simulation of gene network evolution, sexual recombination selected for networks in which mutations were negatively epistatic[12]—the very condition hypothesized to favor sex. Epistatic interactions will have a major role in the rapidly expanding study of gene and protein networks both as a basic property of those networks and as a valuable tool.

COMPETING INTERESTS STATEMENT
The author declares no competing financial interests.

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The human promoter methylome

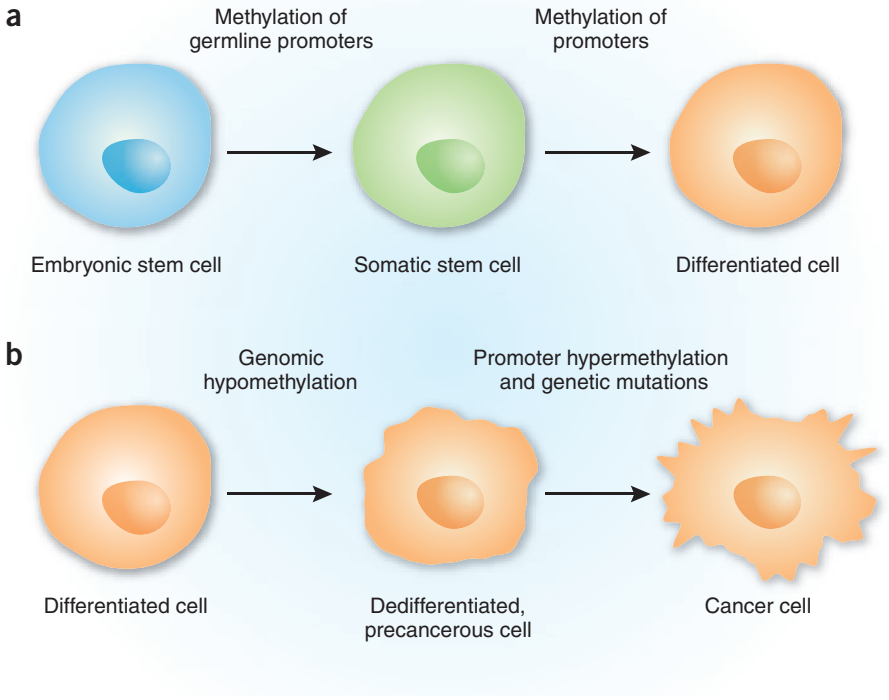
Daniel Zilberman

DNA methylation is a heritable epigenetic mark found in a wide range of eukaryotes. By mapping DNA methylation within the majority of human promoters, the authors of a new study uncover intriguing insights into genome evolution, cellular differentiation and potential links to tumorigenesis.

The completion of the human genome has provided a wealth of information about our genetic wiring. However, there is a great deal of information within the chromatin fiber beyond the DNA sequence. Unlike genetic information, which can be read, but not written, this epigenetic information is actively altered, providing a flexible framework for specifying states of gene activity. DNA methylation, a covalent modification of DNA, is used by organisms ranging from mammals to plants to bacteria to convey epigenetic information[1,2]. Eukaryotes predominantly methylate cytosines in CpG dinucleotides. Because CpG dinucleotides are symmetric, a DNA methyltransferase can scan newly replicated DNA for hemimethylated CpG sites and fill in the missing methylation. This semiconservative replication, akin to that of the DNA sequence, makes methylation an excellent repository of epigenetic information. The first methylome—the profiling of methylation within an entire genome—was recently reported for the plant *Arabidopsis thaliana*[3,4]. Now, Weber *et al.*[5] take the closest step yet toward the human methylome by mapping DNA methylation within the majority of human promoters in somatic cells and mature sperm.

A versatile epigenetic mechanism

In eukaryotes, DNA methylation is associated with transcriptional repression, particularly at gene promoters. DNA methylation is important in a variety of processes, including transposon silencing, X chromosome inactivation and genomic imprinting[1]. Whether changes in somatic DNA methylation have a role in mammalian development has been controversial[6]. Dirk Schübeler's group has recently developed a method that uses antibodies to 5-methylcytosine to enrich methylated DNA[7]. In the current study, they combined this with high-density DNA microarrays to obtain the methylation profile of human promoters[5]. To monitor promoter activity, the authors used the same arrays to map sites of RNA polymerase II binding. Their results suggest that differential methylation is not a general mechanism for regulating gene expression, because most inactive promoters remained unmethylated. However, the promoters of a small subset of genes were methylated in fibroblasts, but not in sperm, indicating that somatic methylation might contribute to differentiation and development. A particularly intriguing finding was that among differentially



Kim Caesar

Figure 1 DNA methylation in development and disease. (**a**) Methylation of key genes during development ensures a stable differentiated phenotype. (**b**) A somatic cell undergoes genome-wide hypomethylation, leading to a population of dedifferentiated, precancerous cells. Inappropriate methylation of tumor suppressors in combination with genetic mutations leads to cancer.

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methylated genes, a large number were germline specific, suggesting that DNA methylation shuts off expression of these genes in somatic tissues.

Considering that DNA methylation is a covalent, heritable modification, the limited use of somatic methylation to control gene expression makes sense. Methylation can be thought of as an intermediate form of regulation: less nimble than transcription factor binding but not as permanent as genetic changes. Methylation would be an unwieldy system to rapidly regulate transcription, but it is ideally suited for long-term gene silencing. Therefore, the most likely regulatory role for methylation is to repress key genes, thereby cementing cell fate (**Fig. 1a**). Germline-specific genes are a good example: these genes must be permanently turned off in somatic cells. Nonetheless, unlike genetic changes, methylation can be reversed, potentially leading to disease (discussed in more detail below).

Evolutionary implications

Despite its utility in silencing genes, DNA methylation carries a cost to the organism, in part because 5-methylcytosine is mutagenic[8]. Deamination of an unmethylated cytosine produces a uracil, which is efficiently removed by the base excision repair machinery, whereas deamination of 5-methylcytosine yields thymine, a normal DNA base. As a result, the human genome contains only about 20% of the expected number of CpG sites. Gene promoters tend to be associated with regions of high CpG density, termed CpG islands[9]. Weber *et al.* found that CpG island–containing promoters are rarely methylated, particularly in the germline. In contrast, promoters without CpG islands are usually methylated. General methylation of promoters without CpG islands suggests that methylation has led to the current sequence composition through loss of methylated CpG sites. Furthermore, by comparing the human and chimpanzee genomes, Weber *et al.* found that promoters methylated in sperm show enhanced loss of CpGs, indicating that methylation continues to shape the evolution of the human genome. Weber *et al.* also found that CpG-rich promoters, but not CpG-poor promoters, are silenced by methylation, probably because the latter no longer have sufficient methylation density to elicit silencing. This phenomenon might explain why Weber *et al.* found promoters that become *de novo* methylated in fibroblasts but essentially did not find any promoters methylated exclusively in sperm. Because methylated CpGs are unstable, promoters methylated in the germline are destined to become low-CpG promoters, which cannot be regulated by methylation.

Development and cancer

Cancer has long been thought to progress through accumulation of genetic mutations. However, it has become increasingly clear that epigenetic mechanisms have a central role in tumorigenesis[10,11]. Tumors frequently show overall hypomethylation combined with hypermethylation of tumor suppressor genes. Perhaps the most striking illustration of the epigenetic basis of cancer was the cloning of a mouse from the nucleus of a melanoma cell[12]. The egg cytoplasm was able to reprogram the cancer nucleus, erasing the melanoma phenotype and allowing the development of a normal mouse, albeit one with a predisposition to cancer. In this case, the major underlying causes of cancer were clearly epigenetic.

The findings of Weber *et al.* provide a plausible mechanism for some of the epigenetic underpinnings of cancer. If methylation of key genes is used to cement a differentiated state, then loss of DNA methylation would result in a dedifferentiated, developmentally plastic phenotype associated with tumors (**Fig. 1b**). Combined with hypermethylation of tumor suppressors, such changes could conceivably drive tumorigenesis with few genetic alterations. Genome-wide analysis of promoter DNA methylation in cancer cells is therefore likely to yield valuable insight into the biology and progression of this disease.

COMPETING INTERESTS STATEMENT
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Drosophila melanogaster neurofibromatosis-1: ROS, not Ras?

James A Walker & André Bernards

A new study suggests that manipulating the expression of a *Drosophila melanogaster* neurofibromatosis-1 ortholog affects organismal lifespan through protein kinase A–mediated regulation of mitochondrial respiration and reactive oxygen species production. These results provide a new and unexpected twist to the study of NF1 signaling.

Neurofibromatosis-1 (NF1) is the most common genetic disease associated with an increased risk of cancer. It is caused by loss of neurofibromin, whose only well-understood function is to serve as a GTPase activating protein for Ras (RasGAP). In the 16 years since the discovery of neurofibromin, virtually all aspects of the phenotype in individuals and mouse models of NF1 have been attributed to aberrant Ras signaling[1,2]. This Ras-centric view, however, has been challenged by findings that most *D. melanogaster NF1* phenotypes are not readily modified by manipulating Ras activity. Based on findings that most *D. melanogaster NF1* phenotypes are enhanced by decreasing signaling through the cAMP-dependent protein kinase A (PKA) pathway and rescued by increasing signaling through this pathway[3,4], it has been suggested that neurofibromin may also positively regulate the cAMP/PKA pathway. Nonetheless, there have been conflicting reports as to whether this role is independent of neurofibromin's role as a RasGAP[5,6].

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