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LIFE SCIENCES DIVISION E-NEWSLETTER

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DOE scientific focus area notes

Low Dose Radiation Research

Low Dose Program Represented

Mary Helen Barcellos-Hoff, Life Sciences Division Deputy Director and the Low Dose Program Chief Scientist, gave an invited presentation on 20 May for the Nuclear Energy Agency's Committee on Radiation Protection and Public Health (CRPPH) at the annual meeting of international delegates in Paris that was dedicated to radiation protection science. Barcellos-Hoff provided a historical perspective of the program research since its inception in 1999, to the current research accomplishments that provide fundamental research in the low dose region.

Mary Helen Barcellos-Hoff, 5/08

GTL-Genomics

Novel High Throughout Strategy Developed

The Protein Complex Analysis Project (PCAP) has just published a paper (Dong et al., 2008) describing a novel high throughout strategy to identify protein complexes. The principal method for purifying and identifying stable protein complexes system-wide in whole cells to date has been Tandem Affinity Purification (TAP). Although highly effective, this approach is laborious and impractical in organisms where genetic manipulation is not possible. In this publication, a novel "tagless" strategy is proposed that combines multidimensional separation of endogenous complexes with mass spectrometric monitoring of their composition. In this procedure, putative protein complexes are identified based on the comigration of collections of polypeptides through multiple orthogonal separation steps. Proof-of-principle evidence for the feasibility of key aspects of this strategy is presented. A majority of Escherichia coli proteins are shown to remain in stable complexes during fractionation of a crude extract through three chromatographic steps. It is also demonstrated that iTRAQ reagent-based tracking can quantify relative migration of polypeptides through chromatographic separation media. LC MALDI MS and MS/MS analysis of the iTRAQ-labeled peptides gave reliable relative quantification of 37 components of 13 known E. coli complexes: 95% of known complex components closely co-eluted and 57% were automatically grouped by a prototype computational clustering method. With further technological improvements in each step, the authors believe this strategy will dramatically improve the efficiency of the purification and identification of protein complexes in cells. A research highlight describing this work was published in the same issue of the journal (Cassidy, 2008).

Mark Biggin, 5/08

Dong M, Yang LL, Williams K, Fisher SJ, Hall SC, **Biggin MD**, Jin J, Witkowska HE. A "Tagless" Strategy for Identification of Stable Protein Complexes Genome-wide by Multidimensional Orthogonal Chromatographic Separation and iTRAQ Reagent Tracking. *Journal of Proteome Research*, 2008 May 2;7(5):1836-1849. PMID: 18336004 [Article] http://pubs.acs.org/cgi-bin/abstract.cgi/jprobs/2008/7/i05/abs/pr700624e.html

Cassidy, L. Protein-complex characterization goes tagless. *Journal of Proteome Research*, 2008 May 2;7(5):1836-1849. PMID: 18336004 [Article] http://pubs.acs.org/subscribe/journals/jprobs/7/i05/html/0508profile2.html

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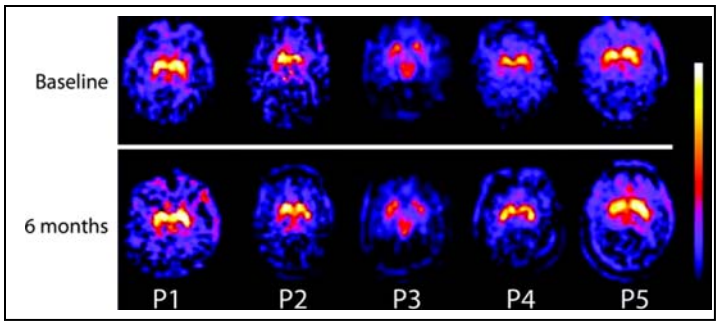
Nuclear Medicine

Report on Gene Therapy with PET in Parkinson's Disease
Investigators in the Department of Molecular Imaging and Neuroscience have just published a paper showing the use of PET scanning in tracking the clinical efficacy of gene therapy in Parkinson's disease (PD). **Jamie Eberling** , lead author of the study (Eberling et al., 2008), led the team that used the PET tracer [18F] flurometatyrosine (FMT) to follow 5 PD patients treated with the gene for the enzyme Aromatic Amino Acid Decarboxylase (AAAD). This enzyme is involved in the synthesis of dopamine, which is depleted in PD patients. Because FMT tracks the activity of AAAD, it can provide an in vivo measure of gene function noninvasively. Eberling's study showed that treatment with the gene for AADC produced an average 25-30% increase in uptake of the tracer. While the study was small and not placebo-controlled, this is the first report of this approach in humans and it paves the way for continued larger and more definitive studies using this form of gene therapy with PET in PD.

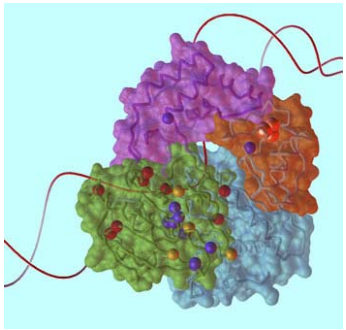


The figure on the left demonstrates results in each of the 5 patients studied at baseline and 6 months after gene therapy. Bright regions are the basal ganglia, the site of most tracer uptake since this is the brain region that synthesizes dopamine. Tracer uptake is greater in all cases at 6 months after gene therapy than it was at baseline before treatment.
Bill Jagust, 5/08

Eberling JL, **Jagust WJ**, Christine CW, Starr P, Larson P, **Bankiewicz** KS, Aminoff MJ. Results from a phase I safety trial of hAADC gene therapy for Parkinson disease. *Neurology*, 2008 May 20;70(21):1980-3. Epub 2008 Apr 9. PMID: 18401019

Scientific news

The Structure of XPD Sheds Light on Cancer and Aging -
The role of a unique DNA-repair protein in multiple diseases
The protein XPD is one component of the essential multi-protein complex TFIIH, which functions both in transcription and in maintaining the integrity of the genome through DNA repair. Now, for the first time, researchers at the U.S. Department of Energy's Lawrence Berkeley National Laboratory and the Scripps Research Institute have solved the crystal structure of XPD, a helicase that unwinds DNA to initiate repair. The structure reveals how discrete flaws in the remarkable architecture of XPD — as seemingly insignificant as a change in either of two adjacent amino acid



The four domains of XPD, different sites of mutations, and strand of unwound DNA shown passing under the arch.

residues — can lead to three different diseases with completely different phenotypes, and gives novel insight into the processes of aging and cancer resulting from defective DNA repair.

The research team, which included Li Fan and **John Tainer** (LBNL and Scripps) and **Jill Fuss**, **Quen Cheng**, and **Priscilla Cooper** (LBNL), report their results in the May 30, 2008 issue of the journal Cell. [Press Release] http://www.lbl.gov/Science-Articles/Archive/LSD-XPD.html
Today at Berkeley Lab, 6/2/08

Fan L, **Fuss JO**, **Cheng QJ**, Arvai AS, Hammel M, Roberts VA, **Cooper PK**, **Tainer JA**. XPD Helicase Structures and Activities: Insights into the Cancer and Aging Phenotypes from XPD Mutations. *Cell*, 2008 May 30;133(5):789-800. PMID: 18510924

The Joint Center for Integrated Bioimaging -
A Vision for a Multiscale Bioimaging Facility at LBNL

Building on the success of the 2007 Gulliver Multiscale Bioimaging Workshop at LBNL, the concept of a Joint Center for Integrated Bioimaging (JCIB) has been further refined under the leadership of **Manfred Auer**, **Jan Liphardt**, and **Joe Gray**. Realizing that "Imaging techniques are dramatically accelerating biological research, revealing organizational and functional details at all levels of spatial and temporal resolution, ranging from single molecules to whole organisms," (Gulliver article: http://www.lbl.gov/Publications/Currents/Archive/Jun-15-2007.html) JCIB will enable biomedical and bioenergy researchers at LBNL and several collaborating institutions to investigate how macromolecular complexes, cells and tissues function with unprecedented resolution, coverage and throughput. The center will accomplish this goal by functioning both as a technology incubator and a service provider. The central insight for the necessity of JCIB is that it is now clear that development and integration of existing and emerging imaging technologies, together with advances in probe development, visualization, feature extraction and data representation as well as in automation, will allow biologists to visualize macromolecular complexes, cells and tissues across different scales with extremely high resolution and simultaneously with wide coverage and high throughput. The historical trade-offs between coverage, throughput and high resolution are not fundamental and properly integrated imaging tools will provide all three, allowing biologists to study processes such as gene regulation, cell differentiation, and tumor growth with high resolution and in a wide cell or tissue context.

As a technology incubator, JCIB's primary mission will be to invent and integrate new imaging technologies in close collaboration with affiliated imaging, biomedical and bioenergy investigators. Principally, these affiliated investigators will be experts in microbiology, cell biology, cancer, low-dose, bioremediation, and bioenergy. Close collaborations of JCIB staff with biomedical and bioenergy researchers will ensure that the technologies developed at JCIB directly address central imaging needs. JCIB's service component will allow the wider DOE and NIH community to apply this resource to a wide range of problems. Four key areas have been identified: (1) imaging: integration of imaging, including specimen preparation and data registration (2) chemistry: development of chemical probes that can be imaged by several different modalities, (3) computer sciences: data visualization, feature extraction and quantitative analysis, data representation and data mining and (4) engineering: automation for high-throughput, experimental manipulation of biological samples, including microfluidics.

Multiscale imaging will help researchers understand how collections of molecules or cells function or cease to function. JCIB will reveal new levels of biological organization of macromolecular machines,

cells and tissues/cellular communities. By superimposing complementary imaging channels, it will be possible to directly correlate ultrastructural context, dynamics and function. Specific scientific pay-offs may include: i) improving cancer detection, diagnosis, and response to therapy ,(ii) characterization of the human microbiome and establishing its involvement in the pathophysiology of chronic diseases such as e.g. Cystic fibrosis and Crohn's disease, (iii) revealing the pathophysiological consequences of exposure to low dose ionizing radiatiomeet its mandate of enabling national energy independence. These research areas are a central part of a larger program in Multi-scale Biosystems Engineering and instrumentation, which is one of LBNL's priority scientific leadership initiatives put forward to DOE in April as a part of its annual planning process.

As of May 2008, two specific pilot research projects have begun to demonstrate the ability of integrated bioimaging to give deeper insight into the biomedical and bioenergy spaces than would be possible from a single imaging modality alone. The goal of the first is to use nature's lignocellulose processing plant, the termite hindgut microbial community, as a platform to understand a process of degradation of cell wall material. The goal of the second is to visualize, with nanometer resolution, the changes in a chromatin organizer that regulates genes when certain cell lines are irradiated.
Liphardt/Auer/Gray, 5/08

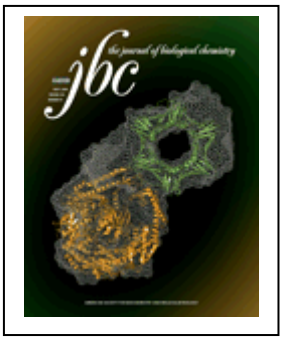
Collaborative Effort Expected to Lead to Development Therapeutic Strategies against Breast Cancer
The NCI Integrative Cancer Biology Program (ICBP) supports a multidisciplinary effort to develop computational and experimental procedures to elucidate the mechanisms governing responses of cell lines to chemotherapy and to develop strategies to predict how drugs should be combined most effectively. This effort engages investigators at LBNL (led by **Joe Gray**), UCSF, UC Berkeley, SRI International, The Netherlands Cancer Institute, MD Anderson Cancer Center, Ohio State University, MIT, Harvard and several private sector partners. It is expected this effort will lead to therapeutic strategies that combine multiple drugs in ways that will take research in the direction of curative therapy for metastatic breast cancer.

One recent, exciting development in this area is that the ICBP has adopted the 50 cell line collection assembled and characterized at LBNL as the "ICBP 50". They are taking steps to make the cell lines available as a set to investigators throughout the world to stimulate research on their characteristics and use. In addition, the ICBP will develop an internet web portal to make information about these cell lines available to researchers around the world and to invite the entire scientific community to contribute to their continued molecular and biological characterization. The web portal design was developed during the course of an ICBP Data Integration Workshop held in San Francisco on May 6 organized by Joe Gray, and Jill Mesirov from the Broad Institute. Their work on these lines effort has already attracted substantial private sector interest with three pharma /biotech partners already signed up to support continued development and use of the cell line resource.
Joe Gray, 5/08

Bay Area Breast Cancer SPORE Renewed
The Bay Area Breast Cancer Specialized Program Of Research Excellence (SPORE) has supported translational breast cancer research in the Bay Area since the initiation of the SPORE program by the National Cancer Institute (NIH/NCI) in 1992. The Bay Area SPORE, led by **Joe Gray**, has just been renewed for an additional 5 years (~$12M over 5 years). Projects in the SPORE include projects to

develop: (a) markers to predict outcome in patients with Ductal Carcinoma in Situ (Karla Kerlikowske and Thea Tlsty co-PIs), (b) molecular markers to predict responses to pathway targeted therapies (Joe Gray, Fred Waldman and Laura Esserman, co-PIs), (c) drug carrying liposomes immunologically targeted to basal subtype breast cancer (John Park and Jim Marks, co-PIs) and (d) nucleic acid therapies aimed at killing cells with active telomerase (Elizabeth Blackburn and John Park, co-PIs. The SPORE also supports cores for tissue collection (Britt Marie Ljung, Fred Waldman and Karla Kerlikowske, Co-PIs), bioinformatics (**Paul Spellman**, PI), biostatistics (Dan Moore PI), and advocacy (Caroline Marks, advocate leader).
Joe Gray, 5/08

Cover Article on the DNA Repair Proteins Muts and PCNA

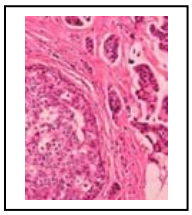


In the cover article of the Journal of Biological Chemistry, Iyer et al. summarize their findings on the DNA Repair proteins MutS and PCNA (Proliferating Cell Nuclear Antigen). MutS is involved in a variety of biochemical processes in the cell including the identification of DNA damage involving mismatched basepairs. PCNA on the other hand is much like a Swiss army knife for DNA related processes. It encircles DNA and binds a variety of proteins with different functions on DNA. The work, conducted at the LBNL Advanced Light Source in collaboration with LBNL staff at the SIBYLS beamline, identifies the 1:1 stoichiometry between the MutS heterodimer and PCNA homotrimer. This result is surprising as PCNA has three equivalent binding sites for MutS. The findings have implications on which processes involve the complex and how these processes occur. The study is part of a Structural Biology of DNA Repair grant, funded by the National Cancer Institute, under the leadership of **John Tainer** and **Priscilla Cooper**.
Gregory Hura, 05/08

Iyer RR, Pohlhaus TJ, Chen S, Hura GL, Dzantiev L, Beese LS, Modrich P. The MutSalpha-proliferating cell nuclear antigen interaction in human DNA mismatch repair. *Journal of Biological Chemistry*, 2008 May 9;283(19):13310-9 PMID: 18326858

Milk Duct Cells Key to DCIS Spreading

When a form of cancer that begins in the milk ducts of the breast invades neighboring tissue to spread



to other parts of the body, the cause lies not in the tumor cells themselves but in a group of abnormal surrounding cells that cause the walls of the duct to deteriorate like a rusty pipe, according to a new study led by Dana-Farber Cancer Institute researchers. The discovery may lead to screening tests to determine whether the disease — known as ductal carcinoma in situ, or DCIS — is likely to spread beyond the ducts, based on genetic abnormalities in cells in the ducts' lining. Berkeley Lab life scientist **Mina Bissell** was a co-author of the study. [Full Story] http://www.dana-farber.org/abo/news/press/2008/cells-lining-milk-ducts-hold-key-to-spread-of-common-form-of-breast-cancer-study-finds.html
Today at Berkeley Lab, 5/9/08

Focus on Pre-Conception Health of Child's Father

When Barbara Bell wanted to have kids, she ditched the birth control pills, popped prenatal vitamins, and got more iron and calcium. Her husband's pre-conception regimen was much easier: "He had a glass of wine," Bell said, laughing. Would-be dads are rarely bombarded with pre-pregnancy tips on diet missteps and toxic exposures, even though sperm DNA is vulnerable to defects. A March study — coauthored by Berkeley Lab life scientist **Andrew Wyrobek** and featured in the March issue of the LSD E-newsletter — added to the growing knowledge. Utilizing new technology to label sperm DNA, men who ate little folate had more sperm with the wrong number of chromosomes. That could lead to miscarriages and birth defects like Down syndrome.
[Full Story] http://origin.mercurynews.com/losgatos/ci_9319441?nclick_check=1
Today at Berkeley Lab, 5/22/08

Blakely to Lead 2008 NASA Space Radiation Summer School

Eleanor Blakely has been appointed as Scientific Director of the 2008 NASA Space Radiation Summer School at Brookhaven National Laboratory. This intensive, three-week course funded by NASA and DOE offers a unique physical and intellectual environment not duplicated in the nation's universities, medical schools or research institutes. Fifteen international students and fellows participate in both classroom activities and scientific experiments, working side-by-side with top space scientists from research organizations such as NASA, Brookhaven Lab, LBNL, Loma Linda University, Columbia University, University of Pennsylvania, and UT Southwestern Medical Center. Experimental creativity and interdisciplinary approaches are emphasized. Studies at the NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratory (http://www.bnl.gov/medical/NASA/LTSF.asp) simulate space radiation to learn how the intense rays may promote the development of cancer, as well as how this radiation can affect the central nervous system and other organ systems of the body. NSRL researchers are also looking at ways to protect against these dangers through shielding and other strategies to minimize the risk to space travelers.
Eleanor Blakely, 5/08

Kronenberg Invited Speaker at International Space Medicine Summit

Amy Kronenberg was an invited speaker in the International Space Medicine Summit held at the James A. Baker III Institute for Public Policy at Rice University May 17-19, 2008. This conference brought together physicians and biomedical scientists from the international group of space-faring nations to expand on the research needed to prevent and/or mitigate the medical and biomedical problems impacting the health of astronauts and cosmonauts during long duration spaceflight and to plan for extended missions to the moon and other planetary surfaces. Kronenberg presented the research needs for minimizing the acute and late effects of exposure to solar particle events as well as the galactic cosmic radiation, focusing on uncertainties in risk associated with low dose-rate exposures and those of intermediate dose-rates as well as the relative biological effectiveness for sparsely ionizing protons across a range of energies. Other sessions during this conference dealt with issues regarding human performance and the practice of space medicine.
Amy Kronenberg, 5/08

Awards

Budinger Elected Secretary for Engineering Academy
The National Academy of Engineering has elected **Thomas Budinger**, a UC Berkeley professor and senior scientist with Berkeley Lab's Life Sciences Division, to a four-year term as home secretary. He was elected to the NAE in 1996 and will oversee the Academy's membership activities. [Full Story]
http://www8.nationalacademies.org/onpinews/newsitem.aspx?RecordID=05152008
Today at Berkeley Lab, 5/21/08

Life Scientists among Keck Award Winners
Judith Campisi and **Christopher Patil**, both with Berkeley Lab's Life Sciences Division, were among the recent recipients of a National Academies Keck Futures Initiative grants. The grants fund research on aging and healthspan. Campisi, Patil and their team members received $75,000 to develop a plan to test hypotheses about biological factors that control lifespan and healthspan, and compare tissues from multiple species of animals. [Full story]
http://www8.nationalacademies.org/onpinews/newsitem.aspx?RecordID=12084
Today at Berkeley Lab, 5/12/08

Dernburg Gets HHMI Investigator Appointment
Four Berkeley Lab researchers have received one of the most sought-after honors in biomedical research: appointment as Howard Hughes Medical Institute (HHMI) investigators with guaranteed research support for five, 10, or more years into the future. They include Christopher Chang (Chemical Sciences), **Abby Dernburg** (Life Sciences), Michael Eisen (Genomics), and Jay Groves (Physical Biosciences) and they are among the 56 scientists selected from among 1,070 applications submitted in a nationwide competition. To evaluate the applications, HHMI assembled review panels of distinguished biomedical scientists.

Photo Abby Dernburg: Noah Berger/AP, ©HHMI

The HHMI, a non-profit medical research organization that ranks as one of the nation's largest philanthropies, plays a powerful role in advancing biomedical research and science education in the United States. In the past two decades HHMI has made investments of more than $8.3 billion for the support, training, and education of the nation's most creative and promising scientists. HHMI values innovation and encourages its investigators to extend the boundaries of science. By appointing scientists as Hughes investigators — rather than awarding research grants — HHMI is guided by the principle of "people, not projects." HHMI investigators have the freedom to explore and, if necessary, to change direction in their research. [Full story] http://www.hhmi.org/news/20080527.html
CG, also in Today at Berkeley Lab, 5/29/08

Bissell Named Winner Recipient DOD Innovator Award Sequel

Mina Bissell

Mina Bissell is the recipient of the sequel to the Department of Defense, Breast Cancer Research Program (BCRP) Innovator Research Project Award of $2 million for direct costs for a 5-year performance period. The Innovator Research Project Award provides individuals who were recipients of FY01 through FY04 Innovator Awards with the opportunity to continue their pursuit of novel, visionary, high-risk ideas that could ultimately have a significant impact on the breast cancer community.
Mina Bissell, 5/08

Bissell Delivers Harvey Lecture

Mina Bissell delivered the Harvey Lecture on March 20, 2008 at The Rockefeller University. The Harvey Society, founded in 1905, sponsors a series of seven lectures annually that are open to the public and are attended by hundreds of scientists from New York City and environs. The Harvey lecture series is one of the most distinguished in the country and the annual books of the lectures are well known throughout the world. The lecturers, selected by the Society's Council, are leading biomedical researchers from around the world. Most Nobel laureates in Physiology or Medicine have presented Harvey lectures.
Mina Bissell, 5/08

Recent publications (selected)

Dong M, Yang LL, Williams K, Fisher SJ, Hall SC, **Biggin MD**, Jin J, Witkowska HE. A "Tagless" Strategy for Identification of Stable Protein Complexes Genome-wide by Multidimensional Orthogonal Chromatographic Separation and iTRAQ Reagent Tracking. *Journal of Proteome Research*, 2008 May 2;7(5):1836-1849. PMID: 18336004

> Tandem affinity purification is the principal method for purifying and identifying stable protein complexes system-wide in whole cells. Although highly effective, this approach is laborious and impractical in organisms where genetic manipulation is not possible. Here, we propose a novel "tagless" strategy that combines multidimensional separation of endogenous complexes with mass spectrometric monitoring of their composition. In this procedure, putative protein complexes are identified based on the comigration of collections of polypeptides through multiple orthogonal separation steps. We present proof-of-principle evidence for the feasibility of key aspects of this strategy. A majority of Escherichia coli proteins are shown to remain in stable complexes during fractionation of a crude extract through three chromatographic steps. We also demonstrate that iTRAQ reagent-based tracking can quantify relative migration of polypeptides through chromatographic separation media. LC MALDI MS and MS/MS analysis of the iTRAQ-labeled peptides gave reliable relative quantification of 37 components of 13 known E. coli complexes: 95% of known complex components closely co-eluted and 57% were automatically grouped by a prototype computational clustering method. With further technological improvements in each step, we believe this strategy will dramatically improve the efficiency of the purification and identification of protein complexes in cells.

Triffo WJ, Palsdottir H, McDonald KL, Lee JK, Inman JL, **Bissell MJ**, Raphael RM**, Auer M**. Controlled microaspiration for high-pressure freezing: a new method for ultrastructural preservation of fragile and sparse tissues for TEM and electron tomography. *Journal of Microscopy*, 2008 May;230(Pt 2):278-87. PMID: 18445158

> High-pressure freezing is the preferred method to prepare thick biological specimens for ultrastructural studies. However, the advantages obtained by this method often prove unattainable for samples that are difficult to handle during the freezing and substitution protocols. Delicate and sparse samples are difficult to manipulate and maintain intact throughout the sequence of freezing, infiltration, embedding and final orientation for sectioning and subsequent transmission electron microscopy. An established approach to surmount these difficulties is the use of cellulose microdialysis tubing to transport the sample. With an inner diameter of 200 microm, the tubing protects small and fragile samples within the thickness constraints of high-pressure freezing, and the tube ends can be sealed to avoid loss of sample. Importantly, the transparency of the tubing allows optical study of the specimen at different steps in the process. Here, we describe the use of a micromanipulator and microinjection apparatus to handle and position delicate specimens within the tubing. We report two biologically significant examples that benefit from this approach, 3D cultures of mammary epithelial cells and cochlear outer hair cells. We illustrate the potential for correlative light and electron microscopy as well as electron tomography.

Kim SH, Davalos AR, Heo SJ, Rodier F, Zou Y, Beausejour C, Kaminker P, Yannone SM, **Campisi J**. Telomere dysfunction and cell survival: roles for distinct TIN2-containing complexes. *Journal of Cell Biology*, 2008 May 5;181(3):447-60. PMID: 18443218

> Telomeres are maintained by three DNA-binding proteins (telomeric repeat binding factor 1 [TRF1], TRF2, and protector of telomeres 1 [POT1]) and several associated factors. One factor, TRF1-interacting protein 2 (TIN2), binds TRF1 and TRF2 directly and POT1 indirectly. Along with two other proteins, TPP1 and hRap1, these form a soluble complex that may be the core telomere maintenance complex. It is not clear whether subcomplexes also exist in vivo. We provide evidence for two TIN2 subcomplexes with distinct functions in human cells. We isolated these two TIN2 subcomplexes from nuclear lysates of unperturbed cells and cells expressing TIN2 mutants TIN2-13 and TIN2-15C, which cannot bind TRF2 or TRF1, respectively. In cells with wild-type p53 function, TIN2-15C was more potent than TIN2-13 in causing telomere uncapping and eventual growth arrest. In cells lacking p53 function, TIN2-15C was more potent than TIN2-13 in causing telomere dysfunction and cell death. Our findings suggest that distinct TIN2 complexes exist and that TIN2-15C-sensitive subcomplexes are particularly important for cell survival in the absence of functional p53.

Eberling JL, **Jagust WJ**, Christine CW, Starr P, Larson P, **Bankiewicz** KS, Aminoff MJ. Results from a phase I safety trial of hAADC gene therapy for Parkinson disease. *Neurology*. 2008 May 20;70(21):1980-3. Epub 2008 Apr 9. PMID: 18401019

> BACKGROUND: In a primate model of Parkinson disease (PD), intrastriatal infusion of an adeno-associated viral (AAV) vector containing the human aromatic l-amino acid decarboxylase (hAADC) gene results in robust gene expression. After gene transfer, low doses of systemically administered l-dopa are converted to dopamine in the transduced striatal neurons, resulting in behavioral improvement without the side effects typically associated with higher doses of l-dopa. These studies led to the initiation of a phase I safety trial. Here we report the findings for the first cohort of five patients. METHODS: Patients with moderate to advanced PD received bilateral infusion of a low dose of the AAV-hAADC vector into the putamen. PET scans using the AADC tracer, 6-[18F]fluoro-l-m-tyrosine (FMT), were performed at baseline and at 1 and 6 months after infusion as an in vivo measure of gene expression. RESULTS: PET results showed an average 30% increase in FMT uptake (K(i)(c)) in the putamen after gene transfer. Preliminary

analysis of clinical data indicates a modest improvement, but absence of a control and the nonblinded analyses make interpretation difficult. CONCLUSIONS: Thus far, this gene therapy approach has been well tolerated and shows PET evidence of sustained gene expression. These initial findings demonstrate the safety of the therapy; higher doses of adeno-associated viral vector containing the human aromatic l-amino acid decarboxylase gene in the next cohort of patients may further increase dopamine production in the putamen and provide more profound clinical benefit.

Fan L, **Fuss JO**, **Cheng QJ**, Arvai AS, Hammel M, Roberts VA, **Cooper PK**, **Tainer JA**. XPD Helicase Structures and Activities: Insights into the Cancer and Aging Phenotypes from XPD Mutations. *Cell*, 2008 May 30;133(5):789-800. PMID: 18510924

Mutations in XPD helicase, required for nucleotide excision repair (NER) as part of the transcription/repair complex TFIIH, cause three distinct phenotypes: cancer-prone xeroderma pigmentosum (XP), or aging disorders Cockayne syndrome (CS), and trichothiodystrophy (TTD). To clarify molecular differences underlying these diseases, we determined crystal structures of the XPD catalytic core from Sulfolobus acidocaldarius and measured mutant enzyme activities. Substrate-binding grooves separate adjacent Rad51/RecA-like helicase domains (HD1, HD2) and an arch formed by 4FeS and Arch domains. XP mutations map along the HD1 ATP-binding edge and HD2 DNA-binding channel and impair helicase activity essential for NER. XP/CS mutations both impair helicase activity and likely affect HD2 functional movement. TTD mutants lose or retain helicase activity but map to sites in all four domains expected to cause framework defects impacting TFIIH integrity. These results provide a foundation for understanding disease consequences of mutations in XPD and related 4Fe-4S helicases including FancJ.

Raphael BJ, Volik S, Yu P, Wu C, Huang G, Linardopoulou EV, Trask BJ, Waldman F, Costello J, Pienta KJ, Mills GB, Bajsarowicz K, Kobayashi Y, Sridharan S, Paris PL, Tao Q, Aerni SJ, Brown RP, Bashir A, **Gray JW**, Cheng JF, de Jong P, Nefedov M, Ried T, Padilla-Nash HM, Collins CC. A sequence-based survey of the complex structural organization of tumor genomes. *Genome Biology,* 2008 Mar 25;9(3):R59. PMID: 18364049

BACKGROUND: The genomes of many epithelial tumors exhibit extensive chromosomal rearrangements. All classes of genome rearrangements can be identified using end sequencing profiling, which relies on paired-end sequencing of cloned tumor genomes. RESULTS: In the present study brain, breast, ovary, and prostate tumors, along with three breast cancer cell lines, were surveyed using end sequencing profiling, yielding the largest available collection of sequence-ready tumor genome breakpoints and providing evidence that some rearrangements may be recurrent. Sequencing and fluorescence in situ hybridization confirmed translocations and complex tumor genome structures that include co-amplification and packaging of disparate genomic loci with associated molecular heterogeneity. Comparison of the tumor genomes suggests recurrent rearrangements. Some are likely to be novel structural polymorphisms, whereas others may be bona fide somatic rearrangements. A recurrent fusion transcript in breast tumors and a constitutional fusion transcript resulting from a segmental duplication were identified. Analysis of end sequences for single nucleotide polymorphisms revealed candidate somatic mutations and an elevated rate of novel single nucleotide polymorphisms in an ovarian tumor. CONCLUSION: These results suggest that the genomes of many epithelial tumors may be far more dynamic and complex than was previously appreciated and that genomic fusions, including fusion transcripts and proteins, may be common, possibly yielding tumor-specific biomarkers and therapeutic targets.

Hwang AB, Franc BL, **Gullberg GT**, Hasegawa BH. Assessment of the sources of error affecting the quantitative accuracy of SPECT imaging in small animals. *Physics in Medicine and Biology*, 2008 May 7;53(9):2233-2252. PMID: 18401059

Small animal SPECT imaging systems have multiple potential applications in biomedical research. Whereas SPECT data are commonly interpreted qualitatively in a clinical setting, the ability to accurately quantify measurements will increase the utility of the SPECT data for laboratory measurements involving small animals. In this work, we assess the effect of photon attenuation, scatter and partial volume errors on the quantitative accuracy of small animal SPECT measurements, first with Monte Carlo simulation and then confirmed with experimental measurements. The simulations modeled the imaging geometry of a commercially available small animal SPECT system. We simulated the imaging of a radioactive source within a cylinder of water, and reconstructed the projection data using iterative reconstruction algorithms. The size of the source and the size of the surrounding cylinder were varied to evaluate the effects of photon attenuation and scatter on quantitative accuracy. We found that photon attenuation can reduce the measured concentration of radioactivity in a volume of interest in the center of a rat-sized cylinder of water by up to 50% when imaging with iodine-125, and up to 25% when imaging with technetium-99m. When imaging with iodine-125, the scatter-to-primary ratio can reach up to approximately 30%, and can cause overestimation of the radioactivity concentration when reconstructing data with attenuation correction. We varied the size of the source to evaluate partial volume errors, which we found to be a strong function of the size of the volume of interest and the spatial resolution. These errors can result in large (>50%) changes in the measured amount of radioactivity. The simulation results were compared with and found to agree with experimental measurements. The inclusion of attenuation correction in the reconstruction algorithm improved quantitative accuracy. We also found that an improvement of the spatial resolution through the use of resolution recovery techniques (i.e. modeling the finite collimator spatial resolution in iterative reconstruction algorithms) can significantly reduce the partial volume errors.

Kuczynski B, Reed B, Mungas D, Weiner M, Chui HC, **Jagust W**. Cognitive and anatomic contributions of metabolic decline in Alzheimer disease and cerebrovascular disease. *Archives of Neurology,* 2008 May;65(5):650-5. PMID: 18474742

BACKGROUND: Alzheimer disease and cerebrovascular disease affect elderly persons through alterations in brain structure and metabolism that produce cognitive decline. Understanding how each disease contributes to dementia is essential from both a pathophysiologic and diagnostic perspective. OBJECTIVE: To elucidate how baseline cognitive function (episodic memory and executive function) and brain anatomy (white matter hyperintensities and hippocampal volume) are associated with baseline (positron emission tomography-1 [PET1]) and longitudinal (PET2) glucose metabolism in 38 subjects older than 55 years ranging from normal cognition, cognitive impairment without dementia, and dementia. DESIGN: Cross-sectional regression analyses across subjects. SETTING: Multicenter, university-based study of subcortical vascular dementia. MAIN OUTCOME MEASURES: Regional cerebral glucose metabolism was the primary outcome, with the major hypotheses that memory and hippocampal volume are related to temporoparietal hypometabolism while executive function and white matter hyperintensities correlate with frontal lobe hypometabolism. RESULTS: Low baseline hippocampal volume predicted longitudinal development (PET2) of medial temporal hypometabolism. Lower memory was associated with parietal and cingulate hypometabolism at PET1, which increased at the 2-year-follow-up (PET2). Executive function was associated with frontal and temporoparietal hypometabolism at PET1 but only with frontal hypometabolism at follow-up. White matter hyperintensities predicted hypometabolism over time in the frontoparietal regions, predicting a rate of metabolic change (PET1 - PET2/time). CONCLUSIONS: Low baseline episodic memory and hippocampal volume predict the metabolic alterations associated with Alzheimer disease, whereas elevated baseline white matter hyperintensities predict a different pattern of metabolic decline that is plausibly associated with cerebrovascular disease.

Krauss SW, Spence JR, Bahmanyar S, Barth AI, Go MM, Czerwinski D, Meyer AJ. Downregulation of protein 4.1R, a mature centriole protein, disrupts centrosomes, alters cell cycle progression, and

perturbs mitotic spindles and anaphase. *Molecular and Cellular Biology, 2*008 Apr;28(7):2283-94. PMID: 18212055

> Centrosomes nucleate and organize interphase microtubules and are instrumental in mitotic bipolar spindle assembly, ensuring orderly cell cycle progression with accurate chromosome segregation. We report that the multifunctional structural protein 4.1R localizes at centrosomes to distal/subdistal regions of mature centrioles in a cell cycle-dependent pattern. Significantly, 4.1R-specific depletion mediated by RNA interference perturbs subdistal appendage proteins ninein and outer dense fiber 2/cenexin at mature centrosomes and concomitantly reduces interphase microtubule anchoring and organization. 4.1R depletion causes G(1) accumulation in p53-proficient cells, similar to depletion of many other proteins that compromise centrosome integrity. In p53-deficient cells, 4.1R depletion delays S phase, but aberrant ninein distribution is not dependent on the S-phase delay. In 4.1R-depleted mitotic cells, efficient centrosome separation is reduced, resulting in monopolar spindle formation. Multipolar spindles and bipolar spindles with misaligned chromatin are also induced by 4.1R depletion. Notably, all types of defective spindles have mislocalized NuMA (nuclear mitotic apparatus protein), a 4.1R binding partner essential for spindle pole focusing. These disruptions contribute to lagging chromosomes and aberrant microtubule bridges during anaphase/telophase. Our data provide functional evidence that 4.1R makes crucial contributions to the structural integrity of centrosomes and mitotic spindles which normally enable mitosis and anaphase to proceed with the coordinated precision required to avoid pathological events.

Le Scolan E, Zhu Q, Wang L, Bandyopadhyay A, Javelaud D, Mauviel A, Sun L, **Luo K**. Transforming growth factor-beta suppresses the ability of Ski to inhibit tumor metastasis by inducing its degradation. *Cancer Research*, 2008 May 1;68(9):3277-85. PMID: 18451154

> c-Ski is an important corepressor of transforming growth factor-beta (TGF-beta) signaling through its ability to bind to and repress the activity of the Smad proteins. It was initially identified as an oncogene that promotes anchorage-independent growth of chicken and quail embryo fibroblasts when overexpressed. Although increased Ski expression is detected in many human cancer cells, the roles of Ski in mammalian carcinogenesis have yet to be defined. Here, we report that reducing Ski expression in breast and lung cancer cells does not affect tumor growth but enhances tumor metastasis in vivo. Thus, in these cells, Ski plays an antitumorigenic role. We also showed that TGF-beta, a cytokine that is often highly expressed in metastatic tumors, induces Ski degradation through the ubiquitin-dependent proteasome in malignant human cancer cells. On TGF-beta treatment, the E3 ubiquitin ligase Arkadia mediates degradation of Ski in a Smad-dependent manner. Although Arkadia interacts with Ski in the absence of TGF-beta, binding of phosphorylated Smad2 or Smad3 to Ski is required to induce efficient degradation of Ski by Arkadia. Our results suggest that the ability of TGF-beta to induce degradation of Ski could be an additional mechanism contributing to its protumorigenic activity.

Brown LA, Kalloger SE, Miller MA, Shih IeM, McKinney SE, Santos JL, Swenerton K, **Spellman PT**, **Gray J**, Gilks CB, Huntsman DG. Amplification of 11q13 in ovarian carcinoma. *Genes, Chromosomes & Cancer,* 2008 Jun; 47(6):481-9. PMID: 18314909

> Amplification at the 11q13 locus is commonly observed in breast, ovarian, head and neck, oral, and esophageal cancer. Studies of this region led to the identification of multiple amplicons containing several potential oncogenes including EMSY, PAK1, RSF1, and GAB2. Here, we investigate the amplification of the above four genes and their prognostic significance in histologically and clinically defined subsets of ovarian cancer. Amplification of all four genes was assessed by fluorescent in situ hybridization in tissue microarrays containing 538 clinically annotated ovarian carcinomas with 12 years of follow-up data. Overall, for the entire cohort, EMSY was amplified in 44 (16%) of 269 cases, PAK1 was amplified in 38

(15%) of 255 cases, RSF1 was amplified in 37 (12%) of 310 cases, and GAB2 was amplified in 41 (16%) of 255 cases. Amplification of EMSY, PAK1, RSF1, and GAB2 were all highly correlated with each other and with a serous histology. Univariate survival analysis showed that tumors with EMSY and RSF1 amplification were associated with a significantly worse outcome. A molecular inversion probe array was then used to study the 11q13 amplicon in 33 high grade serous carcinomas. The core of the amplicon mapped to a 6-Mb region encompassing EMSY, PAK1, RSF1, and GAB2. However, a second more telomeric amplicon was also observed for which no candidate genes have been identified. In summary, amplification of these four putative oncogenes from 11q13 in early ovarian cancer is associated with a serous histology and in the case of EMSY and RSF1 a poor outcome. These findings support the hypothesis that the11q13 amplicon in ovarian cancer is likely driven by a cassette of genes rather than by a single oncogene.

Williams PT. A cohort study of incident hypertension in relation to changes in vigorous physical activity in men and women. *Journal of Hypertension*, 2008 Jun; 26(6):1085-93. PMID: 18475145

OBJECTIVE: To assess the dose-response relationship between changes in vigorous exercise (running distance, Deltakm per week) and physician-diagnosed hypertension. DESIGN: Twenty-four thousand, five hundred and fifty men and 10 113 women were followed prospectively for (mean +/- SD) 7.8 +/- 1.8 and 7.5 +/- 2.0 years, respectively. RESULTS: Among those who maintained their running distance within +/-5 km per week (5841 men), logistic regression showed that the log odds for hypertension was significantly lower for those who ran longer distances (coefficient+/-SE:-0.019 +/- 0.003 per km per week; P < 0.0001) even when adjusted for body mass index (-0.010 +/- 0.003 per km per week; P = 0.002). Analyses of all 24 550 male and 10 113 female runners showed that the log odds for hypertension declined significantly in relation to Deltakm per week in men (-0.009 +/- 0.001; P < 0.0001) and women (-0.006 +/- 0.003; P = 0.03), which remained significant when adjusted for body mass index in men (-0.005 +/- 0.001; P < 0.0001) but not in women (-0.004 +/- 0.003; P = 0.13). In both sexes, the decline was related to the distance run at the end of follow-up but not at baseline. Compared with men who ran less than 8 km per week, the age-specific rate for incident hypertension in those who ran more than 40 km per week at the end of follow-up was 80% lower in those aged between 35 and 44 years, 66% lower in those between 45 and 54 years, 69% lower in those aged between 55 and 64 years (all P < 0.0001), and 57% lower in those older than 65 years (P = 0.08). CONCLUSION: The odds of developing hypertension are reduced in those who remain vigorously active and increased in those whose vigorous activity declined. These effects are dependent on the exercise dose and are due in part to metabolic processes associated with body weight.

Williams PT. Effects of diet, physical activity and performance, and body weight on incident gout in ostensibly healthy, vigorously active men. *The American Journal of Clinical Nutrition,* 2008 May;87(5):1480-7. PMID: 18469274

BACKGROUND: Physical activity and cardiorespiratory fitness are not currently recognized as factors related to preventing gout, nor are risk factors for gout in physically active men well understood. OBJECTIVE: The objective was to identify risk factors for gout in ostensibly healthy, vigorously active men. DESIGN: Incident self-reported gout was compared with baseline diet, body mass index (BMI; in kg/m(2)), physical activity (in km/d run), and cardiorespiratory fitness (in m/s during 10-km footrace) prospectively in 28,990 male runners. RESULTS: Men (n = 228; 0.79%) self-reported incident gout during 7.74 y of follow-up. The risk of gout increased with higher alcohol intake [per 10 g/d; relative risk (RR): 1.19; 95% CI: 1.12, 1.26; P < 0.0001], meat consumption (per servings/d; RR: 1.45; 95% CI: 1.06, 1.92; P = 0.002), and BMI (RR: 1.19; 95% CI: 1.15, 1.23; P < 0.0001) and declined with greater fruit intake (per pieces/d; RR: 0.73; 95% CI: 0.62, 0.84; P < 0.0001), running distance (per km/d; RR: 0.92; 95% CI: 0.88, 0.97; P < 0.001), and fitness (per m/s; RR: 0.55; 95% CI: 0.41, 0.75; P < 0.0001). The RR per 10 g alcohol/d consumed as wine (1.27; P = 0.002), beer (1.19; P < 0.0001), and mixed drinks (1.13; P = 0.18) was not significantly

different from each other. Men who consumed > 15 g alcohol/d had 93% greater risk than abstainers, and men who averaged > 2 pieces fruit/d had 50% less risk than those who ate < 0.5 fruit/d. Risk of gout was 16-fold greater for BMI > 27.5 than < 20. Compared with the least active or fit men, those who ran > or = 8 km/d or > 4.0 m/s had 50% and 65% lower risk of gout, respectively. Lower BMI contributed to the risk reductions associated with distance run and fitness. CONCLUSION: These findings, based on male runners, suggest that the risk of gout is lower in men who are more physically active, maintain ideal body weight, and consume diets enriched in fruit and limited in meat and alcohol.

Williams PT. Vigorous exercise, fitness and incident hypertension, high cholesterol, and diabetes. *Medicine and Science in Sports and Exercise,* 2008 Jun;40(6):998-1006. PMID: 18461008

PURPOSE: Current physical activity guidelines are based in part on studies of cardiorespiratory fitness, however, the effects of fitness may differ from activity. Analyses were undertaken to determine the dose-response relationships of fitness to incident hypertension, hypercholesterolemia, and diabetes independent of activity. METHODS: Self-reported physician-diagnosed incident diabetes, hypercholesterolemia, and hypertension were compared to baseline running distance in 29,139 men and 11,985 women followed prospectively for 7.7 and 7.4 yr, respectively, and compared to cardiorespiratory fitness (m.s 10-km performance) in 85% of men and 76% of women. RESULTS: During follow up, 2342 men (8.53%) and 499 women (4.26%) became hypertensive, 3330 men (12.2%) and 599 women (5.14%) became hypercholesterolemic, and 197 men (0.68%) and 28 women (0.23%) became diabetic. Longer baseline distance predicted lower incident hypertension (men, P < 0.0001; women, P = 0.08), hypercholesterolemia (men and women, P < 0.0001), and diabetes (men, P < 0.001; women, P < 0.01) during follow up. The odds for hypercholesterolemia decreased significantly with each 16 km.wk increment in distance through 64 km.wk in men and 48 km.wk in women. Higher baseline fitness predicted significantly lower odds for incident hypertension (men, P < 0.0001; women, P < 0.001), hypercholesterolemia (men, P < 0.0001; women, P < 0.01), and diabetes (men, P < 0.001; women, P < 0.01), independent of distance. Compared to the least fit men, the fittest men had 62% lower odds for becoming hypertensive, 67% lower odds for becoming hypercholesterolemic, and 86% lower odds for becoming diabetic. When adjusted for BMI, greater fitness predicted significantly lower odds for hypertension, hypercholesterolemia, and diabetes in men. CONCLUSIONS: Higher cardiorespiratory fitness reduces the odds for hypertension, hypercholesterolemia, and diabetes, independent of physical activity and is an important risk factor separate from physical activity.