Control of gene expression by CpG island methylation in normal cells

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Abstract
The role of DNA methylation in the control of mammalian gene expression has been the subject of intensive research in recent years, partly due to the critical role of CpG island methylation in the inactivation of tumour suppressor genes during the development of cancer. However, this research has also helped elucidate the role that DNA methylation plays in normal cells. At present, it is also clear that DNA methylation forms an important part of the normal cell-regulatory processes that govern gene transcription. Methylation, targeted at CpG islands, is an important part of the mechanisms that govern X-chromosome inactivation; it is also essential for the maintenance of imprinted genes and, at least in some cases, is critical in determining the cell-type-specific expression patterns of genes. Study of these examples will be important in identifying the mechanisms that control targeting of DNA methylation and how these processes are disrupted during disease pathogenesis.

Introduction
Methylation at the 5’ position of cytosine residues that form part of CpG dinucleotides is the only commonly occurring covalent modification of DNA and approx. 70% of the CpG sites in the human genome are methylated [1]. CpG dinucleotides are underrepresented throughout the genome, except for short stretches of DNA known as CpG islands. These CpG islands are GC-rich stretches of DNA of up to a few kb in length with approximately the expected number of CpG dinucleotides and are frequently associated with human genes, often mapping to the promoter/first exon of the gene. In contrast with the bulk of DNA, the CpG sites within CpG islands are mostly methylation-free [1]. Studies on the effect of CpG island methylation have clearly demonstrated that increased methylation of CpG islands within gene promoters is associated with transcriptional repression of the gene [2].

Interest in the field of DNA methylation has increased significantly in recent years, since it has become clear that aberrant methylation of CpG islands plays a major role in the inactivation of genes during the development of cancer. Indeed, inactivation of genes due to CpG island methylation is probably as important as genetic changes in the development and progression of cancer [3].

Despite playing an important role in the control of gene expression in tumour cells, DNA methylation is often believed to play only a very limited role in normal cells. Indeed, although DNA methylation was originally proposed as a mechanism for control of tissue-specific expression [4], analysis of the methylation status of CpG islands in human adult somatic tissues determined that CpG islands were almost always methylation-free even in tissues where the associated gene was not expressed [1]. However, there are some sets of genes for which there is a clear correlation between CpG island methylation and transcriptional inactivation in normal cells. Some of these are now well established, whereas other examples are only now coming to light (Figure 1).

X-chromosome inactivation
Female mammals contain two copies of the X chromosome, whereas males contain only a single copy. The problem of dosage compensation is overcome by random inactivation of one X chromosome in females during early development. Inactivation of the X chromosome is associated with widespread methylation of CpG islands throughout the inactivated, but not the active, X chromosome [5]. In addition, the CpG islands associated with the small number of genes that escape inactivation (such as those in the pseudoautosomal region) remain unmethylated on both the active and inactive chromosomes [5]. DNA methylation of CpG islands within the inactive X chromosome is clearly important in the maintenance of inactivation, since treatment of cells with inhibitors of methylation results in re-activation of previously silenced genes on the inactive chromosome [6]. DNA methylation may also play a role in the initiation of X-chromosome inactivation, although this remains to be clearly demonstrated [7].

Imprinting
A number of mammalian genes are known to be imprinted. That is to say that they are functionally haploid, with expression being specifically limited to either the paternally or the maternally inherited allele [8]. DNA methylation seems to be a critical part of the imprinting signal and almost all

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Abbreviation used: DMR, differentially methylated region.

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imprinted genes identified to date are associated with DMRs (differentially methylated regions) [9]. The DMRs can map to the promoter region of the imprinted genes, but are also frequently found in non-promoter sequences. At least in some cases, these non-promoter DMRs appear to regulate the transcription of antisense products that are believed to antagonize the expression of the sense product. For example, the mouse Igf2r gene is maternally expressed and exhibits maternal methylation of a DMR in the second intron of the gene, which blocks transcription of an antisense product. Conversely, the paternal allele exhibits methylation of the Igf2r promoter region, but is not methylated within the second intron and, thus, expresses the antisense but not the sense product. The DMR within the second intron appears to be crucial in the control of the imprinted signal, since deletion of this region results in bi-allelic expression of Igf2r [10].

**Testis-specific genes**

A large group of genes, which are expressed specifically in testis, also appears to be subject to inactivation by CpG island methylation in all somatic tissues [11]. The first of these to be identified was the MAGE1 (melanoma-associated antigen) gene [12], following the discovery that it was frequently re-activated in melanomas. Subsequently, it was found that MAGE1 was part of a number of families of testis-specific genes (such as the GAGE, PAGE and XAGE families), in addition to a number of unique testis-specific genes, all of which exhibit CpG island methylation and transcriptional repression in all somatic tissues [11]. Many of these have been found to be re-expressed in tumour cells, depending on demethylation of their CpG islands, leading to the conclusion that CpG island methylation was the predominant mechanism for transcriptional repression [11].

Recently, however, it has been demonstrated that, for at least a small number of genes, cell-type-specific CpG island methylation is critical in regulating their cell-type-specific expression patterns. The first clear example of such a gene, maspin, has been described by Futscher et al. [13]. The maspin gene was known to be frequently hypermethylated in breast cancers and was supposed to be important in the control of metastasis [14]. However, studies in normal cells found that, although the maspin gene was unmethylated and expressed in cells of epithelial origin, it was methylated and its expression was repressed in mesenchymal and haematopoietic cells. Furthermore, inhibition of DNA methylation resulted in re-expression of maspin, demonstrating that methylation was required for transcriptional repression [13].

Subsequently, a second example of cell-type-specific CpG island methylation and expression, MCJ, was also described [15]. MCJ was originally identified as a gene frequently hypermethylated in ovarian cancer and implicated in chemotherapeutic drug resistance [16]. Unlike maspin, the MCJ gene exhibited CpG island methylation and transcriptional repression in epithelial cells, but was expressed and unmethylated in mesenchymal and haematopoietic cells [15].

At least two other genes exhibit similar cell-type-specific methylation. 14-3-3σ exhibits a very similar pattern of CpG island methylation and expression when compared with maspin, and methylation has been identified in both fibroblast and haematopoietic cells [17], although the two genes do differ in their methylation and expression in ovarian surface epithelial cells (G. Strathdee, A. Sim and R. Brown, unpublished work). Similarly, the HoxA5 gene, a member of the Hox gene family that plays key roles in development and differentiation, exhibits CpG island methylation, specifically in haematopoietic and mesenchymal cells (G. Strathdee, A. Sim and R. Brown, unpublished work).

The mechanisms controlling cell-type-specific DNA methylation remain to be identified. Recent work has identified novel patterns of histone modifications after the methylation of two of these genes (MCJ and HoxA5), which are unlike those observed in imprinted genes or genes hypermethylated in cancer (G. Strathdee, A. Sim and R. Brown, unpublished work), suggesting that specific pathways may exist for the interpretation of DNA methylation signals on these genes.

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**DNA methylation and cell-type-specific expression**

Although the above examples demonstrate that some CpG islands can be methylated in normal cells, these patterns of methylation are essentially identical in all somatic cell types, and CpG island methylation was not generally believed to be involved in tissue- or cell-type-specific gene expression.

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**References**


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