Bethesda: Dawn of the Epigenetics Era

24 June 2010. Epigenetics, the study of how modifications to DNA and chromatin regulate the activity of genes, is the hot new thing in bioscience. Emerging research indicates that epigenetic regulation may play a role in many complex human diseases, including Alzheimer's (AD). The AD field is just beginning to grapple with the question of how to handle epigenetic data, and how to integrate it with existing genetic data. To facilitate this process, the National Institute on Aging convened a workshop of about 60 scientists in Bethesda, Maryland, on 7-8 June 2010. **Suzana Petanceska**, **Marilyn Miller**, and **Tony Phelps** of the NIA organized the gathering, called "An Integrated Epigenetic-Genetic Approach to AD." It brought together AD scientists funded through the NIH Roadmap Epigenomics Program with other researchers working in genetics and gene regulation to share their data, discuss challenges, and promote collaborations.

The five-year NIH Roadmap Epigenomics Program began in 2008 as an "incubator space" for new topics, according to program coordinator **John Satterlee** of the National Institute on Drug Abuse in Rockville, Maryland. This $175 million program has five components, including an initiative to create reference epigenomes of a number of human cell types, a data analysis and coordination center, projects to encourage new technology development, and the discovery of novel epigenetic marks. The centerpiece of the program, Satterlee said, is the effort to enhance our understanding of human health and disease. To this end, NIH funded 22 research projects examining the role of epigenetics in disease, including four projects specific for AD and cognitive decline. Representatives from all four AD studies spoke at the workshop.

The workshop provided a portrait of a burgeoning field still in its infancy. Epigenetics research is exploding, said **Randy Jirtle** of Duke University in Durham, North Carolina. Jirtle showed data indicating the field has entered a stage of exponential growth in the last five years, with the number of epigenetics research papers roughly doubling every two years. "Epigenetics will become synonymous with biological research," Jirtle predicted. Speakers outlined ambitious agendas for extensive mapping and discovery research, but most projects are in their first year, with few data to show yet. One of the main issues that engaged the community was how to manage the flood of data that will be generated, and how to standardize their methods to allow meaningful comparisons among studies. The group also discussed how to integrate epigenetic data into genetics databases, as epigenetics must be viewed in the context of the underlying genome.

The Spotlight Shifts From Genetics to Epigenetics

For many years ApoE4 was the only gene proven to be linked to the common form of non-autosomal-dominant AD, but recently, genomewide association studies and meta-analyses of many association and linkage studies have begun to uncover new AD-associated genes, with several new genes reported in 2009 (see AlzGene for a comprehensive display and Top 10 list). **Allen Roses**, Duke University, described his work on a variable polyT repeat in the TOMM40 gene, which he found to be related to the age of onset of AD (see ARF related news story on Roses et al., 2009). The effect of most of the newly discovered genes is quite small, however, and some of them do not improve the ability of a model to predict AD, according to **Sudha**

Seshadri, Boston University, which means these genes at present are not useful in diagnosis (see ARF related news story on Seshadri et al., 2010). Geneticists continue to mine for new interactions, using both huge studies, such as the Alzheimer's Disease Genetics Consortium reported on by **Gerard Schellenberg**, University of Pennsylvania in Philadelphia, or ADNI, as well as small family studies. For example, **Margaret Pericak-Vance**, University of Miami, Florida, outlined an approach to find rare genetic variants with strong effects by studying families in which several members develop late-onset AD. Nonetheless, one main realization arising out of the field's intensive genetics efforts over the past decade is that genetics alone may not provide a full picture of AD heritability.

Into this impasse, enter epigenetics. These are heritable changes in DNA structure, such as the addition of methyl and acetyl groups, that affect gene expression but do not change the underlying DNA sequence. Jirtle compared epigenetics to the "software" that allows cells to access and interpret the information stored in the DNA "hardware": in effect, a programmable computer within each cell. Because epigenetic modifications control what genes are active in any cell, epigenetic regulation is the primary means of cell differentiation. Each tissue type has a distinct set of epigenetic marks, or pattern of chemical modifications, meaning each person contains over 200 different epigenomes. A particularly intriguing feature of epigenetic regulation is that it acts as an interface between genes and the environment. Epigenetic marks can change over a person's lifespan, either as part of normal aging, or due to environmental factors such as diet, drugs, pesticides, and disease. Epigenetic dysregulation may affect human ailments as diverse as cancer, psychiatric disorders, addiction, autoimmune diseases, asthma, glaucoma, and dementias, Satterlee said.

Another theme Satterlee and other speakers touched on is the potential of epigenetics to identify better biomarkers for the diagnosis or prognosis of AD. Several presenters discussed plans to hunt for AD biomarkers by correlating changes in AD brains with epigenetic markers in CSF or blood.

Although epigenetics is generally believed to hold promise for the development of novel therapeutic strategies, only one presenter discussed a particular therapeutic application. There was also little use of animal models. As epigenetic marks can vary greatly among species, results from animal models may be difficult to extrapolate to humans, Jirtle said. Most of the research reported at the workshop consisted of broad exploratory studies, seeking to pinpoint epigenetic changes between AD brains and normally aged brains. The attendees noted that they still lack definitive proof that epigenetics plays a significant role in AD, and their first job is to firm up the preliminary evidence. Most of the reported research involved the best-studied epigenetic marks—DNA methylation and histone acetylation—while a couple of speakers talked about a potential role in AD for a different form of genetic regulation, that of non-coding RNAs.

Gene Regulation by Non-coding RNAs

Non-coding RNAs make up the majority of the genome and come from what used to be called "junk DNA." They play a major role in the regulation of gene expression. **Claes Wahlestedt**, of The Scripps Research Institute in Jupiter, Florida, described a large exploratory study that is examining changes in non-coding RNAs in the CSF, entorhinal cortex, and hippocampus of AD brains. Initial results indicate that hundreds of non-coding RNAs are increased or decreased in AD brains compared to

normally aged brains. Wahlestedt held out the possibility that some of these CSF transcripts could become biomarkers for AD. He also suggested non-coding RNAs might have value as novel therapeutic targets, if scientists find a way to harness their ability to regulate the levels of harmful proteins such as β-secretase (Faghihi et al., 2008 and Faghihi et al., 2010).

MicroRNAs are small, non-coding transcripts that regulate translation of target genes' mRNA, according to **Peter Nelson**, of the University of Kentucky in Lexington. Many scientists have observed altered patterns of microRNA expression in AD brains, Nelson said. He described a method to directly identify the target transcripts of some of these altered microRNAs to discover what genes are being up- or downregulated in AD brains. This approach fingered a neurodegenerative disease risk factor gene, progranulin, which is targeted potently—and unexpectedly—by a microRNA (miR-107) downregulated in AD (Wang et al., 2010).—Madolyn Bowman Rogers.

Bethesda: The Methylated Brain

25 June 2010. One of the best-studied epigenetic modifications, methylation is the addition of a methyl group to a cytosine residue of DNA, usually at a cytosine paired to a guanine (CpG site). Methylation of gene promoters usually silences gene expression, perhaps by interfering with the binding of transcriptional proteins. The human genome contains around 28 million CpG sites, allowing for an enormous number of possible methylation patterns. Like other epigenetic marks, methylation patterns vary tremendously among different tissue types, according to **Benjamin Tycko**, Columbia University, New York. This means that each person contains hundreds of distinct "methylomes," rendering the complete mapping of the human methylome an overly simplistic goal. More positively, Tycko introduced the idea of methylation patterns being strongly influenced by the genetic makeup of the individual, an emerging theme linking genetics with epigenetics, which was taken up by others at the workshop.

Methylation is relatively easy to study, however, with numerous methods available. Some of the most common include cutting DNA with methylation-sensitive restriction enzymes, precipitating methylated DNA using antibodies, and using bisulfite conversion to mark methylated DNA sites prior to sequencing. **Cristian Coarfa** of Baylor College of Medicine in Houston, Texas, reported on a comparative study of several methylation mapping methods that found 95 percent agreement among their results, with the methods differing primarily in the resolution of the data and the cost.

Several ambitious methylome mapping projects are underway. One of the major achievements of the Roadmap project to date, said **Suzana Petanceska**, program director at the National Institute on Aging, is the single-base resolution mapping of methylation in human embryonic stem cells, reported in Nature (Lister et al., 2009). The authors compared the methylation pattern in stem cells to methylation in differentiated fibroblast cells, and found a unique pattern of non-CpG methylation in the former. This methylation pattern disappeared when the stem cells differentiated, and reappeared in induced stem cells, suggesting that it may be a hallmark of undifferentiated cells.

Several Roadmap-funded studies currently in progress are examining how methylation varies in AD brains in comparison to age-matched controls. One finding

keeps cropping up: global methylation is down in regions of the brain that are affected by AD. Tycko reported that the CA1 neurons of the hippocampus of AD brains show a global loss of methylation, but nearby brain regions do not. Work by **Paul Coleman** of Sun Health Research Institute in Sun City, Arizona, and **Peter Laird** of the University of Southern California in Los Angeles found a loss of global methylation in DNA from the temporal neocortex of AD brains, but not in DNA from the cerebellum, a region spared in AD.

It's unclear, however, what the significance might be of this loss of methylation. Although methylation silences genes, the hypomethylated regions of AD brains show no increase in gene transcription. In future work, Coleman said, they plan to further explore the relationship between methylation and mRNA expression. When Tycko and colleagues looked specifically at promoter methylation, they found few changes between AD brains and controls, suggesting that most of the loss of methylation occurs outside of promoter regions, in intergenic or intragenic sites. But intragenic methylation sites also have promoter activity, producing alternative transcripts, reported **Ting Wang**, Washington University, St. Louis, Missouri. The more methylation at the intragenic site, the lower the gene expression starting from these sites. These sites show a tissue-specific methylation pattern, suggesting that methylation may control the tissue-specific expression of alternative transcripts.

Another type of methylation now generating interest is allele-specific methylation, in which a particular gene allele dictates the nearby presence or absence of a methyl group (Kerkel et al., 2008 and Tycko, 2010). Since a genetic sequence controls the methylation state, this represents an interaction between the genome and the epigenome. Tycko discussed ongoing research into these interactions, which he hopes will allow researchers to extract more information from genomewide association data. For example, **Jonathan Mill**, King's College, London, reported on his finding that differential methylation of the insulin-like growth factor 2 (IGF2) gene is associated with brain weight (Pidsley et al., 2009). Small brains also correlate with AD risk and psychiatric disorders, suggesting that this epigenetic mark might be a risk factor for AD.

Several presenters discussed the impact of the environment on methylation, as demonstrated by studies of identical twins. Coleman reported that in a case of identical twins with similar education, the twin who developed AD had reduced methylation and decreased levels of enzymes responsible for methylation in his brain (see ARF related news story on Mastroeni et al., 2009). This was likely due to a difference in their environment, Coleman said, noting that the AD twin worked with pesticides for many years. Methylation can change dramatically in response to the environment, Mill said, with some people showing large methylation changes over just five years. Although monozygotic twins are 100 percent identical genetically, Mill found that they show much lower concordance in their epigenome. The epigenome may regulate the different disease outcomes of identical twins, Mill said. This is true not just in AD, but in psychiatric disorders as well.

Laura Rozek, University of Michigan in Ann Arbor, is pursuing the hypothesis that the deleterious effects of lead exposure are mediated by epigenetic changes. Lead exposure is associated with cognitive decline and decreasing scores on the Mini-Mental State Exam, as well as with decreasing levels of methylation in the blood. Previous studies in the field have shown that in a rodent model, exposure to lead early

in life led to higher APP expression and higher levels of Aβ late in life (Basha et al., 2005 and Wu et al., 2008). Rozek noted that the APP gene has a promoter rich in CpG sites, suggesting APP expression could be affected by methylation changes. Rozek is currently studying the relationship between lead exposure and methylation in people with AD. She will compare methylation in AD and control brains to methylation levels in blood and lifetime lead exposure, which she infers by measuring bone lead accumulation with x-ray fluorescence.

Despite the ease and appeal of studying methylation, workshop participants noted that methylation maps provide only a grainy picture of what is happening biologically, and methylation is probably less biologically significant than histone acetylation. Also, it appears that methylation changes in AD brains are small, unlike the dramatic methylation shifts scientists see in cancer.—Madolyn Bowman Rogers.

Bethesda: 'Ome' Sweet 'Ome'—Epigenome Joins Genome, Proteome

28 June 2010. Histone acetylation is a particularly intriguing epigenetic mark, in part because it changes dynamically. When histone acetyltransferases (HATs) add acetyl groups to a histone protein, previously coiled DNA opens up, exposing nearby genes for transcription. Histone deacetylases (HDACs) can clip off acetyl groups, turning off genes. Histone acetylation can interact with DNA methylation: for example, methylation-associated proteins can recruit HDACs, leading to gene silencing. HDACs have already garnered intense interest as therapeutic targets. Histone acetylation may have greater biological effects than methylation, according to workshop discussion, but it's harder to study. Several dozen types of histone acetylation sites exist, since acetylation can take place at numerous lysine residues scattered across many different histone proteins. Most studies look at only one or two sites. Despite the interest in histone acetylation, only a few such studies were discussed at the workshop.

One study, reported by **Philip Landfield**, University of Kentucky, Lexington, examined the effects of normal aging in the hippocampus in both rats and rhesus monkeys. Landfield found increased gene transcription in the hippocampus of normally aged brains. Most of this increase in transcription seemed to be in glial cells, rather than neurons. In rats, this appeared to be due to a decrease in HDACs, while in monkeys, Landfield saw an increase in HATs (see ARF related news story and Blalock et al., 2003; Rowe et al., 2007; Kadish et al., 2009; and Blalock et al., 2010). He speculated that these shifts in epigenetic regulators might have potential as therapeutic targets in AD. It's worth noting, however, that Landfield examined only normally aged brains, not brains with dementia. Also, because epigenetic marks vary tremendously by species, these results may not reflect what happens in humans.

An epigenome-wide association study in human AD brains is underway, however. It will use data from two long-running cohort groups, the Religious Orders Study and the Rush Memory and Aging Project, said **David Bennett** of Rush University, Chicago, Illinois. This project will examine acetylation of lysine residue 9 on histone protein 3 (H3K9 site) in prefrontal cortex, using donated AD and control brains, and correlate the results with genetic and clinical information. Starting next year, the study will examine methylation and mRNA expression in the same brains, as well.

Finally, **Stephen Haggarty** of Harvard University discussed a potential therapeutic application for histone acetylation. In a mouse model, knockdown of HDAC1 in neurons leads to DNA damage, cell cycle re-entry, and cell death, perhaps because open, accessible DNA is more vulnerable to damage. Overexpression of HDAC1 can rescue these neurons (see ARF related news story on Kim et al., 2008). Haggarty hypothesized that activation of HDAC1 might therefore prevent cell cycle re-entry and cell death in AD. He is currently conducting a high-throughput assay to discover small molecules that selectively activate HDAC1 and can be administered in vivo. Haggarty said his group has discovered an effective in vitro activator that also reduces DNA damage in vivo in mice.

Dealing With a Deluge of Data

One of the biggest challenges facing the fledgling field of epigenetics, the workshop participants agreed, will be the management of the vast amount of data that is sure to be generated in the coming years. Epigenetics is an extension of the Genome Project, and adds yet another layer of data on top of the already complex map of the genome. What's more, the huge variety of epigenetic marks known to exist make the epigenome more complicated than the genome. These marks vary not only from person to person, but also between tissue types, and even between cells of the same subclass. On top of that, they can change over a person's lifespan.

Epigenome data must also be correlated with expression data and clinical data. The "omes" keep multiplying, in dizzying layer after layer: the genome, the epigenome, the transcriptome, the proteome, the phenome. **Amanda Myers**, University of Miami, Florida, used the term "brainome" to describe the interactions of all of these layers in determining the health of the brain. She reported her work on a "Human Brainome" project that seeks to correlate genetic, expression, and protein data in the brain, using a computer algorithm to identify promising networks that might play a role in AD. She predicted this approach might enable scientists to define subclasses of patients and lead to more precise therapeutic approaches, as well as help identify biomarkers.

Given the importance of epigenome mapping, one of the goals of the Roadmap program is to support four multi-institutional epigenome mapping centers, which will produce epigenome maps of human cell types of interest in disease. Initially, these centers are working to produce high-resolution maps for just five cell types. These maps will include genomewide methylation data, the acetylation state of 53 histone sites, RNA sequencing data, and DNAseI hypersensitive sites. The centers will also produce less detailed maps for more than 100 human cell types. Tissues examined include breast stem cells, blood primary cells, pancreatic islets, and various cell lines. The mapping center led by Joseph Costello at the University of California, San Francisco, plans to create brain-related reference epigenomes using cells from selected regions of fetal and adult brains, said **Ting Wang**, Washington University, St. Louis.

Data from the epigenome mapping centers will be coordinated and analyzed at the Epigenomics Data Analysis and Coordination Center, led by Aleksandar Milosavljevic at Baylor College of Medicine, Houston, Texas. As a start, the center released the first version of the Human Epigenome Atlas on May 14, said **Cristian Coarfa**, also at Baylor College of Medicine. Successive releases will provide more detailed data.

The challenge of cataloguing epigenomic data goes beyond the epigenome maps, however. Researchers would like to integrate epigenomic data with data generated by the Encyclopedia of DNA Elements (ENCODE), an NIH project that seeks to identify every functional element in the human genome. In other words, ENCODE plans to discover the purpose of the vast majority of human DNA code once labeled "junk." Since all of this regulatory DNA may contain epigenetic marks, the merging of these databases would be powerful.

Importantly, data must be presented in a format that is meaningful to biologists. Mere lists of numbers are not helpful, a computational biologist at the workshop pointed out. In the group discussion, the University of California Santa Cruz Human Genome Browser repeatedly came up as an example of the kind of searchable database that researchers would like to see for epigenomics. The current genome browser is not adequate for epigenetics questions, however. In particular, a database for AD research would need to include pathological and clinical data. Several participants suggested they would like to be able to visualize the 3D structure of networks, as well. Wang, who works on the browser, suggested that these features might be added.

Participants also discussed the issue of how to share and compare data meaningfully. Since every experiment uses different methodology, comparisons are problematic. Participants agreed that the field is too new to standardize methods, as it's not yet clear which methods are best. One member suggested creating standard controls for certain experiments to permit comparisons across studies. The group also discussed the feasibility of forming a consortium for AD epigenetics research. In theory, this would facilitate communication and the sharing of data. The consensus seemed to be, however, that it's too early for that.

Workshop participants agreed that a concerted effort must go into building a computational foundation that can support not only the new field of epigenomics, but also the integration of epigenomic data with pathological data. To quote one workshop attendee, "The human disease side [of AD] is going to blow this whole thing open in terms of complexity."—Madolyn Bowman Rogers.