MIT CEHS NEWSLETTER

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Massachusetts Institute of Technology
CENTER FOR ENVIRONMENTAL HEALTH SCIENCES

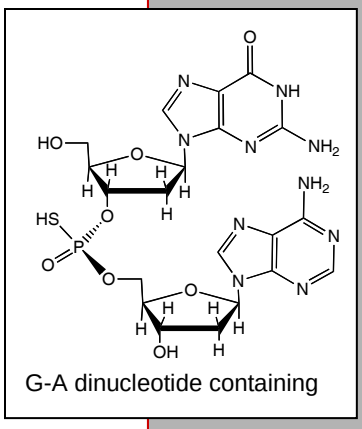
Fall 2008

Rediscovering Nature: CEHS Researchers Identify Phosphorothioate Modifications in Bacterial DNA – Cited as One of the Top 10 Scientific Discoveries of 2007

In the course of scientific invention and discovery, we are often reminded that whatever a human can do, nature has probably done it long before. Such was the case when CEHS researchers recently discovered a novel DNA modification in bacteria: the incorporation of sulfur into the DNA backbone as a phosphorothioate. This variant of normal DNA and RNA, in which one of the nonbridging oxygens is replaced by a sulfur, was "invented" decades ago by synthetic chemists and eventually found extensive application in gene and antisense therapies as a means to make the nucleic acids resistant to nuclease degradation.

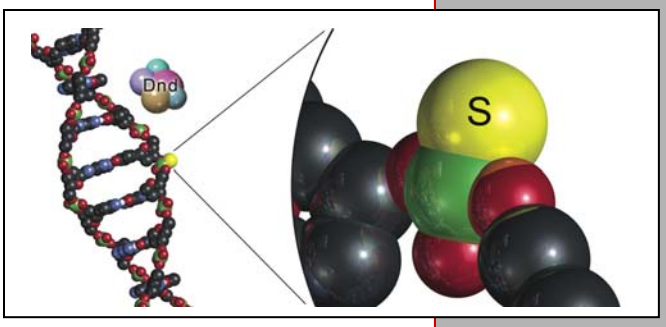
In work reported in *Nature Chemical Biology*,[1] the discovery of phosphorothiaote-modified DNA in bacteria was made by CEHS researchers Peter Dedon, Lianrong Wang, Shi Chen, Koli Taghizadeh, and John Wishnok in collaboration with microbiologists at Shanghai Jiaotong University led by Zixin Deng. Deng and coworkers had previously discovered that sulfur was somehow incorporated into DNA in *Streptomyces lividans* and other bacteria by enzymes encoded by five members of a conserved gene family. Since the sulfur caused the DNA to degrade during electrophoresis in Tris buffer, the gene family was named *dnd* as an acronym for the DNA degradation phenotype conferred by the genes.

The Dedon and Deng research teams solved the elusive structure of the sulfur-containing DNA modification by first labeling the bacterial DNA with radioactive sulfur. Following purification, the DNA was hydrolyzed with nucleases to component nucleosides that were then separated chromatographically. The presence of the radioactive label allowed the researchers to isolate and subsequently identify the structure of nuclease-resistant, phosphorothioate-containing dinucleotides. The structural characterization was accomplished using a variety of mass spectrometry technologies in the Bioanalytical Facilities Core of the CEHS, including exact molecular weight determination by high mass accuracy mass spectrometry, elucidation of the structure and sequence of the phosphorothioate-containing dinucleotides by tandem mass spectrometry and chromatographic determination of the stereochemistry of the phosphorothioates. As shown in the adjacent figure, phosphorothiaotes are inherently chiral (handedness of the configuration of bonds attached to the phosphorous), with the naturally occurring modifications taking the right-handed or R form.



G-A dinucleotide containing

The discovery of phosphorothioate modifications in bacterial DNA raises a host of questions about their function and biochemistry. Of the >90 different base and sugar modifications of DNA and RNA that have been studied for decades, only a few have well understood functions. One clue to the function of phosphorothioate modifications involves their location in the DNA. The CEHS researchers observed that the modification was sequence selective in two bacterial strains, lying between guanine and adenine bases in *Salmonella enterica* and between two adjacent guanines in *Streptomyces lividans.* While the larger sequence context is not currently known, this is analogous to bacterial restriction-modifications systems and suggests a role for phosphorothioates in host protection against foreign DNA. Another possibility is some type of regulatory mechanism for gene expression or DNA replication, with the phosphorothioate serving as a marker for protein recognition or



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UPCOMING EVENT

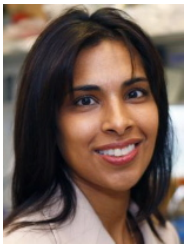
March 19, 2009 William Nelson (Johns Hopkins University School of Medicine) is the guest speaker for the Robert S. Harris Lecture

AWARDS AND HONORS

CEHS Center Members named HHMI Investigators

Five MIT faculty have been named Howard Hughes Medical Institute Investigators, of which two are Center Members.

They are:



Sangeeta N. Bhatia, Professor of Electrical Engineering and Computer Science. She and her colleagues have created tiny colonies of human liver cells that model aspects of the full-size human organ. One of Prof. Bhatia's long term goals is to create a complete implantable liver.

Catherine L. Drennan, Associate Professor of Chemistry, revels in sorting out the architecture and function of metalloproteins—those proteins whose structure contains one or more metal ions.

James G. Fox awarded the AALAS Award

Professor **James G. Fox**, Director of Division of Comparative Medicine, has won the 2008 Charles A. Griffin Award from the American Association for Laboratory Animal Science (AALAS).

The Griffin Award is AALAS' oldest and most prestigious accolade. The Award is presented for outstanding accomplishments in the improvement of care, quality, and environment of animals used in biologic and medical research.

Environmental Health and Safety Award

This fall, the Center for Environmental Health Sciences was awarded the Environmental Health and Safety Award for recognition of outstanding performance related to environmental health and safety issues for demonstrating excellent performance in the EHS Management System and maintaining an exemplary EHS program throughout the year in the small Department, Center, or Lab category.

In addition, the Biological Engineering Department received the Honorable Mention Award.

FACILITIES CORES UPDATES

Genomics and Imaging Facilities Core

We would like to welcome **Stuart Levine** who is Director of the BioMicro Center and is co-Director of the Center's Genomics and Imaging Facilities Core. Dr. Levine came from the Whitehead Institute for Biomedical Research. He has extensive work studying gene regulation in eukaryotes. One of the new technologies the BioMicro Center will be rolling out is massively parallel sequencing using the Illumina/Solexa sequencer. In addition to high-throughput sequencing, several other large changes are underway. They are currently laying the groundwork for a renovation of the BioMicro lab space to make more room to bring in new technologies. They are also planning to completely rework the BioMicro website to both bring it up to date, and to make it much more useful to the users. The new website will be built as a wiki using OpenWetWare where they hope to include not only information about the technology and pricing, but also information about protocols and analysis that should help new users.

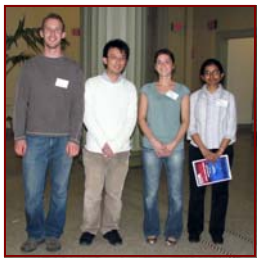
We would also like to welcome **Jennifer Calvo** who is co-Director of the Gemonics and Imaging Facilities Core. She will oversee the Microscope Facility located in Building 16-318. This Core contains both the Metasystems scope and the Compucyte Laser Scanning Cytometer (LSC). You can learn more about the Metasystems scope and its applications at http://www.metasystems.de/. The Compucyte laser scanning cytometer has recently undergone an extensive upgrade. This system combines standard florescent microscopy contains 3 lasers (405, 488, 633), which allows one to examine staining with multiple fluorescent and/or chromatic dyes. Multiple formats are also allowed, including standard slides, 6-well, 96-well, and 384-well plates. This system allows you to scan and image large regions of cells, examine/gate on different populations, and view the morphology of these cells, thereby allowing you to validate the gating/analysis performed. If you have questions regarding experiments on the LSC, or would like to be trained on the system, please contact Jennifer Calvo (jcalvo@mit.edu). You can also learn more about this system at www.compucyte.com. There is a new TechTime account for the LSC, (CEHS: LSC); please contact Jennifer Calvo for training in order to gain access to this resource. The Metasystems microscope is also found on TechTime (CEHS: Metasystems). Make sure you activate your TechTime account before trying to access these resources. To do this you can go to http://calendar.mit.edu and select the "activate your account" link.

CEHS NEWS

CEHS 2008 Poster Session

The CEHS Poster Session, a tradition started in 2004 continues to draw interest from a wide range of CEHS affiliated member labs. This year the poster session was held in May and there were approximately 53 posters presented from several different disciplines. It is the goal of the Center to bring together CEHS members/affiliates labs to highlight their ongoing research projects as well as providing an opportunity for interaction with others and possibly leading to future collaborative, multidisciplinary approaches to research in the area of environmental health science.

Prof. Leona Samson



(from left to right) Jared Toettcher, Clement Yan Chan, Ericka Noonan, and Aarthi Chandrasekaran.

This year the Center offered prizes to poster participants which were selected by our secret panel of judges.

CEHS 2008 Poster Session Prize winners for the Graduate Student Awards are:

1st Place:
Aarthi Chandrasekaran from the Sasisekharan Lab
**Prize - $500 cash award.*

2nd Place (2 way tie): Clement Yan Chan from the Dedon Lab and Ericka Noonan from the Samson Lab.
**Prize - $100 cash award.*

3rd Place: Jared Toettcher from the Engelward Lab.
**Prize: - CEHS T-Shirt and Coffee Mug.*

(from left to right) Johan Peter Svensson, Dragony Fu, Peter Slade, and Scott Knudsen.

CEHS 2008 Poster Session Prize winners for the Postdoc Awards are:

1st Place: Scott Knudsen from the Manalis Lab.
**Prize - $500 cash award.*

2nd Place: Peter Slade from the Tannenbaum Lab.
**Prize - $100 cash award.*

3rd Place (2 way tie): Johan Peter Svensson and Dragony Fu from the Samson Lab.
**Prize: - CEHS T-Shirt and Coffee Mug.*

1st and 2nd prizes were made possible by the Myriam Marcelle Znaty Fund.

(from left to right) Prof. Steven Tannenbaum, Olga Parkin, and Prof. Peter Dedon.

2008-2009 Pilot Projects Awardees

CEHS allocates a significant portion of its NIEHS P30-ES002109 funding to support pilot projects that: provide initial support for investigators to establish new lines of research in environmental health; allow exploration of innovative new directions representing a significant departure from ongoing research for established investigators in environmental health sciences; and stimulate investigators from other fields to apply their expertise to environmental health research.

Current award recipients and their project titles are:

- **Peter C. Dedon**, Professor, Biological Engineering, "Changes in the Spectrum of tRNA Secondary Modifications as Biomarkers of Exposures".
- **Catherine Drennan**, Associate Professor, Chemistry, "Structural Studies of DNA Repair Protein Human Alkyladenine DNA Glycosylase".
- **Susan E. Erdman**, Principal Research Scientist, Division of Comparative Medicine, "Inflammation-associated Prostate Cancer: Development of Mouse Models for Assay of Environmental Contaminants".
- **Jongyoon (Jay) Han**, Associate Professor, Biological Engineering and Electrical Engineering and Computer Science, "Direct Coupling of Nanofluidic Preconcentration System and Conventional Mass Spectrometry".
- **Michael Strano**, Associate Professor, Chemical Engineering, "Detection of Toxic Events in the Liver in vivo using Single Wall Carbon Nanotubes".
- **Bruce Tidor**, Professor, Biological Engineering and Electrical Engineering and Computer Science, "Exploring DNA Damage Response Networks with High-Dimensional Information Theoretic Statistics".

WELCOME

We are delighted to announce the new Center Members:

Jiali Han, Assistant Professor, Harvard University

Stuart Levine, Director of BioMicro Center, Biology

Ravi Thadhani, Co-Director, CTSA

Janelle Thompson, Assistant Professor, CEE

Koli Taghizadeh, Co-Director of Bioanalytical Facilities Core, CEHS

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CEHS NEWS (CONTINUED)

SCIENCE: EDITOR'S CHOICE

***SCIENCE* 7 November 2008: Vol. 322. no. 5903, pp. 823-825**

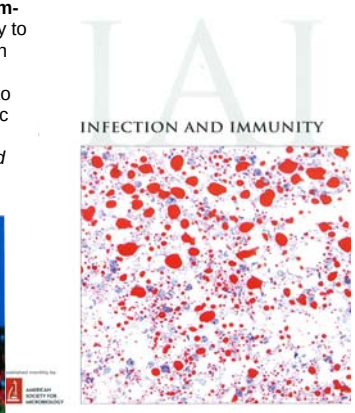
SYSTEMS BIOLOGY : NETWORK FAILURE[1]

Models of metabolic and signaling networks have been characterized, perhaps unfairly, as reannotations of previously discovered interactions. To counter this concern (and the statistical issue of sorting through hundreds of correlations), Janes *et al.* describe an approach called "model breakpoint analysis" that stresses the networks by using nonphysiological inputs in a manner similar to that of engineers performing failure analysis of bridges or cars. They began with their model of cytokine-induced apoptosis and proceeded to introduce implausible data that stretched the dynamic range of the cell (defined as the responsiveness of cell outcomes to incremental changes in cell activation). Surprisingly, network function did not degrade in parallel, but worked perfectly well until a threshold (or breakpoint) was reached, at which point the predictions were no longer useful. Pinpointing the signals and stimuli that were responsible for the system failure enabled them to distinguish epiphenomena from causal factors and to make predictions about the dynamic roles of three kinases (Akt, ERK, and Mk2) in cytokine-induced apoptosis. These predictions were then confirmed in inhibitor– and mutant-based experiments, suggesting that differences in dynamic range can be more important to cellular function than the strength of a particular signal.

[1] K. Janes, H. Reinhardt, M. Yaffe, *Cell* **135** 2008. p. 343-354. Cytokine-Induced Signaling Networks Prioritize Dynamic Range Over Signal Strength

COVER ART!

Garcia A, Ihrig MM, **Fry RC**, Feng Y, Xu S, Boutin SR, **Rogers AB**, Muthupalani S, **Samson LD**, and **Fox JG**. Genetic susceptibility to chronic hepatitis is inherited codominantly in *Helicobacter hepaticus*-infected AB6F1 and B6AF1 hybrid male mice, and progression to hepatocellular carcinoma is linked to hepatic expression of lipogenic genes and immune function-associated networks. *Infection and Immunity*, **76**:1866-1876, 2008.



Hagen SJ, Yang DX, Taylor NS, Tashima K, and **Fox JG**. Epithelial cell expression of BCL-2 family proteins predicts mechanisms that regulate *Helicobacter pylori*-induced pathology in the mouse stomach. Lab Invest 88:1227-1244, 2008.

Professor **Ram Sasisekharan** has been appointed as Director of the Division of Health Sciences and Technology (HST) at MIT. Professor Sasisekharan will also become the Edward Hood Taplin Professor of Biological Engineering and Health Sciences and Technology. We look forward to strong ties between CEHS and HST.

Continued from page 1

binding.

The discovery of phosphorothioates in bacteria was recently selected as one of the top 10 scientific advancements of 2007 by the Chinese edition of *Scientific American*. Both the Dedon and Deng laboratories are continuing their studies of this novel DNA modification, the discovery of which was entirely dependent upon the world-class facilities available to CEHS researchers in the Bioanalytical Facilities Core.

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[1]Wang, L., Chen, S., Xu, T., Taghizadeh, K., Wishnok, J. S., Zhou, X., You, D., Deng, Z. and Dedon, P. C. (2007) Phosphorothioation of DNA in bacteria by dnd genes. *Nat Chem Biol* **3**, 709-710. See the accompanying News and Views commentary by Fritz Eckstein, one of the pioneers of the chemistry and application of phosphorothioates: Eckstein, F. (2007) Phosphorothioation of DNA in bacteria. *Nat Chem Biol* **3**, 689-690.