Transcription-dependent dynamic supercoiling is a short-range genomic force

Fedor Kouzine, Ashutosh Gupta, Laura Baranello, Damian Wojtowicz, Khadija Ben-Aissa, Juhong Liu, Teresa M Przytycka & David Levens

Transcription has the capacity to mechanically modify DNA topology, DNA structure and nucleosome arrangement. Resulting from ongoing transcription, these modifications in turn may provide instant feedback to the transcription machinery. To substantiate the connection between transcription and DNA dynamics, we charted an ENCODE map of transcription-dependent dynamic supercoiling in human Burkitt’s lymphoma cells by using psoralen photobinding to probe DNA topology in vivo. Dynamic supercoils spread ~1.5 kilobases upstream of the start sites of active genes. Low- and high-output promoters handled this torsional stress differently, as shown by using inhibitors of transcription and topoisomerases and by chromatin immunoprecipitation of RNA polymerase and topoisomerases I and II. Whereas lower outputs are managed adequately by topoisomerase I, high-output promoters additionally require topoisomerase II. The genome-wide coupling between transcription and DNA topology emphasizes the importance of dynamic supercoiling for gene regulation.
1. Quiz (~10-15 min?)

2. DNA methylation in eukaryotes

BACKGROUND:
Why do we care?
What is it?
What might it do? (a truly epigenetic mechanism?)
Distribution in eukaryotes and within genomes
What is it bad for (mutations)?
What is it good for?
How is it controlled?
(examples from model systems)
DNA methylation has been linked to cancer

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DNA methylation is required for the control of stem cell differentiation in the small intestine

Karyn L. Shreffler,1,2 Binhun Kim,1 Reina Aoki,1 Ellen N. Elliott,1 Jonathan Schug,1 Lukas Burger,2,3 Dirk Schübeler,2,3 and Klaus H. Kaestner1,2

1Department of Genetics, Institute for Diabetes, Obesity, and Metabolism, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA. 2Frederick M. Frees Laboratory for Biomedical Research, 4000 Basel, Switzerland. 3University of Basel, University of Basel, Switzerland. www.emond.org

The mammalian intestinal epithelium has a unique organization in which crypts harboring stem cells produce progenitors and finally clonal populations of differentiated cells. Remarkably, the epithelium is replaced every 3-5 days throughout adult life. Disrupted maintenance of the intrinsic balance of proliferation and differentiation leads to loss of epithelial integrity or barrier function or to cancer. There is a tight correlation between the epigenetic status of genes and expression changes during differentiation; however, the mechanism of how changes in DNA methylation direct gene expression and the progression from stem cells to their differentiated descendants is unclear. Using conditional gene ablation of the maintenance methyltransferase Dunmt1, we demonstrate that reducing DNA methylation causes intestinal crypt expansion in vivo. Determination of the base-resolution DNA methylation in intestinal stem cells and their differentiated descendants shows that DNA methylation is dynamic at enhancers, which are often associated with genes important for both stem cell maintenance and differentiation. We establish that the loss of DNA methylation at intestinal stem cell gene enhancers causes inappropriate gene expression and delayed differentiation.

Keywords: DNA methylation, intestinal stem cell, Dunmt1

Supplemental material is available for this article.

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Recognition of m’d DNA? Methyl-DNA-binding proteins specifically bind to methylated cytosines

Figure 1. Cytosine Methylation in DNA

(a) Addition of a methyl group (red) at the 5 position of the cytosine pyrimidine ring (black arrow) does not sterically interfere with GC base-pairing (blue lines). DNA methyltransferases associate covalently with the carbon-6 position (green arrow) during methyl group transfer. (b) A model of B-form DNA methylated at cytosines in two self-complementary Cpg sequences. The paired methyl moieties (magenta and yellow) lie in the major groove of the double helix.

Figure 3. Proteins That Bind Methyl-CpG
But methylation may also directly affect properties of DNA; e.g. it favors the Z-form of DNA

(Perhaps because in Z-form, methyl groups fill small hydrophobic depressions, whereas in B-DNA the methyl groups project into the major groove, which is largely surrounded by water)

**Distribution of DNA methylation in eukaryotes**

Absent from some simple eukaryotes (yeasts, *C. elegans*, Drosophila?)

Heavy methylation in plants (up to 30% C's; not limited to CpGs)

Nearly all 5mC in animals is at CpG dinucleotides

In animals, typically, ≈70% of CpGs in genome are methylated

**Overall, not much tissue-specificity:** measurements from mouse:

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Methylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>liver</td>
<td>78%</td>
</tr>
<tr>
<td>brain</td>
<td>75%</td>
</tr>
<tr>
<td>spleen</td>
<td>82%</td>
</tr>
<tr>
<td>sperm</td>
<td>67%</td>
</tr>
<tr>
<td>embryo</td>
<td>77%</td>
</tr>
<tr>
<td>cell line (L)</td>
<td>65%</td>
</tr>
<tr>
<td>3T3 cells</td>
<td>70%</td>
</tr>
</tbody>
</table>

But only ~3% C's methylated

**Why?**
CpG deficiency in a tissue specific human gene (γ-G globin)

Some other genes showing CpG deficiency

- β-Globin gene family: Human, mouse, rabbit, goat
- Placental lactogen: Human
- Nerve growth factor: Human
- α-amylase I and II: Mouse
- Growth hormone: Mouse
- Insulin: Mouse
- Leucocyte interferon (alpha-2): Mouse
- Fibrinogen: Rat
- α-Crystallin: Mouse
- Myoglobin: Seal

Relationship between CpG deficiency and excess of TpGs and CpAs

Fig. 2. Absence of CpG-rich islands in a human gene (like γ-G globin genes) that is tissue-specifically expressed. Sequence data is from Stress. Comparing CpG and GpC maps (upper and lower, respectively) show that CpG is deficient throughout this part of the gene. Other regions of the β-globin locus do not show evidence of CpG clustering. Not all tissue specific genes lack CpG clusters. The human α-globin genes, for example, each have pronounced clusters of CpG (see Table 1).

CpG-rich islands and the function of DNA methylation

Adrian P. Bird

Not effect: demethylation!
Many mammalian genes sport “CpG-islands”

- mouse APRT gene
- mouse L-32 gene

CpG density in human promoters

Implications of islands?
CpG islands are generally unmethylated;
Promoters with low [CpG] typically methylated

Mutation of 5mC: A cost of DNA methylation?

Recall that many genes are methylated (globin, Ig, insulin, actin, crystallin, albumin, etc.) and deficient in CpG.

Could remaining “important” mCs be protected somehow?

Apparently not: ~35% of point mutations causing human genetic disorders occur at CpGs and >90% of these are C to T (or G to A)

hMSH1 and hMSH2 (responsible for 90% of hereditary colon cancer) responsible for repairing GT mismatches. So methylation would be responsible for even more mutations if it weren’t for repair systems.

The lability of mC suggests:
1. methylation must be doing something worth this cost;
2. whatever “good” mC’s do is probably done in a “cooperative” way
What is the function of DNA methylation?

DNA methylation is frequently correlated with gene inactivity

Does methylation cause gene inactivation?
Does gene inactivation cause methylation?
Are both methylation and inactivity manifestations of something else?
Note: These are not mutually exclusive possibilities.

DNA methylation can inhibit gene expression

What would constitute evidence for this?

1. Effect of methylation in transfection experiments
2. Effect of abolishing DNA methylation in vivo

Inhibition of DNA Methylation and Reactivation of Silenced Genes by Zebularine

Journal of the National Cancer Institute, Vol. 95, No. 5, March 5, 2003
**Neurospora crassa**

**Vegetative**

Macroconidia (asexual spores)

Vegetative hyphae

**Sexual**

Inhibition of DMTase causes activation of methylated transgene

S=Sau3A (inhibited by mC)

D=DpnII (not inhibited by mC)

5'-GATC

3'-CTAG

$S^hph$ (methylated DNA)

$S^hyg$
Myotube formation in mouse embryonic fibroblast 10T1/2 cells

Derepression of a tumor suppressor gene (p16) that had become silenced by methylation
What is primary function of DNA methylation?

Note: ~90% of the 5-mC in human DNA is in the retroposons and endogenous retroviruses that represent > 35% of the mammalian genome (Yoder et al. 1997)

Little evidence that DNA methylation widely used in gene regulation

Main function of DNA methylation may be to suppress selfish DNA
(although also involved in inactivating genes in imprinting and X-inactivation)

Transcription of IAP endogenous retroviruses is constrained by cytosine methylation

**Fig. 1** Demethylation and transcriptional activation of IAP retroviruses in homozygous Demt<sup>−/−</sup> mutant mouse embryos. **a**. Demethylation of genomic DNA. DNA from wild-type (lane 1) and Demt<sup>−/−</sup> mutant embryos (lane 2) was digested with the methylase-sensitive restriction endonucleases HpaII and MspI. Mean fragment sizes of mutant embryo DNA were lower than in the control. **b**. Low levels of IAP RNA in mutant embryos. Autoradiography around the disrupted exon in the N allele of Demt<sup>−/−</sup> used in these studies allows the production of small (approximately 5%) of wild-type (approximately 95%) amounts of enzymatically active Direct protein<sup>17</sup>. c. High-level expression of IAP RNA in Demt<sup>−/−</sup> embryos. The IAP shown in (b) was stripped and probed with a 3<sup>2</sup>P-labeled IAP probe originally isolated from an IAP insertion allele of the Axin gene<sup>17</sup>. Densitometry indicated an approximately 50–100-fold increase in steady state IAP transcript levels. At bottom is the same blot probed for 18S RNA to confirm equal loading. d. In situ hybridization of wild-type and Demt<sup>−/−</sup> mutant embryos with an IAP probe. IAP transcripts are detected in all regions of the mutant embryo; the heart wall probe lightly due to its thickness.
DNA methylation may interfere with transcription initiation or elongation

Transcripts only seen if methylation abolished

But run-on assays show initiation occurs in presence of methylation

Rountree & Selker, 1997

Some observations about role of DNA methylation in humans from genomic analyses of methylation and promoter function*

Most (~96%) CpG island promoters unmethylated.

Methylation is sufficient but not necessary to silence CpG-island promoters.

Lack of methylation may result from presence of H3K4 dimethylation.

Promoters with low [CpG] are typically methylated but can still be active.

Repression requires high [5mC] but perhaps low [5mC] inhibits spurious TRXN.

Some methylated CpG island promoters in somatic cells are for germline genes.

Human/chimp comparisons show high CpG loss in promoters methylated in sperm.

* e.g. see
Control of DNA methylation: The Holliday/Riggs model (1975):

1. De novo
2. Maintenance

PREDICTIONS:

1. Stable methylation only at CpG’s (clonal lines should not have partial methylation of sites).
2. CpG’s either totally methylated or unmethylated, rarely hemimethylated.
3. DMTases should prefer hemimethylated substrates (and be specific for symmetrical sites).
4. Unmethylated DNA introduced into cells should stay unmethylated and methylated DNA should stay methylated (but only at CpG’s).

Some evidence supports this!

Dnmt1 is complexed with PCNA & SRA* protein (NP95) that binds hemi-methylated DNA


Figure 3. Dynamics of 5mC/5hmC/5fC/5cC in paternal and maternal genomes during preimplantation development. DNA demethylation of the zygote, gauged by 5mC levels, occurs by a passive mechanism in the female pronucleus, diluting the marks with the passage of every cell cycle. The male pronucleus genome becomes demethylated actively by the action of the Tet enzymes. Tet3 is expressed in the oocyte and zygote. After fertilization, Tet3 is relocated from the cytoplasm to the paternal nucleus to convert 5mC to 5hmC/5fC/5cC. Subsequently, paternal and maternal genomes undergo replication-dependent dilution of 5mC. 5hmC is males and 5mC is females. It is possible that replication-independent active DNA demethylation may occur in a loci-specific manner in zygotes, but the exact mechanism is currently unclear. DNA methylation patterns in ECM are reestablished by de novo DNA methylation enzymes Dnmt3a and Dnmt3b at the blastocyst stage. ECM, inner cell mass; TE, trophectoderm.
Figure 6. Model of Tet-initiated DNA demethylation pathways. DNA methylation (5mC) is established and maintained by DNMT. 5mC can be oxidized by Tet family of dioxygenases to generate 5hmC, 5fC, and 5caC. Because the oxidized 5mC derivatives cannot serve as substrates for DNMT1, they can be lost by replication-dependent passive demethylation. 5hmC can be demethylated by AID/APOBEC to become 5hmU, which together with 5fC and 5caC can be excised by glycosylases such as TDG, followed by DNA repair to generate C. Alternatively, a putative decarboxylase may convert 5caC to C.