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.