

EMERGING SCIENCE FOR ENVIRONMENTAL HEALTH DECISIONS

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Use of Emerging Science and Technologies to Explore Epigenetic Mechanisms Underlying the Developmental Basis for Disease

On July 30–31, 2009, the National Academies Standing Committee on the Use of Emerging Science for Environmental Health Decisions held a public workshop to further understanding of the implications of epigenetic effects on public health and of the research that would be most important for efforts to inform public health leaders about epigenetic effects of chemicals.

Framing the Issue

When we think of how chemicals affect genes, we tend to think of their potential to cause DNA mutations or deletions and, as a result, to mediate cancer and other diseases. But we must now also consider the potential epigenetic effects of chemicals on gene expression—effects on how genes are regulated that are distinct from their effects on DNA sequence. What are the human health outcomes when genes (such as a tumor-suppressor gene) are turned on or off at different stages in life and in various tissues? Scientific evidence on animals and humans suggests that epigenetic changes are important and may be passed from one generation to the next.

We have not determined whether we need to have screening tests for potential epigenetic effects of environmental stressors. Many questions remain about epigenetics: Are humans more sensitive to epigenetic effects during particular developmental periods? How much of morbidity and mortality might these effects account for? Are the effects reversible? We do not yet have tests designed to screen for potential epigenetic effects. Workshop participants took up those and other topics in a spirited discussion of this emerging field.

Thomas Gasiewicz (University of Rochester), chair of the standing committee, summed up the purpose of the workshop in his opening remarks, saying that participants would examine scientific and technologic approaches to identifying and quantifying epigenetic effects of environmental agents and to assessing their potential effects on human health. The participants—in academia, government agencies, industry, and environmental groups—would

also consider the strengths and weaknesses and the costs and benefits of looking at epigenetic effects of environmental agents by using new approaches and technologies.

Ultimately, what we are looking for in this discussion is a better understanding of the health implications of epigenetic changes, explained William Farland.

At the meeting, experts in epidemiology, toxicology, molecular biology, public health, pharmacology, reproductive biology, and genetics discussed

- Epigenetic mechanisms
- Screening tools
- How to improve the state of the science
- Continuing scientific controversies

They also addressed

- Implications of the emerging science of epigenetics for those who strive to protect public health, including regulatory experts
- The current regulatory paradigms
- The how and why of communicating findings with the media and the public.

Day 1 of the workshop was devoted to the complex science of epigenetics, and day 2 to the no less complicated topic of possibly regulating exposure to chemicals and other potentially controllable stressors that have epigenetic effects.

Defining the Terms

We can thank Conrad Waddington, professor of animal genetics at the University of Edinburgh, for coining the term epigenetics in the middle 1900s. He defined it as “the science concerned with the causal analysis (gene action) of development”. Its meaning has evolved as researchers’ understanding of how genes are regulated has grown. Richard Meehan (Medical Research Council, Scotland, University of Edinburgh) explained how epigenetics is now commonly defined as heritable changes in gene function that occur without a change in the sequence of the nuclear DNA—in other words, something is being done to the DNA or how it is packaged and modified that alters its biological activity but does not change its sequence.

The major modifications of DNA that occur in animals are addition of a methyl group to cytosine and unique chemical alterations (signatures) in chromatin—the combination of DNA and protein that comprise chromosomes. The modifications change how the DNA is compacted in the nucleus. The epigenetic status of DNA packaged into chromatin in different tissues is collectively referred to as the epigenome, in the same way that the genome refers to the sequence of all our genes. The epigenome is a dynamic system that determines whether genes are in an active or inactive state. A major role of epigenetic modifications in somatic cells is maintaining 98% of the genome in an inactive (heterochromatic) state. The epigenetic state is in fact maintaining genomic stability by preventing genomic rearrangements.

Epigenetic changes are also normal and necessary, pointed out Karl Kelsey (Brown University). They facilitate tissue- and cell-specific differentiation. By remodeling the chromatin, epigenetic changes control gene expression, silence noncoding repetitive elements, and inactivate the X chromosome during the development of the female embryo.

How Epigenetics Works: The Mechanics

Wan-Yee Tang (University of Cincinnati) delved into the specifics of epigenetic changes in humans and their effects. She described how an assortment of processes, either together or alone, determines whether a gene is silent or activated. By affecting the access of translation machinery to DNA (by affecting chromatin, DNA folding, and nucleosome positioning) or the stability of the RNA transcript, epigenetic changes, such as DNA methylation and histone modification, turn a gene on or off. In the

Differences Between Genetics and Epigenetics During Animal Development

How does epigenetics differ from genetics? The short answer: “genetics proposes and epigenetics disposes,” the Medawars wrote in their *Philosophical Dictionary of Biology* (Medawar and Medawar, 1983). Another clue: In Greek, *epi* means *over, above, in addition to*.

Genetic	Epigenetic
Blueprint (DNA) for development	Additional instructions (chemical tags on chromatin)
Stable (rarely reversed)	Reversible (imprinting)
Resistant to environmental influences (except mutagens)	Potentially susceptible to environmental perturbation

fetus, epigenetic modification is influenced by the mother’s diet and other exposures, such as smoking. Dysregulation of the epigenetic process leads to disease.

Dr. Meehan said that it is important to consider which happens first—DNA methylation, gene silencing, or histone modification. An understanding of those events is important to define triggering events that may lead to epigenetic alterations that result in some functional change, the mechanism by which environmental agents may act, and potential targets of prevention and intervention. John Greally (Albert Einstein College of Medicine) and some of the other participants believe that although DNA methylation is less variable and may eventually prove to be a reliable marker of some defined molecular changes, histone modifications currently appear to yield more information on functional changes in gene regulation.

DNA Methylation, Histone Modifications and Transgenerational Potential of Effects

One topic that was addressed throughout the meeting was how long epigenetic changes endure. Are they permanent, like the mutations that change a gene sequence, or, as Dr. Meehan asked, are they transient and perhaps reset when germ cells are passed to a new generation? Moshe Szyf (McGill Medical School) described DNA methylation as having several time scales. Some methylation changes in a cell occur and disappear within a few hours. Some changes take place on an evolutionary time scale in which methylation patterns evolve over billions of years. Researchers are most concerned about changes that last a lifetime (because we do not understand them) and may affect children and grandchildren of the exposed.

Researchers discussed study results which suggest that epigenetic changes may endure into the next generation, being passed from mother to child. Dr. Kelsey described what he called an important example of such transgenerational effects occurring in humans: a change that leads to hereditary nonpolyposis colorectal cancer

(HNPCC), an autosomal dominant, early-onset form of colon cancer. That cancer has long been described as the result of an inherited mismatch-repair gene mutation, he said. But we now know that the disease can be caused by an in utero event in which the same phenotype occurs by epigenetic inactivation of a specific gene (the MLH1 gene), he said. Studies have not been conducted on gametes to definitively show that this is heritable, but he thinks that we can infer that heritability is the case.

In addition to the human data that Dr. Kelsey described, animal data suggest that epigenetic changes can be inherited, said Linda Birnbaum (director, National Institute of Environmental Health Sciences and the National Toxicology Program). Dr. Birnbaum noted that we have long been aware that the developing fetus and neonate are extremely sensitive to perturbation by chemicals, and we know that the developmental period is the most sensitive to epigenetic alterations. As Dr. Kelsey put it, “you are what your mother eats” because the epigenetic marks are set in utero. Most of our work now addresses the question of the chronic or long-term effects of what happens in utero. However, the epigenomic signature

or the status of various epigenetic modifications in the genome is going to be very different in different cells, at different times, and in different people.

From Nickel to Motherly Love—Possible Causes and Outcomes of Epigenetic Changes

Participants discussed how environmental chemicals, estrogenic compounds, and even social factors, such as child abuse and maternal care, may cause epigenetic changes. They discussed the diseases, such as cancer and asthma, that might result from the changes.

In his presentation, Max Costa (New York University) described his cell studies of the effects of heavy metals, particularly nickel, on epigenetic programming. His group has been studying nickel carcinogenesis since the late 1970s. In the late 1980s, they discovered that DNA hypermethylation is induced by nickel, and they have continued to study metals and methylation.

Moderator William Farland summarized the meeting as follows:

Understanding the Epigenome

- Participants described the epigenome as a dynamic system, in contrast with gene sequences. Crosstalk occurs between DNA methylation and histone acetylation, between genes and the environment. To understand what is abnormal or adverse, we need to understand the normal state of the epigenome.
- How epigenetic modulators change a cell depends on the state of the cell itself—for example, is the cell hypoxic?
- Not all the changes in the epigenome that we can measure will persist; changes need to occur in the germ cell to be passed on to the next generation.
- It is not always obvious which genes are being affected by epigenetic changes. Some epigenetic changes occur far from the gene that is subsequently affected; this suggests that epigenetic changes may involve a large segment of DNA.
- The potential effects of epigenetic changes can be as varied as cancer, asthma, and behavioral changes, and they occur at different rates, from rapid to the evolutionary scale.

Testing for Epigenetic Changes

- Everything from environmental pollutants to maternal care may affect the epigenome. For example, social impacts may translate into chemical signals that

change the expression of genes in the brain or wherever the effect is seated.

- We need to determine which epigenetic modulators are the most likely to affect human health and then focus our epigenomic studies on them while considering the cost of technologies and the power of different types of novel and existing studies to detect responses.
- A systems biology approach may best enable us to develop practical assays to predict effects of epigenetic changes. Using that approach, we would look not just at a single event, such as a change in methylation patterns in a particular cell, but at how the change affects cell function in the context of an organ or other parts of the body.
- We are looking for biomarkers that tell us when epigenetic processes are in play; there may be increases in enzymes that acetylate histones, for example.
- We need screens that will help to group chemicals or agents into action or activity classes as we begin to look at linking diseases, chemicals, and pathways.
- We need to mine existing datasets or resources, including National Toxicology Program (NTP) tissue repositories, ToxCast and Tox21, the National Children’s Study, and tissue repositories related to specific diseases.

Dr. Meehan described an important finding that linked environmental exposures and epigenetics. In the late 1990s, scientists showed that there was an association between dietary changes and changes in DNA methylation in mice, changes that appeared to have phenotypic consequences.

Nurturing Your Epigenome

Research on epigenetic changes may improve understanding of how nurture alters nature, according to Dr. Szyf. By

Richard Meehan pointed out important questions in the field:

- How do we determine which epigenetic effects cause changes of concern and which merely correlate with such changes?
- What happens first—DNA methylation, gene silencing, or histone modification?
- The special state of a somatic cell is not unchangeable—it can be reversed experimentally by exposure of cells to a cocktail of transcription factors. How does that happen?

licking their pups, mother rats alter their offspring's epigenetic programming. Dr. Szyf and Michael Meaney, also of McGill University, showed that maternal behavior affects DNA methylation of the glucocorticoid receptor gene in the hippocampus of rat offspring. Rat pups that receive little maternal nurturing, including licking, have unusual methylation patterns and are more anxious than rats that receive more nurturing. It is consistent with that observation that injections of the chemical trichostatin A, which methylates the gene, change the anxious rats' behavior.

Dr. Szyf went on to describe how Gustavo Turecki, director of the Quebec Brain Bank, and Dr. Szyf's group conducted a small study of people who had committed suicide. The study found a significant difference between the methylation of ribosomal RNA in those who committed suicide and were abused as children and a control group of people who committed suicide but were not abused during childhood. They also saw more methylation in rhesus monkeys that were reared with a mother than in monkeys that were not reared with a mother. Other research that his team has contributed to found differences in methylation patterns between adults who, as children, experienced extreme high and very low social adversity. As a point of comparison, Dr. Szyf noted the effects of childhood social adversity on overall methylation patterns were more pronounced than the effects of having a mother who smoked.

Epigenetic Changes, Cancer, and Asthma

Dr. Kelsey described epigenetic changes and cancer. The study of cancer has pushed epigenetics forward to the extent that cancer patients now receive epigenetic treatment. He emphasized that every epigenetic change known to occur can happen in tumors, but the origin of epigenetic changes in tumors is difficult to understand, and one must look at how in utero exposures or events could be associated with tumors. Abnormal imprinting, global methylation, and other changes in the epigenetic state are all associated with events that occur in utero and with tumors. But Dr. Kelsey said that it is important to remember that most epigenetic alterations in tumors that have been studied to date are thought to be of somatic origin (as opposed to mutations that occurred in germ cells in utero).

Dr. Tang and her colleagues are making the first attempt to study the epigenetic markers of transplacental exposure to the polycyclic aromatic hydrocarbons (PAHs) in traffic-related air pollution and the relationship of these markers to childhood asthma. By studying umbilical-cord white blood cells, they have identified more than 30 DNA sequences that had a methylation status that depended on the magnitude of maternal PAH exposure. For example, their research suggested that maternal PAH exposure is associated with methylation of the ACSL3 gene and asthma status of children. If those results are confirmed in future studies, methylated ACSL3 in umbilical-cord white blood cell DNA could be a marker for assessing

One of the most important and challenging questions that future studies need to address is how polymorphisms in asthma-related genes interact with epigenetic mechanisms to confer higher susceptibility to environmental influences, noted Wan-Yee Tang.

transplacental PAH exposure or predicting childhood asthma. More generally, these studies demonstrate how a chemical in the environment can affect disease risk via gene methylation.

How To Identify Stressors That May Have Epigenetic Effects

Dr. Greally provided an overview of tools and approaches used to demonstrate epigenetic effects and the challenges in studying epigenomic dysregulation in human disease. He explained how the existence of many types of regulators of epigenomic and transcriptional organization that could be analyzed creates a challenge in selecting which one to

look at. Theoretically, many molecular regulators could be affected in various ways, including alteration of epigenetic marks on the DNA molecule itself and histone modifications and alterations of the physical structure of DNA or chromatin.

Transcriptional assays are commonly used and provide a readout of various effects on the genome, but nuclear RNA or the primary transcript is probably a better representation of the effects of transcriptional regulatory changes. Assessing cytosine methylation is a common approach for looking at the actual regulators rather than a readout. Cytosine methylation can be studied with various assays that differ in strength. The appropriate tradeoffs depend on the objective: discovery mode with low resolution but genomewide, comprehensive assays for quantitative results and nucleotide resolution, and population studies that require high throughput but do not need to be genomewide and are instead targeted to the most informative loci in the genome. In summarizing the assay comparisons, Dr. Greally noted that there is a risk of missing important information if one limits human-disease studies to the study of cytosine methylation—a very limited component of the entire epigenomic regulatory panoply. Chromatin-immunoprecipitation assays are relatively difficult to apply in clinical practice but remain feasible in cell-culture systems.

After reviewing assay technologies, Dr. Greally analyzed the pros and cons of different study designs for studies of epigenomewide associations. With respect to study design, we have to be able to look genomewide and quantitatively, said Dr. Greally. We have to look carefully at our cohorts to ensure that we get good cell samples. In addition, we probably need to have a two-stage design: genomewide screening and then more quantitative work on the single loci identified as candidates for dysregulation.

Dr. Greally said that his most important take home message was that some of the epigenetic changes that

occur in disorders other than cancer are quite subtle, and our studies and our technology have to be sensitive enough to detect them.

During the discussion, Trevor Archer (NIEHS) said that instead of taking an expensive approach, such as looking for the location of potentially hundreds of epigenetic alterations on chromatin, we might want to look at the enzymes that are responsible for the alterations. That approach would be amenable to traditional screening processes, such as those used in drug companies' pharmaceutical screening.

Questions about the Study of Epigenetic Effects

Biomarkers of Disease Susceptibility

The “imprint” left by developmental programming, such as altered methyl markers, may be useful for identifying exposed individuals and as a biomarker of disease susceptibility, said Dr. Birnbaum. With regard to biomarkers, Dr. Kelsey observed that in cancer, for example, there may be many measurable epigenetic signals that are powerful biomarkers, but they might not have been inciting the disease process. Determining when a potential biomarker came onto the scene could pose a challenge but will help us to understand what the biomarker is telling us about its relationship with cancer.

Animal Models for Studying Epigenetics

Participants asked about the most appropriate animal models for predicting human risk. As we study the effects of environmental stressors on the epigenome, are there substantial differences in epigenome regulation that we need to be aware of when comparing model systems? Dr. Meehan responded that scientists have to base their selection of models on what they want to know. For example, plants are extremely plastic, so epigenetic changes in them might be easily propagated, but it is

Sample Requirements, Resolution, and Quantitative Capabilities of Assays

Assay	Sample Preparation Issues	Cell Numbers Required	Quantitative Capability of Genome-Scale Assay	Resolution of Genome-Scale Assay
Chromatin immunoprecipitation	Needs immediate sample processing	10^5 – 10^7	Poor	Hundreds of base pairs
Nucleosome positioning	Needs immediate sample processing	$<10^5$ – 10^7	Poor	Hundreds of base pairs
Transcription	Sample should be immediately flash-frozen or processed	$<10^4$ – 10^6	Good	Exon-specific
Cytosine methylation	Sample should be frozen	$<10^4$ – 10^6	Fair	One to hundreds of base pairs

difficult to measure responses to DNA methylation with a mouse model because they are intertwined with other changes that normally occur at the same time.

George Daston (Proctor & Gamble) questioned the appropriateness of rodent models for other reasons. A rodent has multiple litters and a short reproductive cycle, so epigenetic changes in a single litter that result from environmental conditions to which the pregnant mother is exposed might be adaptive. That level of adaptation may not occur in humans, inasmuch as humans have few offspring and a long reproductive cycle.

We would like a DNA-methylation assay that looks at each area that is rich in CG nucleotides individually and quantitatively and that is cost-efficient enough to permit a high sample throughput, but this sort of assay is not now available, John Greally pointed out.

Martin Stephens (Humane Society of the United States) said that if we do need a testing program to assess potential deleterious epigenetic effects of chemicals, he would prefer to see animal testing only as the final, conditional step in a tiered evaluation program. That is, before any animal testing, the process should include an evaluation of chemical class, structure, exposure magnitude, and so on, as well as the use of in vitro and in silico methods.

Sample Repositories for Further Study

In the discussion of how to assess epigenetic effects of various chemicals, Dr. Birnbaum and Chris Portier (NIEHS) noted that a large repository of frozen, archived mouse and rat tissue samples from repeat-dose, 90-day, and 2-year studies collected over the last 30 years at NTP is available to scientists. Susan Fisher (University of California, San Francisco) said that scientists could use existing banks of human samples to test hypotheses about relative magnitudes of exposure and associations with disease.

Screening Tools

Participants discussed whether existing screening tools, such as animal assays, can detect epigenetic responses and are efficient enough for testing the many chemicals that agencies might be expected to screen if screening for epigenetic effects were to go forward. David Dix (EPA) said that we must ask why we would screen and how we would interpret the results of research and the subsequent screening. He believes that those are critical questions that should be answered up front. If screening were to occur, screens could look for changes in methylation, chromatin structure, and microRNA, Dr. Dix proposed.

Low-Dose Responses

Desmond Bannon (US Army Center for Health Promotion and Preventive Medicine) said that we need to ask what happens at the low end of the dose–response spectrum; in other words, what is the difference between a normal pharmacologic response to something that we eat or drink and a toxic response.

Defining Normal

We need to understand the normal variability from cell to cell as a foundation of knowledge if we are to be able to interpret epigenetic changes, Dr. Greally said. The epigenetic state is likely to be different in each tissue, and we are only now beginning to get some clues about how these differences are related to the histone code. The interplay between DNA methylation and the chromatin state is a subject of enormous ignorance and a fertile ground for research, according to Dr. Kelsey.

Interplay Between Genetics and Epigenetics

Dr. Tang noted that one of the most important and challenging questions that future studies will need to address is how polymorphisms in asthma-related genes interact with epigenetic mechanisms to confer higher susceptibility to environmental influences. Echoing that point, Dr. Kelsey said that merging the investigation of epigenetics with genetics would be important. The two forms of alterations of genes are not independent—one will inform the other, and we need to begin to look at them together. He believes that is beginning to happen, but few data are now available.

Epigenetic Changes as Exposure Markers

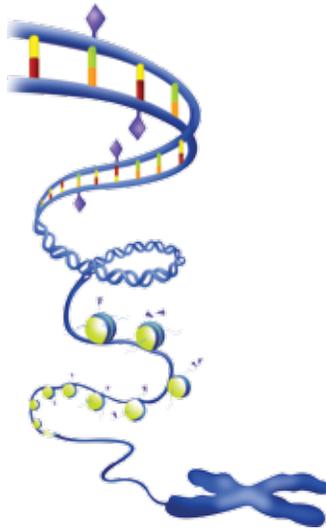
Through its Roadmap Epigenomics Program, the National Institutes of Health (NIH) is supporting the development of reference epigenomic maps in a variety of human tissues, said Fred Tyson (NIEHS). Genomewide maps of histone modifications and methylations in a number of cell types are being constructed, including those for human embryonic stem cells. NIH is also supporting a new set of grants to look at the possible role of the epigenome in various diseases.

William Slikker Jr. (FDA) and Lynn Goldman (Johns Hopkins University) pointed out that regulators will have to make decisions about many data related to epigenetic pathways. Continuing discussions like those at this meeting may help to prepare regulators for those decisions. Dr. Birnbaum said that the NTP is already talking about the incorporation of epigenetic measures into some studies and may ask other agencies to discuss what it should be doing in this regard. Epigenetic tools will be useful for screening but also useful for creating a crosswalk to better relate animal data to human risk, said Dr. Goldman.

Communicating Future Findings

One of the goals of the meeting was to discuss strategies for communicating to the public conclusions that are emerging on the epigenetic mechanisms underlying disease.

From her vantage point as a science reporter, Rebecca Renner noted that epigenetics is already producing dramatic results and that scientists and government agencies need to be prepared for the public's eventual, and probably large, demand for information on these findings. If experts in the field are talking to each other and have made connections with members of the press, the chance that accurate, useful information will get out when the public does start asking questions increases. As William Farland (Colorado State University) noted, Web sites that provide objective information on epigenetics and advances in the field would be useful to the community and to decision-makers. But when and how information should be released need to be determined.



Epigenetic code
National Institute of General Medical
Sciences

biotechnology would move forward quickly and meet our needs in epigenetics. Over the last 7 or 8 years, our understanding of biology and of the gene-expression changes that result from exposure has accelerated, Dr. Daston said. He attributes much of the progress made so far to technologic improvements, such as the advent of commercially available, high-quality microarrays. Since the advent of those microarrays and the start of toxicogenomic experiments, we have discovered that a large component of regulatory control comes from microRNAs and methylation and acetylation reactions. He expects technology will continue to increase our understanding of epigenetics.

What discoveries await researchers in epigenetics? An epigenome can potentially provide a readout of a person's recent and ancestral environmental history, Dr. Meehan said; epigenetic profiling is possible, and epigenetic immunology is on the way. Clearly, researchers are exposing the secret workings of the epigenome for all to see.

Where the Science Is Going

Meeting participants were excited about the future of epigenetics research, and Dr. Daston expressed that enthusiasm in his comments about the prospects that

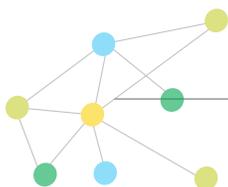
*This summary was prepared by Tina Adler,
with contributions by
Marilee Shelton-Davenport
and Norman Grossblatt*

Related News and Publications

Hilemann B. Chemicals can turn genes on and off; new tests needed, scientists say. Environmental Health News. 2009; August 3

About the Committee

At the request of the National Institute of Environmental Health Sciences, the National Academies forms the Standing Committee on Use of Emerging Science for Environmental Health Decisions to facilitate communication among government agencies, industry, environmental groups, and the academic community about scientific advances that may be used in the identification, quantification, and control of environmental impacts on human health.



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HEALTH DECISIONS NEWSLETTER

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