]

Development of model systems that recapitulate the molecular heterogeneity observed amongst GBM tumors will expedite the testing of targeted molecular therapeutic strategies for GBM treatment. In this study, we profiled DNA copy number and mRNA expression in 21 independent GBM tumor lines maintained as subcutaneous xenografts (GBMX), and compared GBMX molecular signatures to those observed in GBM clinical specimens derived from The Cancer Genome Atlas (TCGA). The predominant copy number signature in both tumor groups was defined by chromosome-7gain/chromosome-10loss, a poor prognosis genetic signature. We also observed, at frequencies similar to that detected in TCGA GBMs genomic amplification and overexpression of known GBM oncogenes such as EGFR, MDM2, CDK6 and MYCN, and novel genes including NUP107, SLC35E3, MMP1, MMP13 and DDX1. The transcriptional signature of GBMX tumors, which was stable over multiple subcutaneous passages, was defined by overexpression of genes involved in M-phase, DNA Replication, and Chromosome organization (MRC) and was highly similar to the poor-prognosis mitosis-and-cell-cycle-module (MCM) in GBM. Assessment of gene expression in TCGA-derived GBMs revealed overexpression of MRC cancer genes AURKB, BIRC5, CCNB1, CCNB2, CDC2, CDK2, and FOXM1, which form a transcriptional network important for G2/M-progression and/or -checkpoint activation. In conclusion, our study supports propagation of GBM tumors as subcutaneous xenografts as a useful approach for sustaining key molecular characteristics of patient tumors, and highlights therapeutic opportunities conferred by this GBMX tumor panel for testing targeted therapeutic strategies for GBM treatment.

Xiao Z, Dunn E, Singh K, Khan IS, **Yannone SM**, Cowan MJ. A non-leaky Artemis-deficient mouse that accurately models the human severe combined immune deficiency phenotype, including resistance to hematopoietic stem cell transplantation. *Biology of Blood and Marrow Transplantation*, 2009 Jan;15(1):1-11. PMID: 19135937

Two Artemis-deficient (mArt(-/-)) mouse models, generated independently on 129/SvJ backgrounds, have the expected T(-)B(-)NK(+) severe combined immune deficiency (SCID) phenotype but fail to mimic the

human disease because of CD4(+) T cell leakiness. Moreover, immune reconstitution after hematopoietic stem cell transplantation is achieved more readily in these leaky mouse models than in Artemis-deficient humans. To develop a more clinically relevant animal model, we backcrossed the mArt(-/-) mutation onto the C57Bl/6 (B6) background (99.9%), which resulted in virtually no CD4(+) T cell leakiness compared with 129/SvJ mArt(+/-) mice (0.3% +/- 0.25% vs 19.5% +/- 15.1%, $P < .001$). The nonleaky mouse also was uniquely resistant to engraftment using allogeneic mismatched hematopoietic stem cells, comparable to what is seen in human Artemis deficiency. The genetic background also influenced Artemis-associated radiation sensitivity, with differing degrees of x-ray hypersensitivity evident in 129/SvJ and B6 backgrounds with both the mArt(-/-) and mArt(+/-) genotypes. Our results indicate that immunogenic and DNA repair phenotypes associated with Artemis deficiency are significantly altered by genetic background, which has important implications for the diagnosis and treatment of SCID. Moreover, the B6 mArt(-/-) mouse provides a more accurate model for the human disease and a more appropriate system for studying human Artemis deficiency and for developing improved transplantation and gene therapy regimens for the treatment of children with SCID.

Xiao Z, **Yannone SM**, Dunn E, Cowan MJ. A novel missense RAG-1 mutation results in T-B-NK+ SCID in Athabascan-speaking Dine Indians from the Canadian Northwest Territories. *European Journal of Human Genetics*, 2009 Feb;17(2):205-12. Epub 2008 Aug 13. PMID: 18701881

DNA double-strand repair factors in the non-homologous end joining (NHEJ) pathway resolve DNA double-strand breaks introduced by the recombination-activating gene (RAG) proteins during V(D)J recombination of T and B lymphocyte receptor genes. Defective NHEJ and subsequent failure of V(D)J recombination leads to severe combined immunodeficiency disease (SCID). We originally linked T(-)B(-)NK(+) SCID in Athabascan-speaking Native Americans in the Southwestern US and Northwest Territories of Canada to chromosome 10. However, despite a common ancestry, the null mutation in the Artemis gene that we found to be causal in the SCID among the Navajo and Apache Indians was not present in the Dine Indians in the Northwest Territories. We now report a novel homozygous missense mutation (R776W) in RAG-1 in three children with T(-)B(-)NK(+) SCID from two related families of Athabascan-speaking Dine Indians in the Canadian Northwest Territories. As expected, we found no increased sensitivity to ionizing radiation in patient fibroblasts. The impaired activity of this RAG-1 mutant in V(D)J recombination was confirmed by the EGFP-based V(D)J recombination assays. Overexpression of wild type RAG-1 in patient fibroblasts complemented V(D)J recombination, with recovery of both coding and signal joint formation. Our results indicate that the novel R776W missense mutation in RAG-1 is causal in the T(-)B(-)NK(+) SCID phenotype in Athabascan-speaking Dine Indians from the Canadian Northwest Territories.