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Charting the DNA-methylation landscape

In the late 1980s, it became apparent that cytosine methylation within CpG dinucleotides is sufficient to block the binding of transcription factors to DNA, thereby inhibiting transcription. To understand the role of this DNA modification *in vivo*, it became necessary to determine its frequency and location.

The first breakthrough came in 1992, with the development of bisulphite sequencing. This method exploits the fact that sodium bisulphite treatment induces a conversion of unmethylated (but not methylated) cytosine to uracil. DNA is then PCR amplified and sequenced. Because the resulting DNA strands are no longer complementary, PCR primers can be designed to yield strand-specific methylation patterns.

Bisulphite sequencing was especially useful for studies of individual loci, but with the dawn of the genomic era more global approaches were required. Developed in 2005, methylated DNA immunoprecipitation (MeDIP) provided a crucial advance in this regard. Here, an antibody specific for methylated cytosines is used to capture methylated DNA fragments, which can then be analysed in a range of standard ways, including by hybridization to DNA microarrays. Unlike previous restriction-based approaches, methylation detection using MeDIP is unbiased by the restriction enzyme-recognition sequence. In their 2005 paper, Weber *et al.* used arrays of human bacterial artificial chromosome (BAC) clones to generate chromosomal maps of methylation of the human genome, with an average tiling resolution of 80 kb. They also used this approach to carry out a global comparison of CpG island methylation in normal and colon cancer cells, revealing specific sites of hypermethylation in the latter.

Immunoprecipitation-based approaches were subsequently used to characterize genome-wide methylation in *Arabidopsis*. Zhang *et al.* and Zilberman *et al.* used antibodies against methylated cytosines and then hybridized the resulting samples to high-density tiling arrays. The resolution of the arrays (only 35 base pairs between the oligos) used by Zhang *et al.* allowed them to generate a particularly high-resolution genome-wide methylation map for *Arabidopsis*. Together, these two studies generated a wealth of data on the distribution of cytosine methylation in relation to functional elements within the genome, such as open reading frames and promoters. They also revealed a crucial interdependence between methylation and transcription.

Since 2000, the Human Epigenome Project has been identifying, cataloguing and interpreting genome-wide DNA-methylation patterns of human genes in major tissues and cell types. The recently initiated international Alliance for the Human Epigenome and Disease (AHEAD) project will extend this work to other epigenetic marks, and their roles in development and disease, including cancer. As always, biological discoveries will no doubt go hand in hand with further technological breakthroughs.

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ORIGINAL RESEARCH PAPERS Frommer, M. *et al.* A genomic sequencing protocol that yields a positive display of 5-methylcytosine residues in individual DNA strands. *Proc. Natl Acad. Sci. USA* **89**, 1827–1831 (1992) | Weber, M. *et al.* Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. *Nature Genet.* **37**, 852–862 (2005) | Zhang, X. *et al.* Genome-wide high-resolution mapping and functional analysis of DNA methylation in *Arabidopsis*. *Cell* **126**, 1189–1201 (2006) | Zilberman, D. *et al.* Genome-wide analysis of *Arabidopsis thaliana* DNA methylation uncovers an interdependence between methylation and transcription. *Nature Genet.* **39**, 61–69 (2007)