



LIFE SCIENCES DIVISION E-NEWSLETTER

November/December, 2008

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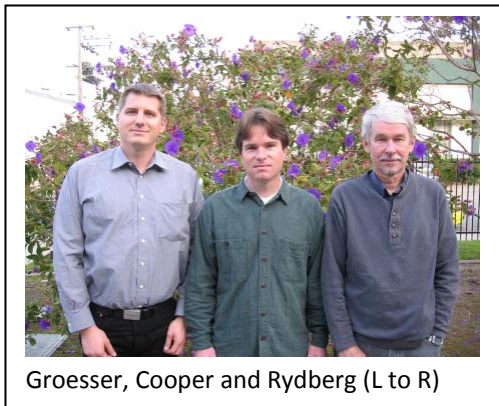
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DOE scientific focus area notes

Low Dose Radiation Research

Bystander Effects Paper Published in *Radiation Research*

Life Sciences **Torsten Groesser**, **Brian Cooper** and **Bjorn Rydberg** have published their study on radiation-induced bystander effects in the December issue of *Radiation Research*. The work was funded by NASA and by the Low Dose program.



The aim of this work was to study radiation-induced bystander effects for early cytogenetic end points in various cell lines using the medium transfer technique after exposure to high- and low-LET radiation. Cells were exposed to 20 MeV/ nucleon nitrogen ions, 968 MeV/nucleon iron ions, or 575 MeV/nucleon iron ions followed by transfer of the conditioned medium from the irradiated cells to unirradiated test cells. The effects studied included DNA double-strand break induction, γ -H2AX focus formation, induction of chromatid breaks in prematurely condensed chromosomes, and micronucleus formation using DNA repair-proficient and -deficient hamster and human cell lines

(xrs6, V79, SW48, MO59K and MO59J). Cell survival was also measured in SW48 bystander cells using X rays. Although it was occasionally possible to detect an increase in chromatid break levels using nitrogen ions and to see a higher number of γ -H2AX foci using nitrogen and iron ions in xrs6 bystander cells in single experiments, the results were not reproducible. After all the data was pooled, no significant bystander effect for any of these end points could be verified. Also, no significant bystander effect for DSB induction or micronucleus formation in these cell lines or for clonogenic survival in SW48 cells was detected. The data suggest that DNA damage and cytogenetic changes are not induced in bystander cells. In contrast, data in the literature show pronounced bystander effects in a variety of cell lines, including clonogenic survival in SW48 cells and induction of chromatid breaks and micronuclei in hamster cells. To reconcile these conflicting data, it is possible that the epigenetic status of the specific cell line or the precise culture conditions and medium supplements, such as serum, may be critical for inducing bystander effects.

Groesser, T, Cooper, B and Rydberg, B. Lack of Bystander Effects from High-LET Radiation for Early Cytogenetic End Points. *Radiation Research*, 2008 Dec. 170, 794–802.

Torsten Groesser, 12/08

International Meeting on Carcinogenesis and Low Dose Radiation Exposure

Francesco Marchetti was an invited speaker at the International Symposium on Carcinogenesis and Genetic Effects of Low Dose Radiation Exposure organized by the Institute for Environmental Sciences of Japan and held at the Cultural Exchange Plaza in Rokkasho, Aomori, Japan on October 7-8, 2008. The symposium brought together scientists from Japan, the U.S. and Europe to exchange up-to-date information on the carcinogenic and genetic effects of low dose radiation exposure. The non-

carcinogenic and transgenerational effects of low dose radiation were also discussed during the symposium. Marchetti presented his work on the effects of DNA repair during the early stages of mammalian development in preventing transmissible genetic defects.

Francesco Marchetti, 11/08

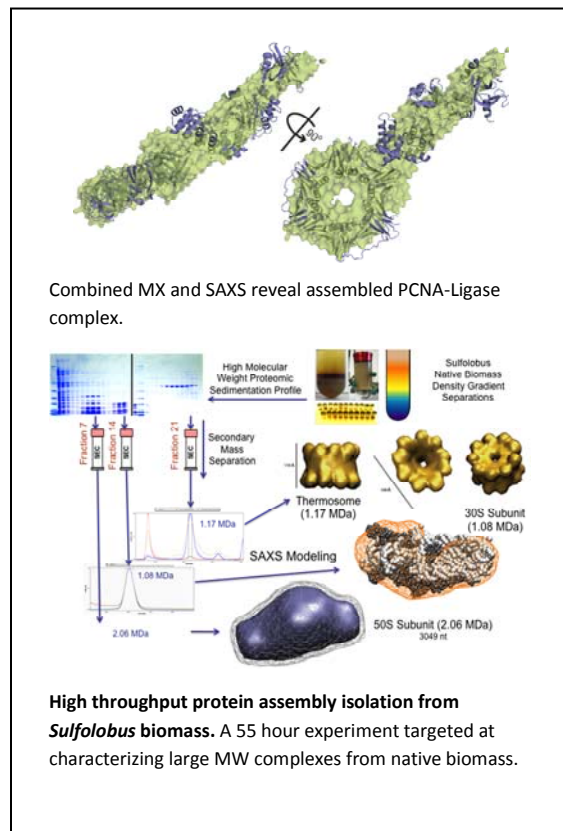
GTL-Genomics

Re-Weaving Cell Networks for GTL at Berkeley Lab

Isaac Newton is known for his famous dissection of sunlight, but it was his ability to recombine the separated colors to reform white light that provided the deep insight into sunlight and colors. At Berkeley Lab, MAGGIE program investigators are working with program leader **John Tainer** to understand how the protein threads are woven into metabolic networks critical to cell biology, such as those that act in DNA replication and the repair of DNA damage.

In prior results, the MAGGIE project used SAXS at the SIBLYS beamline at the ALS to define the flexible, extended ligase complex with PCNA. In the last month, Berkeley Lab team members **Robert Rambo** and **Brian Chapados** were able to successfully re-constitute a heterotrimeric PCNA complex and then bind this to both the flap endonuclease called FEN and the DNA Ligase (top figure). This complex forms a key protein network for both DNA replication and DNA repair by removing primers and displaced flaps and ligating DNA pieces. *Sulfolobus* project leader **Steve Yannone** is also working to isolate these PCNA complexes directly from cells and together with Robert Rambo have developed new methods to separate large complexes without tags (bottom figure). "The ability to re-weave protein networks will allow us to test our understanding of basic biological processes and ultimately permit the rational redesign of microbial networks to accomplish DOE goals in bio-energy and bio-remediation", noted Steve Yannone.

Steve Yannone, 12/08



Nuclear Medicine

BER Workshop

Six Life Science Division researchers helped organize and participated in a special BER workshop, held on November 5 and 6, 2008 in Washington, DC, entitled "New Frontiers of Science in Radiochemistry and Instrumentation for Radionuclide Imaging: Creating the Tools for Research Advances in Biology, Environmental Sciences and Nuclear Medicine." This workshop was organized by Dr. Prem Srivastava of

BER as an initial step in the redirection of the nuclear medicine chemistry and instrumentation program of DOE toward applications to plant, microbial and environmental research. The goal of the new direction is to assist the U.S. efforts in biofuels development and in mitigation and decontamination processes.

The theme of the workshop was to have radiotracer chemists and instrumentation specialists share information with biologists and environmental scientists with respect to what major scientific problems or questions their respective fields have that might be amenable to imaging methods. Principal questions addressed during the conference were: How does the information provided by radionuclide imaging compare to that from other imaging modalities and detection methods? What are the opportunities for improvements in chemical methods and instrumentation that will enable investigations by biological and environmental scientists? In the past there have been substantial progress made in biological discovery including photosynthesis enabled by the Berkeley Radiation Lab and Donner Lab scientists since the 1940s. But the world literature contains less than 100 scientific papers since that time probably due to limited availability of short-lived radionuclides such as those of carbon, nitrogen, and oxygen, and the accessibility to specialized instruments such as positron tomography. The intent of BER is to help the nation's efforts in alternative fuel development and mitigation of environmental hazards by encouraging a partnership between radiotracer scientists and scientists involved in biological and environmental research.

Thomas Budinger of Life Sciences co-chaired the workshop with John Katzenellenbogen from Washington University. Other Life Sciences Division participants were **Steven Denzeno, Bill Moses, Jim O'Neil, Bahram Parvin** and **Scott Taylor**. The workshop report, developed by Berkeley Lab scientists and other workshop participants, is currently in the final review process.

Thomas Budinger, 12/08

Scientific news

Study Reveals Cell Senescence is a Double-Edged Sword

Judy Campisi, of the Life Sciences Division, led a study in which it was revealed that cellular senescence, the process by which biological cells stop dividing in response to DNA damage, is a double-edged sword. While this process effectively acts to prevent cancer in younger persons, it was shown to actually promote cancer in older individuals. Campisi's findings hold implications for chemotherapy, the deleterious effects of old age, and a theory on evolution.

More > <http://newscenter.lbl.gov/press-releases/2008/12/02/cellular-senescence/>

Today at Berkeley Lab, 12/3/08



Broccoli Compound Targets Key Enzyme in Cancer

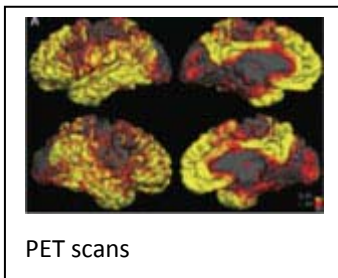
An anti-cancer compound found in broccoli and cabbage works by lowering the activity of an enzyme associated with rapidly advancing breast cancer, according to a UC Berkeley study by **Gary Firestone**, with Berkeley Lab's Life Sciences Division, and Leonard Bjeldanes. The compound, indole-3-carbinol, is

already undergoing clinical trials in humans because it was found to stop the growth of breast and prostate cancer cells in mice. More >

http://www.berkeley.edu/news/media/releases/2008/12/02_indole.shtml

Today at Berkeley Lab, 12/5/08

New Clue in the Search to Predict Alzheimer's Disease



Scientists have identified a cluster of events that could signal Alzheimer's disease. In some cases, an elderly person with increased amyloid plaque in the brain is more likely to have a smaller hippocampus and poorer episodic memory. More >

<http://newscenter.lbl.gov/feature-stories/2008/12/16/predict-alzheimers-disease/>

Berkeley Lab Feature Story, 12/16/08

Life Sciences Departments Reorganized

To better support the Life Sciences Division scientific programs, Joe Gray, in consultation with Division leaders, has reorganized the division's departments. The four new departments are as follows: Bioenergy/GTL & Structural Biology: Kenneth Downing, Dept Head; Cancer & DNA Damage Responses: Priscilla Cooper, Dept Head and Andrew Wyrobek, Deputy Dept Head; Genome Dynamics: Susan Celniker, Dept Head and Terumi Kohwi-Shigematsu, Deputy Dept Head; Radiotracer Development & Imaging Technology: Stephen Derenzo, Dept Head and William Jagust, Deputy Dept Head. While most Dept Heads and Deputy Dept Heads already served in a leadership role prior to the reorganization, both Kohwi-Shigematsu and Celniker, the latter recently promoted to Senior Scientist, are new to this role.

Research in the Division continues to support the Department of Energy (DOE) in a number of its Scientific Focus Areas, namely: Radiochemistry & Instrumentation, Low Dose Radiation, and Genomics/GTL, and many areas of basic and applied biology. The new departmental structure now aligns with these scientific themes. Also, the revised organization is intended to keep Division members well-informed about Berkeley Lab and Life Sciences Divisional issues, developments, opportunities and challenges, including a strong emphasis on safety awareness and best practices. It will also support Division management's understanding of the accomplishments, needs and challenges of all of our research programs. It is expected the new organization will result in a stronger, more interactive Life Sciences Division. Org chart >

http://www.lbl.gov/lsd/People_& Organization/Organization_Chart/index.html

CG, 12/08

New Division Assignments and Safety Coordinator Hire

To strengthen the Divisional research infrastructure for the benefit of all of its laboratories, Damir Sudar, Tony Linard and Scott Taylor have accepted important new assignments: **Damir Sudar** will be serving as the new Life Sciences Division Deputy for Technology to assist the division in building a much-improved technological base for the divisional research. Sudar will serve as a principal contact point, spokesperson and source of information on the integration of cutting-edge technology in support of

multiple collaborative programs within Life Sciences and Berkeley Lab. He will work closely with Joe Gray and other Division leaders to develop strategies for scientific growth involving large programs and new resources for the Division, the Biosciences area, and Laboratory's DOE/Office of Biological and Environmental Research (OBER) programs. **Tony Linard** will be serving as the new Division Facilities and Resources Manager. Working closely with Sudar, Linard will provide high-level project management for divisional capital projects (e.g., Building 74 seismic upgrade and lab modernization, construction of a new research bldg, etc) and renovation projects (e.g., Building 83, Potter, etc), along with routine maintenance and repairs for all divisional space. He will also contribute his expertise to the management of the Divisions core research resources and facilities. **Scott Taylor** has agreed to serve as the new Division Safety Officer, assisting Joe Gray with the oversight of safety in the Division.

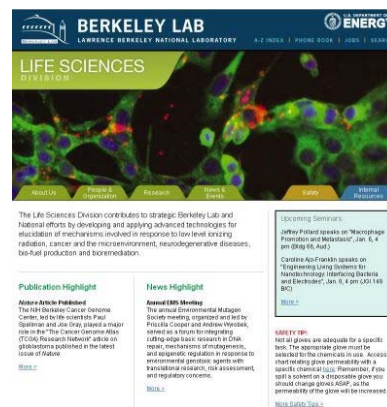
A recruitment effort for a new Life Sciences Safety Coordinator with a professional EH&S background has resulted in the hire of **Andrew Peterson, MPH, CIH**, who will begin supporting the Division's safety program January 20, 2009. Peterson is an accomplished Health, Safety and Environmental professional with 10 years of experience, including extensive program development and implementation. Peterson previously served as EH&S Manager of Xilinx, Inc. where he was responsible for the development, implementation and management of the environment, health and safety management program for Xilinx.

CG, 11/08

New Division Website Now Live

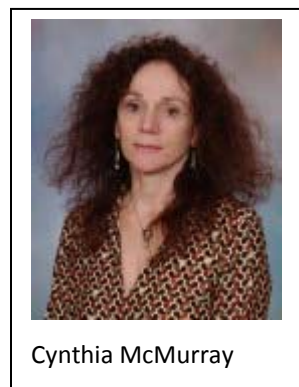
In an effort to revitalize the Life Sciences Division's presence on the World Wide Web and to reflect the new departmental structure of the Division, a new divisional website has been launched at <http://www.lbl.gov/lsd/>

CG, 12/08



McMurray Joins Division

The Life Sciences Division welcomes **Cynthia T. McMurray** as Senior Staff Scientist in the Genome Dynamics Department. The appointment of McMurray, a leader in the fields of neurodegenerative



Cynthia McMurray

disease, DNA repair and molecular machine analysis technology, was approved by the Lab Director after review of her appointment recommendation by the Division Staff Committee, Joe Gray, and the Laboratory Staff Committee. Since 1999, McMurray has held the position of Professor of Molecular Pharmacology, Experimental Therapeutics, Biochemistry and Molecular Biology at the Mayo Medical Foundation in Rochester, MN. Her research focuses on mechanisms of neurodegenerative disease with emphasis on Huntington's disease. She has clearly demonstrated the role of triplet repeat expansion as a causative factor in the disease and has pioneered the use of high resolution molecular machine analysis including multi-scale imaging to elucidate how mismatch repair proteins interact with DNA to produce the triplet repeat expansions.

McMurray's stature in the scientific community is supported by her appointment to the Scientific Advisory Board for the National Institute of Environmental Science, the NSF Neuronal and Glial Mechanics study section and the NIH NIDDS study section and her designation as a Distinguished Mayo Investigator in 2001. She has demonstrated scientific leadership/management skills and a collaborative spirit that will serve Berkeley Lab well. To that end she has agreed to organize and lead a Flow and Image core at Berkeley Lab.

CG, 11/08

Renovation of Building 83 Completed

The renovation of Building 83 has been completed, and the research group of **Cynthia McMurray** has started to move in. McMurray is a new recruit to the Life Sciences Division and comes from the Mayo Clinic in Rochester, MN. The Division is also looking forward to having **John Tainer's** research group in this space, when he moves to Berkeley Lab from The Scripps Research Institute in San Diego. Tainer, who has held a Visiting Faculty position in the Life Sciences Division since 2000, has been working closely with Berkeley Lab researchers over the past years. Building 83 was built in 1979 specifically for Mina Bissell's groundbreaking research in cell-microenvironment

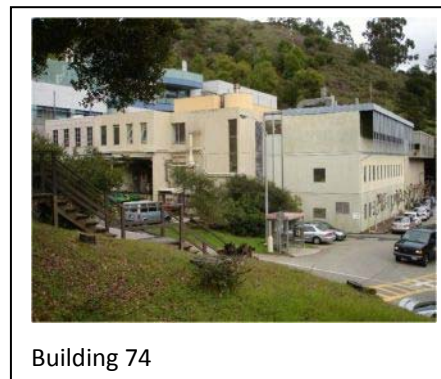


Interior of Building 83

interactions and has served Life Sciences' research very well. The just-completed, 18-month renovation has resulted in a modern, open floor plan and flexible lab space where research can be conducted safely and productively. The building has a state-of-the-art microscopy suite for live cell imaging, a crystal-growing facility, and tissue culture space. The renovation was completed on schedule and under budget by the Berkeley Lab Facilities Dept working closely with the Division's **Tony Linard**.

Damir Sudar, 12/08

Seismic Upgrade of Building 74 to Start



Building 74

Life Sciences Building 74 has been vacated as planned in time for the seismic upgrade of B74 to begin January 26, 2009. Life Sciences members attended an excellent presentation given to the Division by Jack Heffernan of the Berkeley Lab Facilities Dept informing members about potential impacts from extensive construction activities in and around the building. The presentation included background information about seismic risk assessment at Berkeley Lab and the specific seismic problems and fixes planned for B74. Members are being informed and are warned to exercise caution when traveling in the Strawberry Canyon area nearby B74, as the construction lay-down begins and the work progresses over the next years. The seismic upgrade phase is expected to be completed by October 2009, followed by a full renovation phase according to the current plans. During the construction period research is being conducted in adjacent life sciences buildings.

CG, 12/08

January BOP Retreat and Poster Session

The UCSF Helen Diller Cancer Center's Breast Oncology Program (BOP), co-led by **Joe Gray**, will hold a retreat on January 29-30, 2009 at the Jewish Community Center of San Francisco, featuring the latest research in a number of areas. A poster session will also be held, and investigators are encouraged to submit a poster in one of four categories: molecular markers, early detection, clinical trials, and experimental therapeutics. Online registration details and poster entry will be available soon. The Breast Oncology Program at UCSF is a longstanding program that has served as a model for other translational research programs within the Cancer Center. Involving nearly 60 faculty scientists, the program supports and stimulates basic, clinical, and population research in breast cancer and facilitates translation of these findings into improved cancer management and control. Program research is supported by major grants from the National Cancer Institute (NCI), the U.S. Department of Defense, and other agencies.

CG, 12/08

Awards

Life Sciences Student Among First-Place Winners of Energy Competition



Kiran Rangaraj

Kiran Rangaraj, with the Lab's Life Sciences Division's **Cooper Lab**, was among six national science students to win first-place honors at the DOE's inaugural Science and Energy Research Challenge (SERCh) at Oak Ridge National Laboratory. Student projects focused primarily on energy solutions, with competition categories in biological sciences, materials science and engineering, chemistry, physics, and environmental science. The first-place winners each won \$3,000 scholarships. The Science and Energy Research Challenge is part of DOE's ongoing educational outreach efforts. More >

[http://www.ornl.gov/info/press_releases/get_press_release.cfm?ReleaseNumber=mr](http://www.ornl.gov/info/press_releases/get_press_release.cfm?ReleaseNumber=mr20081111-00)

[20081111-00](#)

Today at Berkeley Lab, 11/13/08

Recent publications (selected)

Ghajar CM, **Bissell MJ**. Extracellular matrix control of mammary gland morphogenesis and tumorigenesis: insights from imaging. *Histochemistry and Cell Biology*, 2008 Dec; 130(6):1105-18. PMID: 19009245

The extracellular matrix (ECM), once thought to solely provide physical support to a tissue, is a key component of a cell's microenvironment responsible for directing cell fate and maintaining tissue specificity. It stands to reason, then, that changes in the ECM itself or in how signals from the ECM are presented to or interpreted by cells can disrupt tissue organization; the latter is a necessary step for malignant progression. In this review, we elaborate on this concept using the mammary gland as an example. We describe how the ECM directs mammary gland formation and function, and discuss how a cell's inability to interpret these signals—whether as a result of genetic insults or physicochemical

alterations in the ECM-disorganizes the gland and promotes malignancy. By restoring context and forcing cells to properly interpret these native signals, aberrant behavior can be quelled and organization re-established. Traditional imaging approaches have been a key complement to the standard biochemical, molecular, and cell biology approaches used in these studies. Utilizing imaging modalities with enhanced spatial resolution in live tissues may uncover additional means by which the ECM regulates tissue structure, on different length scales, through its pericellular organization (short-scale) and by biasing morphogenic and morphostatic gradients (long-scale).

Ponomarev AL, **Costes SV**, Cucinotta FA. Stochastic properties of radiation-induced DSB: DSB distributions in large scale chromatin loops, the HPRT gene and within the visible volumes of DNA repair foci. *International Journal Radiation Biology*, 2008 Nov;84(11):916-29. PMID: 19016140

Purpose: We computed probabilities to have multiple double-strand breaks (DSB), which are produced in DNA on a regional scale, and not in close vicinity, in volumes matching the size of DNA damage foci, of a large chromatin loop, and in the physical volume of DNA containing the HPRT (human hypoxanthine phosphoribosyltransferase) locus. Materials and methods: The model is based on a Monte Carlo description of DSB formation by heavy ions in the spatial context of the entire human genome contained within the cell nucleus, as well as at the gene sequence level. Results: We showed that a finite physical volume corresponding to a visible DNA repair focus, believed to be associated with one DSB, can contain multiple DSB due to heavy ion track structure and the DNA supercoiled topography. A corrective distribution was introduced, which was a conditional probability to have excess DSB in a focus volume, given that there was already one present. The corrective distribution was calculated for 19.5 MeV/amu N ions, 3.77 MeV/amu alpha-particles, 1000 MeV/amu Fe ions, and X-rays. The corrected initial DSB yield from the experimental data on DNA repair foci was calculated. The DSB yield based on the corrective function converts the focus yield into the DSB yield, which is comparable with the DSB yield based on the earlier PFGE experiments. The distribution of DSB within the physical limits of the HPRT gene was analyzed by a similar method as well. Conclusion: This corrective procedure shows the applicability of the model and empowers the researcher with a tool to better analyze focus statistics. The model enables researchers to analyze the DSB yield based on focus statistics in real experimental situations that lack one-to-one focus-to-DSB correspondance.

Bhalla N, Wynne DJ, Jantsch V, **Dernburg AF**. ZHP-3 acts at crossovers to couple meiotic recombination with synaptonemal complex disassembly and bivalent formation in *C. elegans*. *PLoS Genetics*, 2008 Oct;4(10):e1000235. PMID: 18949042

Crossover recombination and the formation of chiasmata normally ensure the proper segregation of homologous chromosomes during the first meiotic division. *zhp-3*, the *Caenorhabditis elegans* ortholog of the budding yeast ZIP3 gene, is required for crossover recombination. We show that ZHP-3 protein localization is highly dynamic. At a key transition point in meiotic prophase, the protein shifts from along the length of the synaptonemal complex (SC) to an asymmetric localization on the SC and eventually becomes restricted to foci that mark crossover recombination events. A *zhp-3::gfp* transgene partially complements a null mutation and reveals a separation of function; although the fusion protein can promote nearly wild-type levels of recombination, aneuploidy among the progeny is high, indicating defects in meiotic chromosome segregation. The structure of bivalents is perturbed in this mutant, suggesting that the chromosome segregation defect results from an inability to properly remodel chromosomes in response to crossovers. *smo-1* mutants exhibit phenotypes similar to *zhp-3::gfp* mutants at higher temperatures, and *smo-1; zhp-3::gfp* double mutants exhibit more severe meiotic defects than either single mutant, consistent with a role for SUMO in the process of SC disassembly and bivalent differentiation. We propose that coordination of crossover recombination with SC disassembly and bivalent formation reflects a conserved role of Zip3/ZHP-3 in coupling recombination with SC morphogenesis.

Grosser, T, Cooper, B and Rydberg, B. Lack of Bystander Effects from High-LET Radiation for Early Cytogenetic End Points. *Radiation Research*, 2008 Dec. 170, 794–802.

The aim of this work was to study radiation-induced bystander effects for early cytogenetic end points in various cell lines using the medium transfer technique after exposure to high- and low-LET radiation. Cells were exposed to 20 MeV/nucleon nitrogen ions, 968 MeV/nucleon iron ions, or 575 MeV/nucleon iron ions followed by transfer of the conditioned medium from the irradiated cells to unirradiated test cells. The effects studied included DNA double-strand break induction, γ -H2AX focus formation, induction of chromatid breaks in prematurely condensed chromosomes, and micronucleus formation using DNA repair-proficient and -deficient hamster and human cell lines (xrs6, V79, SW48, MO59K and MO59J). Cell survival was also measured in SW48 bystander cells using X rays. Although it was occasionally possible to detect an increase in chromatid break levels using nitrogen ions and to see a higher number of γ -H2AX foci using nitrogen and iron ions in xrs6 bystander cells in single experiments, the results were not reproducible. After we pooled all the data, we could not verify a significant bystander effect for any of these end points. Also, we did not detect a significant bystander effect for DSB induction or micronucleus formation in these cell lines or for clonogenic survival in SW48 cells. The data suggest that DNA damage and cytogenetic changes are not induced in bystander cells. In contrast, data in the literature show pronounced bystander effects in a variety of cell lines, including clonogenic survival in SW48 cells and induction of chromatid breaks and micronuclei in hamster cells. To reconcile these conflicting data, it is possible that the epigenetic status of the specific cell line or the precise culture conditions and medium supplements, such as serum, may be critical for inducing bystander effects.

Mukherjee S, Pelech S, Neve RM, **Kuo WL, Ziyad S, Spellman PT, Gray JW**, Speed TP. Sparse combinatorial inference with an application in cancer biology. *Bioinformatics*, 2008 Nov 27. [Epub ahead of print] PMID: 19038985

MOTIVATION: Combinatorial effects, in which several variables jointly influence an output or response, play an important role in biological systems. In many settings, Boolean functions provide a natural way to describe such influences. However, biochemical data using which we may wish to characterize such influences are usually subject to much variability. Furthermore, in high-throughput biological settings Boolean relationships of interest are very often sparse, in the sense of being embedded in an overall dataset of higher dimensionality. This motivates a need for statistical methods capable of making inferences regarding Boolean functions under conditions of noise and sparsity. RESULTS: We put forward a statistical model for sparse, noisy Boolean functions and methods for inference under the model. We focus on the case in which the form of the underlying Boolean function, as well as the number and identity of its inputs are all unknown. We present results on synthetic data and on a study of signalling proteins in cancer biology.

Matthews L, Gopinath G, Gillespie M, Caudy M, Croft D, de Bono B, Garapati P, Hemish J, Hermjakob H, Jassal B, Kanapin A, **Lewis S**, Mahajan S, May B, Schmidt E, Vastrik I, Wu G, Birney E, Stein L, D'Eustachio P. Reactome knowledgebase of human biological pathways and processes. *Nucleic Acids Research*, 2008 Nov 3. [Epub ahead of print] PMID: 18981052

Reactome (<http://www.reactome.org>) is an expert-authored, peer-reviewed knowledgebase of human reactions and pathways that functions as a data mining resource and electronic textbook. Its current release includes 2975 human proteins, 2907 reactions and 4455 literature citations. A new entity-level pathway viewer and improved search and data mining tools facilitate searching and visualizing pathway data and the analysis of user-supplied high-throughput data sets. Reactome has increased its utility to the model organism communities with improved orthology prediction methods allowing pathway inference

for 22 species and through collaborations to create manually curated Reactome pathway datasets for species including Arabidopsis, Oryza sativa (rice), Drosophila and Gallus gallus (chicken). Reactome's data content and software can all be freely used and redistributed under open source terms.

Erhardt S, **Mellone BG**, Betts CM, **Zhang W**, **Karpen GH**, Straight AF. Genome-wide analysis reveals a cell cycle-dependent mechanism controlling centromere propagation. *The Journal of Cell Biology*, 2008 Dec 1;183(5):805-18. PMID: 19047461

Centromeres are the structural and functional foundation for kinetochore formation, spindle attachment, and chromosome segregation. In this study, we isolated factors required for centromere propagation using genome-wide RNA interference screening for defects in centromere protein A (CENP-A; centromere identifier [CID]) localization in *Drosophila melanogaster*. We identified the proteins CAL1 and CENP-C as essential factors for CID assembly at the centromere. CID, CAL1, and CENP-C coimmunoprecipitate and are mutually dependent for centromere localization and function. We also identified the mitotic cyclin A (CYCA) and the anaphase-promoting complex (APC) inhibitor RCA1/Emi1 as regulators of centromere propagation. We show that CYCA is centromere localized and that CYCA and RCA1/Emi1 couple centromere assembly to the cell cycle through regulation of the fizzy-related/CDH1 subunit of the APC. Our findings identify essential components of the epigenetic machinery that ensures proper specification and propagation of the centromere and suggest a mechanism for coordinating centromere inheritance with cell division.

Rabinovici GD, **Jagust WJ**, Furst AJ, Ogar JM, Racine CA, **Mormino EC**, **O'Neil JP**, Lal RA, Dronkers NF, Miller BL, Gorno-Tempini ML. Abeta amyloid and glucose metabolism in three variants of primary progressive aphasia. *Annals of Neurology*, 2008 Oct;64(4):388-401. PMID: 18991338

OBJECTIVE: Alzheimer's disease (AD) is found at autopsy in up to one third of patients with primary progressive aphasia (PPA), but clinical features that predict AD pathology in PPA are not well defined. We studied the relationships between language presentation, Abeta amyloidosis, and glucose metabolism in three PPA variants using [11C]-Pittsburgh compound B ([11C]PIB) and [18F]-labeled fluorodeoxyglucose positron emission tomography ([18F]FDG-PET). METHODS: Patients meeting PPA criteria (N = 15) were classified as logopenic aphasia (LPA), progressive nonfluent aphasia (PNFA), or semantic dementia (SD) based on language testing. [11C]PIB distribution volume ratios were calculated using Logan graphical analysis (cerebellar reference). [18F]FDG images were normalized to pons. Partial volume correction was applied. RESULTS: Elevated cortical PIB (by visual inspection) was more common in LPA (4/4 patients) than in PNFA (1/6) and SD (1/5) ($p < 0.02$). In PIB-positive PPA, PIB uptake was diffuse and indistinguishable from the pattern in matched AD patients ($n = 10$). FDG patterns were focal and varied by PPA subtype, with left temporoparietal hypometabolism in LPA, left frontal hypometabolism in PNFA, and left anterior temporal hypometabolism in SD. FDG uptake was significant asymmetric (favoring left hypometabolism) in PPA ($p < 0.005$) but not in AD. INTERPRETATION: LPA is associated with Abeta amyloidosis, suggesting that subclassification of PPA based on language features can help predict the likelihood of AD pathology. Language phenotype in PPA is closely related to metabolic changes that are focal and anatomically distinct between subtypes, but not to amyloid deposition patterns that are diffuse and similar to AD.

Holcomb VB, **Rodier F**, Choi Y, Busuttill RA, Vogel H, Vijg J, **Campisi J**, Hasty P. Ku80 deletion suppresses spontaneous tumors and induces a p53-mediated DNA damage response. *Cancer Research*, 2008 Nov 15;68(22):9497-502. PMID: 19010925

Ku80 facilitates DNA repair and therefore should suppress cancer. However, ku80(-/-) mice exhibit reduced cancer, although they age prematurely and have a shortened life span. We tested the hypothesis that Ku80 deletion suppresses cancer by enhancing cellular tumor-suppressive responses to inefficiently

repaired DNA damage. In support of this hypothesis, Ku80 deletion ameliorated tumor burden in APC(MIN) mice and increased a p53-mediated DNA damage response, DNA lesions, and chromosomal rearrangements. Thus, contrary to its assumed role as a caretaker tumor suppressor, Ku80 facilitates tumor growth most likely by dampening baseline cellular DNA damage responses.

Garcin ED, Arvai AS, Rosenfeld RJ, Kroeger MD, Crane BR, Andersson G, Andrews G, Hamley PJ, Mallinder PR, Nicholls DJ, St-Gallay SA, Tinker AC, Gensmantel NP, Mete A, Cheshire DR, Connolly S, Stuehr DJ, Aberg A, Wallace AV, **Tainer JA**, Getzoff ED. Anchored plasticity opens doors for selective inhibitor design in nitric oxide synthase. *Nature Chemical Biology*, 2008 Nov;4(11):700-7. PMID: 18849972

Nitric oxide synthase (NOS) enzymes synthesize nitric oxide, a signal for vasodilatation and neurotransmission at low concentrations and a defensive cytotoxin at higher concentrations. The high active site conservation among all three NOS isozymes hinders the design of selective NOS inhibitors to treat inflammation, arthritis, stroke, septic shock and cancer. Our crystal structures and mutagenesis results identified an isozyme-specific induced-fit binding mode linking a cascade of conformational changes to a new specificity pocket. Plasticity of an isozyme-specific triad of distant second- and third-shell residues modulates conformational changes of invariant first-shell residues to determine inhibitor selectivity. To design potent and selective NOS inhibitors, we developed the anchored plasticity approach: anchor an inhibitor core in a conserved binding pocket, then extend rigid bulky substituents toward remote specificity pockets, which become accessible upon conformational changes of flexible residues. This approach exemplifies general principles for the design of selective enzyme inhibitors that overcome strong active site conservation.

Williams RS, Moncalian G, Williams JS, Yamada Y, Limbo O, Shin DS, Grocock LM, Cahill D, Hitomi C, Guenther G, Moiani D, Carney JP, Russell P, **Tainer JA**. Mre11 dimers coordinate DNA end bridging and nuclease processing in double-strand-break repair. *Cell*, 2008 Oct 3;135(1):97-109. PMID: 18854158

Mre11 forms the core of the multifunctional Mre11-Rad50-Nbs1 (MRN) complex that detects DNA double-strand breaks (DSBs), activates the ATM checkpoint kinase, and initiates homologous recombination (HR) repair of DSBs. To define the roles of Mre11 in both DNA bridging and nucleolytic processing during initiation of DSB repair, we combined small-angle X-ray scattering (SAXS) and crystal structures of *Pyrococcus furiosus* Mre11 dimers bound to DNA with mutational analyses of fission yeast Mre11. The Mre11 dimer adopts a four-lobed U-shaped structure that is critical for proper MRN complex assembly and for binding and aligning DNA ends. Further, mutations blocking Mre11 endonuclease activity impair cell survival after DSB induction without compromising MRN complex assembly or Mre11-dependant recruitment of Ctp1, an HR factor, to DSBs. These results show how Mre11 dimerization and nuclease activities initiate repair of DSBs and collapsed replication forks, as well as provide a molecular foundation for understanding cancer-causing Mre11 mutations in ataxia telangiectasia-like disorder (ATLD).

Edwards RA, Lee MS, **Tsutakawa SE**, Williams RS, **Tainer JA**, Glover JN. The BARD1 C-terminal domain structure and interactions with polyadenylation factor CstF-50. *Biochemistry*, 2008 Nov 4;47(44):11446-56. PMID: 18842000

The BARD1 N-terminal RING domain binds BRCA1 while the BARD1 C-terminal ankyrin and tandem BRCT repeat domains bind CstF-50 to modulate mRNA processing and RNAP II stability in response to DNA damage. Here we characterize the BARD1 structural biochemistry responsible for CstF-50 binding. The crystal structure of the BARD1 BRCT domain uncovers a degenerate phosphopeptide binding pocket lacking the key arginine required for phosphopeptide interactions in other BRCT proteins. Small angle X-ray scattering together with limited proteolysis results indicates that ankyrin and BRCT domains are linked

by a flexible tether and do not adopt a fixed orientation relative to one another. Protein pull-down experiments utilizing a series of purified BARD1 deletion mutants indicate that interactions between the CstF-50 WD-40 domain and BARD1 involve the ankyrin-BRCT linker but do not require ankyrin or BRCT domains. The structural plasticity imparted by the ANK-BRCT linker helps to explain the regulated assembly of different protein BARD1 complexes with distinct functions in DNA damage signaling including BARD1-dependent induction of apoptosis plus p53 stabilization and interactions. BARD1 architecture and plasticity imparted by the ANK-BRCT linker are suitable to allow the BARD1 C-terminus to act as a hub with multiple binding sites to integrate diverse DNA damage signals directly to RNA polymerase.