Meddling with methylation

Chih-Lin Hsieh and Peter A. Jones

Covalent modification of DNA by methylation is crucial for maintaining the stability and structure of chromatin. Reports of the effects that altered methylation has for cancer development stress the importance of balanced genomic methylation.

Until now, almost all activity in the field of cancer epigenetics has been focused on the role of increased cytosine methylation in specific gene promoters as a mechanism for initiating or enforcing the silencing of tumour suppressors[1]. Despite the fact that we have known for almost 25 years that the genomic content of 5-methylcytosine is often decreased, rather than increased, in animal and human cancers[2–4], the functional importance of hypomethylation was never clear. Now, two papers in *Science* by Eden *et al.*[5] and Gaudet *et al.*[6] show that decreased methylation in knockout mice results in an increased rate of chromosomal instability and also in the development of lymphomas. On the other hand, another paper by Sansom *et al.*[7] in *Nature Genetics* shows that cancer-prone *min* mice, which also lack MBD2 (a protein that binds methylated cytosines and results in transcriptional silencing), develop fewer intestinal adenomas. These papers underscore a central role for DNA methylation in cancer, but seem to have different messages — in the first case, decreased methylation promotes cancer; in the second, failure to interpret the methylation signal is associated with a decreased incidence of cancer.

Eden *et al.*[5] use a clever method to show that mouse embryonic fibroblasts derived from *Npcis* mice (mice carrying mutations in both the neurofibromatosis and p53 tumour suppressor genes), which also carry a hypomorphic allele of the DNA methyltransferase, *Dnmt1*, have a roughly twofold increase in the rate of loss of heterozygosity (LOH). This LOH assessment utilized a chromosome with loci that can be selected in a tissue culture system. These tumour-prone mice also showed an increased rate of soft-tissue tumours, including sarcomas. Previous studies have evaluated the effect of reduced methylation on chromosomal instability in embryonic stem cells, but the importance of the current work is that it uses somatic cells that are probably more relevant to carcinogenesis. Because instability of chromosomes is of such fundamental importance to the generation of animal and human cancers, this experiment demonstrates a causal relationship between DNA methylation and chromosomal loss in somatic cells. Consistent with the study from Eden *et al.*, Gaudet *et al.*[6] show that reducing the level of Dnmt1 by 90% results in genome-wide hypomethylation in all tissues and that such mice develop aggressive T-cell lymphomas accompanied by a high frequency of chromosome 15 trisomy at 4–8 months of age. Together, these studies suggest that reduced methylation results in chromosomal instability and increased risk of cancer.

How, then, does DNA hypomethylation contribute to the loss of heterozygosity and to production of sarcomas and lymphomas? Perhaps the answer lies in the known ability of altered DNA methylation and chromatin structure to directly affect chromosomal stability. Patients with ICF syndrome (for immunodeficiency, centromere instability and facial anomalies), who have mutations in the DNA methyltransferase 3b, show specific decondensation of the centromeric and pericentromeric regions of particular chromosomes, and this is associated with decreased DNA methylation in these regions[8]. Methylation of cytosines, as well as methylation of specific lysine residues on histones, may well be involved in the stabilization of heterochromatic regions of the chromosomes. The elegant work of the Jenuwein laboratory[9] has already demonstrated that loss of the *Su(var)3-9* histone methyltransferase, which governs H3-K9 methylation at pericentric heterochromatin, results directly in

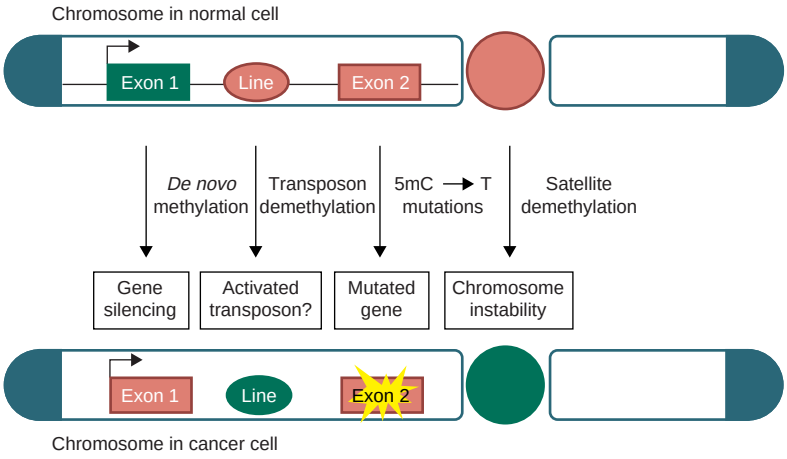

Figure 1 Changes in the patterns of DNA methylation on different DNA elements contribute to the genesis of altered chromosomes in cancer. Most repetitive DNA, including satellite DNA in centromeric regions and dispersed repetitive DNAs (such as LINE elements) are extensively methylated (red) on normal chromosomes but become hypomethylated during cancer (green). Potentially, this could result in chromosomal instability, possibly through altered heterochromatinization. Recent work shows that decreasing overall methylation increases the rate of chromosomal loss and lymphomagenesis[5,6]. Although not discussed in detail here, the coding regions of most genes contain 5-methylcytosine and this can contribute directly to the formation of inactivating mutations (yellow), such as those seen in p53 (ref. 17). Many tumours contain increased cytosine methylation in the promoters and genes that can initiate or reinforce their heritable silencing[1]. Cytosine methylation in such promoters is recognized by proteins such as MBD2 — if this interpreter is missing, cancer-prone mice develop fewer adenomas[7].

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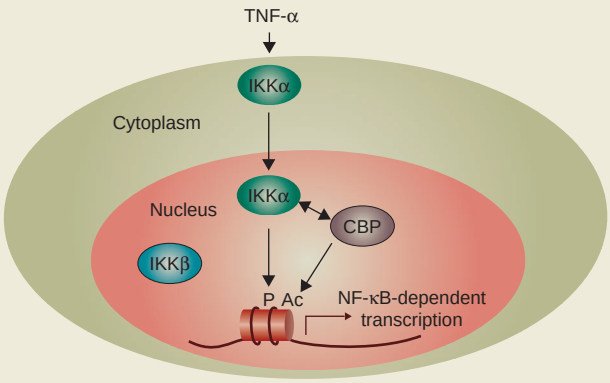
IKKα: a chromatin modifier

Activation of the transcription factor NF-κB depends on inducible phosphorylation and subsequent degradation of NF-κB inhibitors, known as IκBs. Two reports by Anest *et al.* (*Nature* DOI: 10.1038/nature01648) and Yamamoto *et al.* (*Nature* DOI: 10.1038/nature01576) now describe how IκB kinase (IKK) provides an additional layer of regulation through NF-κB-mediated transcription. They show that a subunit of IκB kinase can phosphorylate histone H3 and may therefore modulate chromatin accessibility at NF-κB-responsive promoters.

IKK is composed of two catalytic subunits, IKKα and IKKβ, and is crucial for cytokine-induced IκB degradation and subsequent activation of NF-κB. Previous studies have shown that IKKβ is essential for degradation of IκB. In contrast, IKKα, although not necessary for proteolysis of IκB, is still important for NF-κB-dependent transcription. However, exactly how IKKα regulates NF-κB-dependent transcription is unclear. Previous work showing that IKKα shuttles between the nucleus and the cytoplasm hinted at the possibility of a novel nuclear function for IKKα.

Here, both Anest *et al.* and Yamomoto *et al.* start by confirming that IKKα is a nuclear protein. To address whether nuclear IKKα regulates cytokine-inducible NF-κB gene transcription, the groups used chromatin immunoprecipitation (ChiP) assays to examine the promoter occupancy of NF-κB target genes. These experiments showed that after cytokine stimulation, IKKα is recruited to NF-κB target-promoters in a NF-κB-dependent manner. Furthermore, they showed that the kinetics of IKKα recruitment parallel phosphorylation of histone H3-Ser 10 at the promoter, an event that is known to correlate with active gene expression. Both studies also showed that IKKα is most probably the physiological kinase responsible for cytokine-induced phosphorylation of histone H3, as phosphorylation of histone H3 at Ser 10 was markedly reduced in *IKKα*$^{-/-}$ murine embryonic fibroblasts and IKKα directly phosphorylated Ser 10 *in vitro*. Thus, these findings suggest that IKKα may affect gene expression by regulating chromatin structure at promoters.



Nuclear IKKα is necessary for histone H3 phosphorylation at NF-κb-dependent promoters.

Histone phosphorylation at Ser 10 is often accompanied by increased acetylation at histone H3-Lys 14. Indeed, both reports show that in addition to decreased levels of phosphorylated H3-Ser 10 in *IKKα*$^{-/-}$ cells, levels of H3 acetylation are also reduced at NF-κB-responsive promoters. Furthermore, Yamomoto *et al.* find that IKKα interacts with the histone acetyl transferase, CBP, but that recruitment of CBP to the promoter is not dependent on IKKα. Collectively, these data suggest that phosphorylation of histone H3 at Ser 10 by IKKα is required for subsequent H3-Lys 14 acetylation by CBP and that both events are necessary for efficient activation of NF-κB-mediated transcription.

It is unclear whether phosphorylation of histone H3 at Ser 10 by IKKα will be important for transcription of all, or only a subset of, NF-κB targets. Whether IKKα will be required for NF-κB-independent gene transcription also remains an open question. Furthermore, Anest *et al.* find that IKKβ is also recruited to NF-κB promoters, but that IKKβ does not seem to phosphorylate histone H3 at Ser 10, hinting at a potentially novel function for IKKβ in the NF-κB pathway.

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chromosomal instabilities that are associated with increased risk of B-cell lymphomas[9]. Again, this points to the importance of maintaining an appropriate heterochromatic structure. Experiments have shown that flies with abnormal methylation induced by the mouse DNA methyltransferase 3a show inappropriate chromosome condensation that could be subsequently rescued by knocking out *Su(var)3-9* (ref. 11). So, it seems probable that the crosstalk between cytosine methylation and histone modification is important for the maintenance of chromosomal stability on the basis of increasing evidence that these two systems are mechanistically linked[10].

The paper by Sansom *et al.*[7] demonstrates for the first time that lack of the MBD2 protein, which is a methyl-CpG-binding protein and a potential transcriptional repressor, inhibits intestinal adenoma development in *min* mice. This is consistent with earlier work from the Jaenisch laboratory[12], in which decreased methylation resulted in less intestinal tumorigenesis in *min* mice. Thus, the failure to methylate DNA, or the failure to interpret the methylation signal by one of the methylated DNA-binding proteins, results in fewer epithelial-derived tumours. At first glance this result seems to be at odds with the two papers discussed above, which demonstrated that reduced methylation increases soft-tissue sarcoma and risk of developing lymphomas. So why should perturbing DNA methylation or reading of the methylation signal result in increased tumour formation in some cases and decreased tumour formation in others? In addition, why do the *min* mice not develop lymphomas when they have less methylation[12] or defects in methylation signal interpretation[7]? Perhaps the answer lies in the fact that the reduction of methylation level in the earlier study[12] was significantly less than that found with the new hypomorphic *Dnmt1* allele used in the studies by Eden *et al.* and Gaudet *et al.* Similarly, the failure of *MBD2* knockout mice to develop lymphomas may be a result of a lower penetrance because of redundancy with other proteins that might be more relevant to heterochromatic regions than to promoters. It is noteworthy that MBD2 has been implicated in the silencing of tumour suppressor genes in both human colorectal carcinoma and prostate cancer cells[13,14]. Another possibility is that reduction of methylation and deficiency of MBD2 in *min* mice affects genes downstream in the tumorigenesis pathway or

upregulates other genes, resulting in less tumour formation. It is interesting to note that mice deficient in MLH1 (one of the mismatch repair proteins) carrying the hypomorphic *Dnmt1* mutation have a reduced incidence of adenomas, whereas the risk of lymphoma in these mice is increased[15].

Experiments in these three papers provide evidence that several of the changes in cytosine methylation noted in human cancers are directly involved in tumorigenesis (Fig. 1). For example, abnormal cytosine methylation in promoters is known to be associated with altered chromatin structure and the binding of proteins, such as MBD2, that results in suppression of gene expression. Similarly, methylation can be involved in the silencing of transposons[16] and has been implicated in the production of point mutations by de-amination within the coding regions of genes[17]. The demethylation of satellite sequences in tumours has also been well documented and studied over many years[4]. These methylation changes have been implicated in tumorigenesis. However, they tend to be more restricted to specific genes or regions of chromosomes in specific human tumours, unlike the artificial situation in the mouse studies in which genome-wide changes are engineered into every somatic cell. For example, human satellites become demethylated, whereas promoters tend to become increasingly methylated as a function of tumorigenesis. Perhaps the formation of some tumours, such as sarcomas and lymphomas, depends more on chromosomal instability caused by satellite demethylation than the epithelial tumours, which are a feature of the *min* mouse and of most human cancers. The MBD2 pathway might be involved in silencing of tumour suppressor genes, which is a pathway more intimately involved with the generation of the epithelial tumours, whereas a global decrease in DNA methylation might contribute to the chromosomal instability that can result in the generation of sarcomas and lymphomas. The balance of DNA methylation is rather delicate, and tipping it can be protective by way of one pathway yet harmful by way of another. Hence, the determination of the physiologically safe range seems to be critical for the development of any cancer therapy based on perturbation of DNA methylation. □

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CELL OF THE MONTH

Crumbs and Moesin define *Drosophila* photoreceptor apical domains

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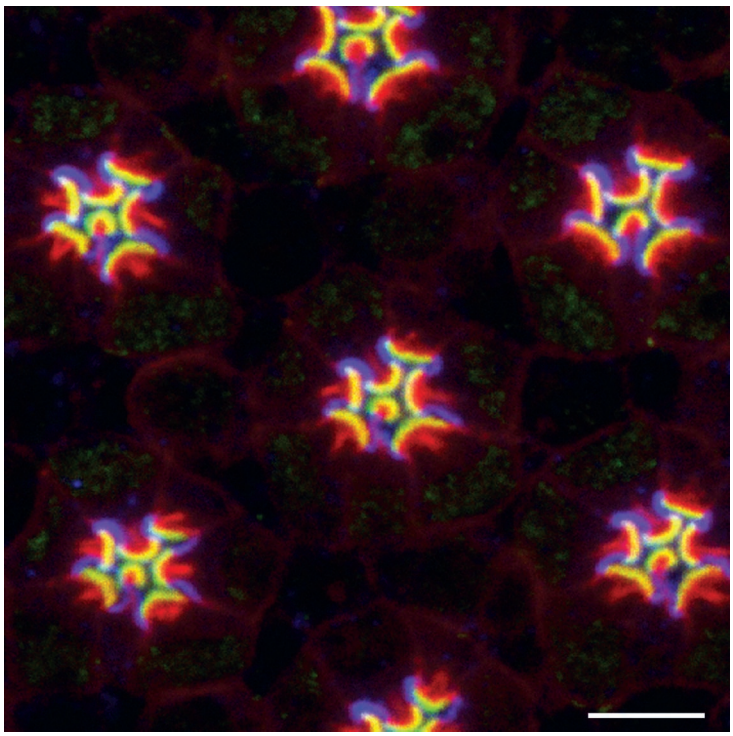

This month's winning image shows a *Drosophila melanogaster* retina that has been immunostained for Crumbs and Moesin.

Blue and green fluorescence represents Crumbs and Moesin immunostaining, respectively. The actin cytoskeleton was stained with rhodamine-conjugated phalloidin (red), highlighting the dense filamentous actin bundles of the rhabdomere terminal web. The image was acquired using a confocal microscope (MRC 1024, BioRad). Bar, 10 μm.

Sue Karagiosis would like to acknowledge the help of Donald Ready (Purdue University, (dready@bilbo.bio.purdue.edu)).

Advisors: Ariel Ruiz i Altaba (Developmental Genetics Program, New York Medical Center) and Lelio Orci (Department of Morphology, University Medical Center, Geneva).