

## MOLECULAR BIOLOGY

## Phylogeny of Methylomes

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The DNA of most species is methylated, containing the modified base 5-methylcytosine. This modification has a role in silencing gene expression, among other important functions (1, 2). Advances in sequencing methods have allowed measurement of the first complete genome-wide DNA methylation map (“methylome”) of the model plant *Arabidopsis thaliana* and human cells (3–5). Studies by Feng *et al.* (6) and by Zemach *et al.* on page 916 of this issue (7) now expand this list by providing genome-wide methylomes for 20 additional species, revealing important conserved features and phylogenetic relationships of the methylation machinery.

Human DNA shows a dense and genome-wide methylation at CpG sites (cytosine and guanine dinucleotides) (1–3), including methylation of transposable elements, which suppress their transcription and recombination. Unmethylated CpG sites are mainly located in so-called CpG islands, regions with large CpG content. These islands often lie in the promoter regions of genes, and if methylated, they repress gene expression. Plants share features with mammalian methylation, such as methylation of transposons and lack of methylation at promoters of expressed genes, but they possess three methylation systems specific for CpG, CHG, and CHH (where H is the nucleotide adenine, thymine, or cytosine). In addition, methylation of gene bodies (the exons and introns of the genes) has been observed in mammals and plants (3, 4, 8, 9). However, not all species show high DNA methylation: The eukaryotic model organisms *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Drosophila melanogaster*, and *Caenorhabditis elegans* show zero or very little DNA methylation. As a consequence, DNA methylation was long considered an interesting phenomenon but not of general importance.

For animals, Feng *et al.* and Zemach *et al.* show strong preference for methylation at CpG sites due to high specificity of the DNA methyltransferase 1 (Dnmt1) enzymes. Non-CpG methylation is introduced by Dnmt3 enzymes, fungi-specific

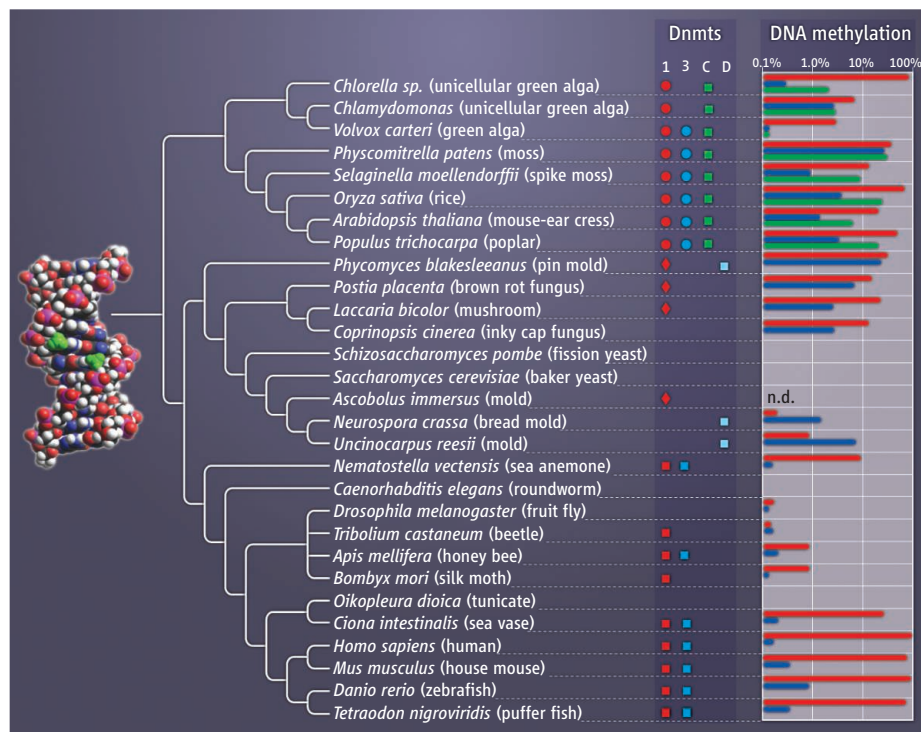
Dim-2-type enzymes, and perhaps additional families of Dnmts. In plants, CpG methylation is maintained by the plant subfamily of Dnmt1 enzymes called Met1; CHG methylation is mediated by the plant-specific chromomethylases; and CHH methylation is introduced by the DRM enzymes (see the figure) the plant subfamily of Dnmt3 enzymes. Conservation of the different Dnmts and DNA methylation levels suggests that the last common ancestor of eukaryotes contained a functional DNA methylation system with secondary expansion and the loss of methylation in some lineages. Primitive methylation likely occurred at low to intermediate levels and was targeted to gene bodies and transposable elements, leaving gene promoters unmethylated.

Zemach *et al.* and Feng *et al.* found that methylation of the gene body (3, 4, 8, 9) is highly conserved and likely was present in the last common ancestor. It might be involved in the prevention of transcriptional initiation within the gene body. Interestingly, gene body

The patterns of DNA methylation across the genomes of 20 eukaryotes reveal conserved features and specific roles during evolution.

methylation shows a parabolic correlation with gene expression, with genes expressed at an intermediate level having the highest amounts of gene body methylation (7). This suggests the coexistence of two opposing targeting mechanisms that increase or decrease methylation with gene expression. Two interesting details of this process are that exons tend to be more highly methylated than introns (as in human DNA) (3, 10) and that the end of the gene shows a similar drop in methylation as the gene’s promoter region. These findings imply general roles of DNA methylation in transcriptional elongation, termination, and perhaps alternative splicing.

One trend observed in all kingdoms is that highly developed multicellular organisms show increased DNA methylation. This may be due to the need for more efficient control of transposons because of outbreeding sexual propagation (7), or the need for additional epigenetic regulation to control the development of many different cell types. Lower methyla-



**Methylomes and methylation machineries.** The phylogenetic tree was made with National Center for Biotechnology Information taxonomy and the Interactive Tree of Life. Dnmt1 (red), Dnmt3 (blue), chromomethylase (green), and Dim-2 families (light blue) are shown. DNA methylation data (6, 7) are averaged. Only plant CMT methyltransferases display a preference for CHG sites; CHG methylation is shown only for plants. Methylation data for human (10) and *N. crassa* (23) are included. *A. immersus* contains DNA methylation (24), but genome-wide distribution has not been determined (n.d.). DNA methylated at a CpG site (green) is on the left.

tion of gene promoter regions is observed in most species. It is correlated to gene expression in plants and animals, which suggests that the role of promoter methylation in gene regulation may be derived. In mammals and flowering plants, this process has been developed into the imprinting system that generates parent of origin–dependent methylation marks on a small subset of genes and leads to their monoallelic expression (11, 12).

On the other hand, several species underwent loss of DNA methylation to a large degree, such as the nematode *C. elegans*, the insect *D. melanogaster*, and the yeasts *S. pombe* and *S. cerevisiae*; hence, DNA methylation is not essential under all conditions of life, and its loss may even be beneficial. This could be connected to the resources allocated to global DNA methylation or to the fact that 5-methylcytosine is mutagenic, because its deamination is repaired less efficiently than deamination of unmethylated cytosine.

Human DNA methylation patterns vary with cell type and developmental stage (3, 10, 13–18), with disease (10, 15, 16), between alleles (11, 19–22), and among individuals (14, 15). Future methylome studies may reveal similar features in the other model organisms as well. Functional investigations of the properties of Dnmts and their interaction with chromatin and additional factors will clarify the mechanisms by which methylation patterns are set and maintained. Other questions yet to be resolved surround the means by which repeats and transposable elements are identified, the processes that target DNA methylation to gene bodies in an expression-dependent manner, the biological function of gene body methylation that led to its high stability in evolution, and the mechanisms to generate and modify tissue-specific patterns of promoter methylation in mammals.

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## CLIMATE

# A Paleoclimatic Enigma?

William F. Ruddiman

Major glaciations began in the Northern Hemisphere around 2.75 million years ago, after a long prior interval of climatic cooling. Numerous observations reveal how climate cooled before glacial onset, but our understanding of the driving forces behind the cooling remains incomplete.

Climate had been cooling from pole to pole for 50 million years before northern glacial onset (see the figure). Arctic forests changed from frost-intolerant evergreens to temperate deciduous trees to cold-adapted spruce and larch and eventually to tundra near the time of glacial onset (1). Antarctica was mostly ice-free until 34 million years ago; glaciers of varying size then existed on the continent until 14 million years ago, after which a large and relatively stable ice sheet formed. The gradual shift toward heavier  $\delta^{18}\text{O}$  values in  $\text{CaCO}_3$  shells of sea-floor foraminifera since 50 million years ago documents a combined deep-ocean cooling and increase in Antarctic ice (2, 3).

Until a decade ago, most paleoclimate modelers attributed this ongoing bipolar cooling to a gradual reduction in the  $\text{CO}_2$  con-

centration in the atmosphere. This inferred  $\text{CO}_2$  decrease was ascribed to a combination of reduced volcanic  $\text{CO}_2$  input to the ocean and atmosphere because of a slowing rate of sea-floor spreading (4) and increased  $\text{CO}_2$  removal by enhanced chemical weathering in tectonically uplifting regions like Tibet (5).

Methods devised to reconstruct the  $\text{CO}_2$  concentration of the atmosphere on longer time scales later tested this conclusion. One method relied on the  $^{13}\text{C}$  composition of marine organic molecules called alkenones (6), another on boron-isotopic values in marine carbonate sediments (7). Results from both methods suggested estimated  $\text{CO}_2$  concentrations of around 1000 parts per million (ppm) or more during much warmer climates tens of millions of years ago, compared with ice-core values of just 180 to 300 ppm during the glacial cycles of the past 800,000 years.

In a broad sense, this long-term  $\text{CO}_2$  decrease provided some support for the idea that  $\text{CO}_2$  has been the long-term driver of global cooling, but a closer look revealed major problems. By 22 million years ago, the alkenone and boron isotope data both showed that estimated  $\text{CO}_2$  concentrations were already within the range typical of the glacial cycles of the past 800,000 years. If  $\text{CO}_2$  concentrations of 180 to 300 ppm have played an integral role in allowing glacial cycles in the

What caused the onset of major Northern Hemisphere glaciation about 2.75 million years ago?

past 800,000 years, why did comparably low  $\text{CO}_2$  values 22 million years ago not initiate glacial cycles? And if the average  $\text{CO}_2$  trend has not fallen in the past 22 million years, what caused the substantial bipolar cooling during that time?

Other proposed causes seem insufficient to explain large-scale cooling. Gradual plate motions and falling sea level have extended the northern margins of circum-Arctic continents into cooler near-polar latitudes (8), but models suggested that these factors were not enough to explain the major cooling observed. Closing of the Isthmus of Panama, about two million years before the onset of northern glaciations, has been proposed as a causal factor in glacial inception. Results from coupled ocean-atmosphere models indicate, however, that isthmus closure would have sent greater amounts of sensible heat northward in the Atlantic and melted more snow and ice, rather than promoting ice growth by delivering more moisture (9).

In the past 15 million years, broad-scale uplift of plateaus and mountains has occurred in the northern and eastern Tibetan Plateau, the east-central Andes and Altiplano, the east African rift valley, and the northern Canadian Rockies. Elevation of these rock surfaces to cooler levels in the atmosphere would have cooled the uplifted terrain and nearby regions,

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