



Life Sciences Division

E-Newsletter September 28, 2007

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***E.coli* and the uncovering of novel protein-protein interactions**

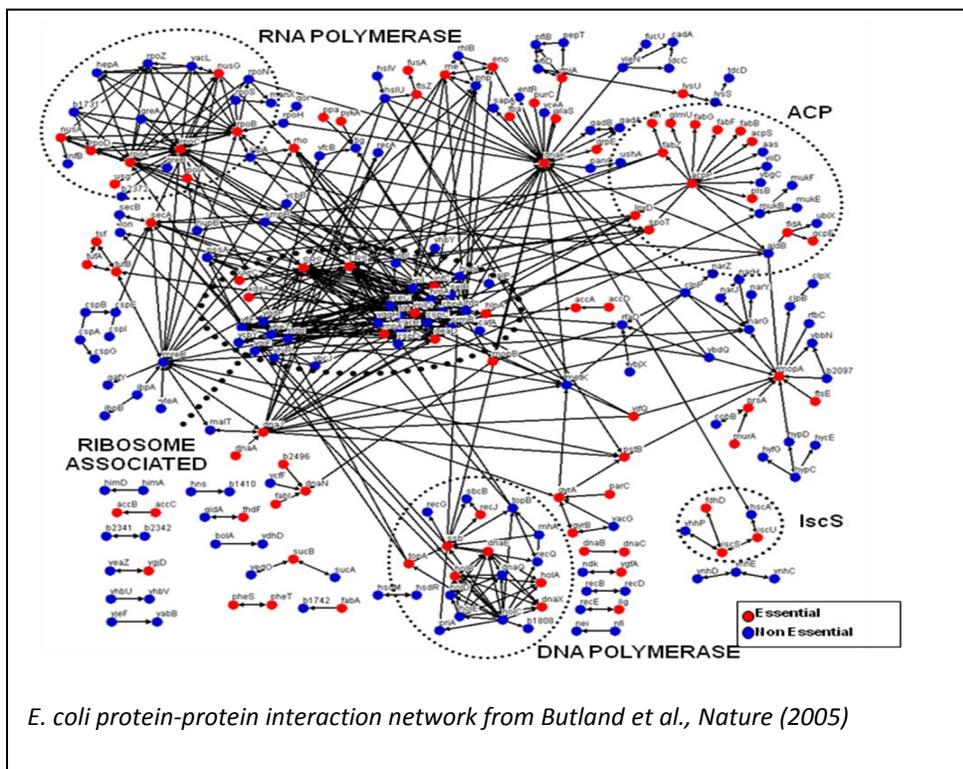
Over the last several years, the birth of functional genomics and high throughput technologies have enabled researchers to apply standard molecular genetic and biochemical approaches on a genome-wide scale. It has become clear from transcriptomic, proteomic and high throughput genetic studies that there is a high degree of organization even in prokaryotic cells which is mediated by various forms of molecular interaction. Physical interactions, such as protein-protein interactions, reveal how individual polypeptide chains come together to form protein complexes which perform many of the biochemical processes in a cell. Other types of interactions, such as functional interactions, propose organizational relationships between gene products and protein complexes within the cell. For example, epistatic (genetic) interactions, one of the most commonly encountered forms of functional interaction, exist between genes which encode proteins involved in parallel pathways or overlapping processes.

E. coli, due to its genetic tractability, has recently been the focus of a high throughput screen for protein-protein interactions. This work uncovered many novel interactions and implicated many as yet uncharacterized proteins as interacting with known complexes in the cell, indicating there is a lot we do not understand about the composition and roles of many protein complexes even in the most well characterized model bacterium. Our goal is to further characterize some of these novel interactions and uncover the roles of as yet uncharacterized proteins in these complexes. We are using a combination of traditional biochemistry and a novel methodology we have developed in collaboration with the University of Toronto to screen for genetic interactions on a genome wide scale. It is our hope that integration of the physical and genetic interaction data will give accurate predictions of the roles of these uncharacterized proteins in the cell.

Many of these technologies are also being applied to *Desulfovibrio vulgaris*, as part of the Genomics:GTL PCAP project. One of the main goals of PCAP is to characterize protein complexes present in this sulfate reducing bacterium and the roles

they play in stress response and heavy metal reduction. As part of this effort, we are applying affinity purification and mass spectrometry approaches to isolate low abundance and labile protein complexes from this organism. It is our hope that larger protein complexes isolated using these methods will be amenable to structural characterization by electron microscopy.

Gareth Butland, 9/19/07



Women-in-Science Event to Feature Bissell Talk

The East Bay Association for Women in Science presented a light dinner and talk by breast cancer researcher **Mina Bissell, Ph.D.**, Distinguished Scientist in the Life Sciences Division, on Thursday, Sept. 27 from 6:30 to 8 p.m. at Novartis in Emeryville. Scientists and science enthusiasts, men and women, were welcome. Meetings are free and open to non-AWIS members.

Today at Berkeley Lab, 9/18/07

Life Scientist Gray on Advisory Board

Joe Gray, associate Lab director and Life Sciences Division director, was recently selected to sit on the scientific advisory board of Cepheid, an on-demand molecular diagnostics company that develops, manufactures, and markets fully integrated systems for genetic analysis in the clinical, industrial and bio-threat markets.

Today at Berkeley Lab, 9/13/07

Runaround Veteran Preps Next Generation of Race Sponsors to Ensure Continued Success

Life scientist **Steve Derenzo**, known by many as "Mr. Runaround," was there for the debut of the annual Lab race back in 1978. Under the tutelage of then-employee Harvey Levy, Derenzo learned the ins and outs of organizing the event. With this wisdom under his belt, Derenzo took over the reins in 1980 and has been the driving force behind the Runaround ever since (with help from numerous volunteers over the years, of course). Continue reading: <http://www.lbl.gov/Publications/Currents/archive/#2>
Berkeley Lab View, 9/21/07

New Hope for Prostate Cancer Patients



A new first-of-its-kind machine is being tested where it was assembled in Building 55 - the Center for Functional Imaging - that could make a major impact on the diagnosis and treatment of prostate cancer. Research scientist Jenny Huber is shown here running project scientist Brian Reutter through a Prostate PET (positron emission tomography) camera. With scanners and detectors in a torso shape around the body - as opposed to the bulky mechanics of a traditional PET scanner - scientists can more precisely guide the image to perform a biopsy or administer treatment to the affected area. It will also track post-treatment analysis faster than ever before.

This three-year project that began in April is funded by the Departments of Energy and Defense.

Berkeley Lab View, 9/21/07

What goes on...? A random look inside the Not-So Familiar Buildings of Berkeley Lab

Filling Out the Fly Blueprint

With the successful sequencing of the human genome and a variety of plant and animal species, the world has virtually forgotten the fruit fly - *Drosophila melanogaster* - the most studied organism in biological research, especially in genetics and developmental biology.

They haven't forgotten this in Building 64, where work continues on fly embryos in the tireless search for transcription factors that dictate the organism's development. With more than 60 percent of the fly's genes shared with humans, insights gained about behavior, development and disease systems in the fly could translate someday into real-world applications - some consider it the Rosetta Stone for human genetics. Since 2000, when the fly's genome was first articulated, genetic pioneers like **Susan Celniker** (front in picture) have worked with NIH grants to add to the growing database of gene expression. Here, student assistant Caitlin Barale tracks an embryo on a monitor attached to a digital electron microscope, with research scientist Ann Hammonds.



Berkeley Lab View, 9/21/07

What goes on...? A random look inside the Not-So Familiar Buildings of Berkeley Lab

Lab's Gray to Speak at Breast Cancer Event

Associate Laboratory Director **Joe Gray** was among the featured speakers at this year's California Breast Cancer Research Program (CBCRP) symposium, its sixth biennial event, in Los Angeles Sept. 7-9. The CBCRP, administered by the University of California, hosts the gathering for researchers, advocates, and the public. Gray will be featured in a plenary session, "New Directions in Breast Cancer Treatment," on Sept. 8 at the St. Bonaventure Hotel. For more information on the symposium, go <http://www.cbcrp.org/media/pr/071807.php>

Today at Berkeley Lab, 8/31/07

Soil to shield astronauts

An LBNL-led team is studying the feasibility of using lunar regolith (soil) to shield astronauts on future lunar missions from space radiation. Samples of lunar mare and highland regolith returned by the Apollo missions were exposed to high energy heavy ions at the NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratory and the Heavy Ion Medical Accelerator at Chiba (HIMAC) at the Japanese National Institute of Radiological Sciences, and radiation dose reduction was measured as a function of regolith thickness. This is the first such study to use actual (rather than simulated) lunar soil.

Maximum reduction of dose is reached after approximately 15-20 cm of regolith. Studies of this type will help mission planners determine the efficacy of lunar regolith as shielding against galactic cosmic ray (GCR) heavy ions. The results suggest that use of in situ resources on the lunar surface holds promise for radiation protection, with modest amounts of lunar soil providing substantial protection against GCR.

First results were presented at the 17th Annual NASA Space Radiation Health Investigators' Meeting, Sonoma, California, July 13-15, 2007. The study's authors are L. Heilbronn, **J. Miller**, **C. Zeitlin** (LBNL); A. Rusek, M. Sivertz (BNL); M. DiGiuseppe (Northrop-Grumman Corp.); G. Sanders (NASA-Johnson Space Center); B. Eimer, L. Taylor (Univ. of Tenn. Planetary Geosciences Institute)

Jack Miller, 09/07

Blakely to serve on SACRR

Eleanor Blakely of Life Sciences has been selected to be a Member of the Brookhaven National Laboratory Scientific Advisory Committee for Radiation Research (SACRR) (2007-2010). The committee advises the Associate Director for Nuclear and Particle Physics on the scientific feasibility of radiation research-related experiments involving the use of particle accelerator beams in the AGS complex at Brookhaven National Laboratory. The Committee consists of seven members; three are staff members of Brookhaven National Laboratory and four are from the national community of radiation scientists.

Recent publications (selected)

C. Zeitlin, S. Guetersloh, L. Heilbronn, J. Miller, A. Fukumura, Y. Iwata, T. Murakami (2007). Fragmentation cross sections of 290 and 400 MeV/nucleon ¹²C beams on elemental targets. Physical Review C 76: 014911-1 to 014911-21.

This paper describes the interactions of carbon ion beams at the energies used in cancer therapy at facilities in Japan and Germany. Previously very few relevant data were available on the pertinent fragmentation cross sections. Better knowledge of these can help optimize treatment planning. The data are also of interest for space radiation protection as carbon ions are ubiquitous in the galactic cosmic rays. Comparisons to current models of nuclear interactions reveals shows poor agreement, indicating that the models need substantial revision.

Persson S, Paredez A, Carroll A, Palsdottir H, Doblin M, Poindexter P, Khitrov N, **Auer M**, Somerville CR. Genetic evidence for three unique components in primary cell-wall cellulose synthase complexes in Arabidopsis. Proc Natl Acad Sci U S A. 2007 Sep 18; [Epub ahead of print]. PMID: 17878302

In higher plants, cellulose is synthesized at the plasma membrane by the cellulose synthase (CESA) complex. The catalytic core of the complex is believed to be composed of three types of CESA subunits. Indirect evidence suggests that the complex associated with primary wall cellulose deposition consists of CESA1, -3, and -6 in Arabidopsis thaliana. However, phenotypes associated with mutations in two of these genes, CESA1 and -6, suggest unequal contribution by the different CESAs to overall enzymatic activity of the complex. We present evidence that the primary complex requires three unique types of components, CESA1-, CESA3-, and CESA6-related, for activity. Removal of any of these components results in gametophytic lethality due to pollen defects, demonstrating that primary-wall cellulose synthesis is necessary for pollen development. We also show that the CESA6-related CESAs are partially functionally redundant.

Schaber JA, Triffo WJ, Suh SJ, Oliver JW, Hastert MC, Griswold JA, **Auer M**, Hamood AN, Rumbaugh KP. Pseudomonas aeruginosa forms biofilms in acute infection independent of cell-to-cell signaling. Infect Immun. 2007 Aug;75(8):3715-21. Epub 2007 Jun 11. PMID: 17562773

Biofilms are bacterial communities residing within a polysaccharide matrix that are associated with persistence and antibiotic resistance in chronic infections. We show that the opportunistic pathogen Pseudomonas aeruginosa forms biofilms within 8 h of infection in thermally injured mice, demonstrating that biofilms contribute to bacterial colonization in acute infections as well. Using light, electron, and confocal scanning laser microscopy, P. aeruginosa biofilms were visualized within burned tissue surrounding blood vessels and adipose cells. Although quorum sensing (QS), a bacterial signaling mechanism, coordinates differentiation of biofilms in vitro, wild-type and QS-deficient P. aeruginosa strains formed similar biofilms in vivo. Our findings demonstrate that P. aeruginosa forms biofilms on specific host tissues independently of QS.

Labarge MA, Petersen OW, **Bissell MJ**. Of microenvironments and mammary stem cells. Stem Cell Rev. 2007;3(2):137-46. PMID: 17873346

In most adult tissues there reside pools of stem and progenitor cells inside specialized microenvironments referred to as niches. The niche protects the stem cells from inappropriate expansion and directs their critical functions. Thus guided, stem cells are able to maintain tissue homeostasis throughout the ebb and flow of metabolic and physical demands encountered over a lifetime. Indeed, a pool of stem cells maintains mammary gland structure throughout development, and responds to the physiological demands associated with pregnancy. This review discusses how stem cells were identified in both human and mouse mammary glands; each requiring different techniques that were determined by differing biological needs and ethical constraints. These studies together create a robust portrait of mammary gland biology

and identify the location of the stem cell niche, elucidate a developmental hierarchy, and suggest how the niche might be manipulated for therapeutic benefit.

Le Beyec J, Xu R, Lee SY, Nelson CM, Rizki A, Alcaraz J, **Bissell MJ**. Cell shape regulates global histone acetylation in human mammary epithelial cells. *Exp Cell Res*. 2007 Aug 15;313(14):3066-75. Epub 2007 Apr 27. PMID: 17524393

Extracellular matrix (ECM) regulates cell morphology and gene expression in vivo; these relationships are maintained in three-dimensional (3D) cultures of mammary epithelial cells. In the presence of laminin-rich ECM (lrECM), mammary epithelial cells round up and undergo global histone deacetylation, a process critical for their functional differentiation. However, it remains unclear whether lrECM-dependent cell rounding and global histone deacetylation are indeed part of a common physical-biochemical pathway. Using 3D cultures as well as nonadhesive and micropatterned substrata, here we showed that the cell 'rounding' caused by lrECM was sufficient to induce deacetylation of histones H3 and H4 in the absence of biochemical cues. Microarray and confocal analysis demonstrated that this deacetylation in 3D culture is associated with a global increase in chromatin condensation and a reduction in gene expression. Whereas cells cultured on plastic substrata formed prominent stress fibers, cells grown in 3D lrECM or on micropatterns lacked these structures. Disruption of the actin cytoskeleton with cytochalasin D phenocopied the lrECM-induced cell rounding and histone deacetylation. These results reveal a novel link between ECM-controlled cell shape and chromatin structure and suggest that this link is mediated by changes in the actin cytoskeleton.

Campisi J, d'Adda di Fagagna F. Cellular senescence: when bad things happen to good cells. *Nat Rev Mol Cell Biol*. 2007 Sep;8(9):729-40. PMID: 17667954

Cells continually experience stress and damage from exogenous and endogenous sources, and their responses range from complete recovery to cell death. Proliferating cells can initiate an additional response by adopting a state of permanent cell-cycle arrest that is termed cellular senescence. Understanding the causes and consequences of cellular senescence has provided novel insights into how cells react to stress, especially genotoxic stress, and how this cellular response can affect complex organismal processes such as the development of cancer and aging.

Andarawewa KL, Erickson AC, Chou WS, Costes SV, **Gascard P**, Mott JD, **Bissell MJ**, **Barcellos-Hoff MH**. Ionizing Radiation Predisposes Nonmalignant Human Mammary Epithelial Cells to Undergo Transforming Growth Factor {beta} Induced Epithelial to Mesenchymal Transition. *Cancer Res*. 2007 Sep 15;67(18):8662-70. PMID: 17875706

Transforming growth factor beta1 (TGFbeta) is a tumor suppressor during the initial stage of tumorigenesis, but it can switch to a tumor promoter during neoplastic progression. Ionizing radiation (IR), both a carcinogen and a therapeutic agent, induces TGFbeta activation in vivo. We now show that IR sensitizes human mammary epithelial cells (HMEC) to undergo TGFbeta-mediated epithelial to mesenchymal transition (EMT). Nonmalignant HMEC (MCF10A, HMT3522 S1, and 184v) were irradiated with 2 Gy shortly after attachment in monolayer culture or treated with a low concentration of TGFbeta (0.4 ng/mL) or double treated. All double-treated (IR + TGFbeta) HMEC underwent a morphologic shift from cuboidal to spindle shaped. This phenotype was accompanied by a decreased expression of epithelial markers E-cadherin, beta-catenin, and ZO-1, remodeling of the actin cytoskeleton, and increased expression of

mesenchymal markers N-cadherin, fibronectin, and vimentin. Furthermore, double treatment increased cell motility, promoted invasion, and disrupted acinar morphogenesis of cells subsequently plated in Matrigel. Neither radiation nor TGFbeta alone elicited EMT, although IR increased chronic TGFbeta signaling and activity. Gene expression profiling revealed that double-treated cells exhibit a specific 10-gene signature associated with Erk/mitogen-activated protein kinase (MAPK) signaling. We hypothesized that IR-induced MAPK activation primes nonmalignant HMEC to undergo TGFbeta-mediated EMT. Consistent with this, Erk phosphorylation was transiently induced by irradiation and persisted in irradiated cells treated with TGFbeta, and treatment with U0126, a MAP/Erk kinase (MEK) inhibitor, blocked the EMT phenotype. Together, these data show that the interactions between radiation-induced signaling pathways elicit heritable phenotypes that could contribute to neoplastic progression

Steidl U, Steidl C, Ebralidze A, Chapuy B, Han HJ, Will B, Rosenbauer F, Becker A, Wagner K, Koschmieder S, Kobayashi S, Costa DB, Schulz T, O'brien KB, Verhaak RG, Delwel R, Haase D, Trumper L, Krauter J, **Kohwi-Shigematsu T**, Griesinger F, Tenen DG. A distal single nucleotide polymorphism alters long-range regulation of the PU.1 gene in acute myeloid leukemia. *J Clin Invest*. 2007 Sep 4;117(9):2611-2620. PMID: 17694175

Targeted disruption of a highly conserved distal enhancer reduces expression of the PU.1 transcription factor by 80% and leads to acute myeloid leukemia (AML) with frequent cytogenetic aberrations in mice. Here we identify a SNP within this element in humans that is more frequent in AML with a complex karyotype, leads to decreased enhancer activity, and reduces PU.1 expression in myeloid progenitors in a development-dependent manner. This SNP inhibits binding of the chromatin-remodeling transcriptional regulator special AT-rich sequence binding protein 1 (SATB1). Overexpression of SATB1 increased PU.1 expression, and siRNA inhibition of SATB1 downregulated PU.1 expression. Targeted disruption of the distal enhancer led to a loss of regulation of PU.1 by SATB1. Interestingly, disruption of SATB1 in mice led to a selective decrease of PU.1 RNA in specific progenitor types (granulocyte-macrophage and megakaryocyte-erythrocyte progenitors) and a similar effect was observed in AML samples harboring this SNP. Thus we have identified a SNP within a distal enhancer that is associated with a subtype of leukemia and exerts a deleterious effect through remote transcriptional dysregulation in specific progenitor subtypes.

Jagust W, Reed B, Mungas D, Ellis W, Decarli C. What does fluorodeoxyglucose PET imaging add to a clinical diagnosis of dementia? *Neurology*. 2007 Aug 28;69(9):871-7. PMID: 17724289

Few studies have compared the accuracy of [(18)F]fluorodeoxyglucose (FDG) PET to the accuracy of clinical and pathologic diagnosis in dementia patients. **METHODS:** Forty-four individuals with dementia, cognitive impairment, or normal cognitive function underwent clinical initial evaluation (IE) and PET scanning and were followed up for approximately 4 years until a final evaluation (FE) and 5 years until death and autopsy. Clinical, pathologic, and imaging diagnoses were categorized as Alzheimer disease (AD) or not AD. **RESULTS:** Sensitivity of the IE for the pathologic diagnosis of AD was 0.76, and specificity was 0.58; PET had values of 0.84 and 0.74, and FE had values of 0.88 and 0.63. Positive predictive values for IE, PET, and FE were 0.70, 0.81, and 0.76. Negative predictive values were 0.65, 0.78, and 0.80. The diagnosis of AD was associated with a 70% probability of detecting AD pathology; with a positive PET scan this increased to 84%, and with a negative PET scan this decreased to 31%. A diagnosis of not AD at IE was associated with a 35% probability of AD pathology, increasing to 70% with a positive PET scan. **CONCLUSIONS:** As a diagnostic tool, PET is superior to a baseline clinical evaluation and

similar to an evaluation performed 4 years later. Although the addition of [(18)F]fluorodeoxyglucose PET to a clinical diagnosis provides useful information that can affect the likelihood of detecting Alzheimer disease pathology, the value of this technique in the current clinical environment with limited therapeutic options is likely to be modest.

Foster NL, Heidebrink JL, Clark CM, **Jagust WJ**, Arnold SE, Barbas NR, Decarli CS, Turner RS, Koeppe RA, Higdon R, Minoshima S. FDG-PET improves accuracy in distinguishing frontotemporal dementia and Alzheimer's disease. *Brain*. 2007 Aug 18; PMID: 17704526

Distinguishing Alzheimer's disease (AD) and frontotemporal dementia (FTD) currently relies on a clinical history and examination, but positron emission tomography with [(18)F]fluorodeoxyglucose (FDG-PET) shows different patterns of hypometabolism in these disorders that might aid differential diagnosis. Six dementia experts with variable FDG-PET experience made independent, forced choice, diagnostic decisions in 45 patients with pathologically confirmed AD (n = 31) or FTD (n = 14) using five separate methods: (1) review of clinical summaries, (2) a diagnostic checklist alone, (3) summary and checklist, (4) transaxial FDG-PET scans and (5) FDG-PET stereotactic surface projection (SSP) metabolic and statistical maps. In addition, we evaluated the effect of the sequential review of a clinical summary followed by SSP. Visual interpretation of SSP images was superior to clinical assessment and had the best inter-rater reliability (mean kappa = 0.78) and diagnostic accuracy (89.6%). It also had the highest specificity (97.6%) and sensitivity (86%), and positive likelihood ratio for FTD (36.5). The addition of FDG-PET to clinical summaries increased diagnostic accuracy and confidence for both AD and FTD. It was particularly helpful when raters were uncertain in their clinical diagnosis. Visual interpretation of FDG-PET after brief training is more reliable and accurate in distinguishing FTD from AD than clinical methods alone. FDG-PET adds important information that appropriately increases diagnostic confidence, even among experienced dementia specialists.