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A new synthesis in epigenetics: towards a unified function of DNA methylation from invertebrates to vertebrates

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Abstract. DNA methylation is generally limited to CpG doublets located at the gene promoter with an involvement in gene silencing. Surprisingly, two recent papers showed an extensive methylation affecting coding portions of transcriptionally active genes in human and plants prompting a rethink of DNA methylation in eukaryotes. Actually, gene body methylation is not surprising since it has been repeatedly reported in invertebrates, where it interferes with transcriptional elongation preventing aberrant transcription initiations. As a whole, the published data suggest that the most ancestral function of DNA methylation is the control of genes that are susceptible to transcriptional interference and not to gene silencing. The recruitment of DNA methylation for silencing represents a successive tinkered use. In view of this additional function, the invertebrate-vertebrate transition has been accompanied by new constraints on DNA methylation that resulted in the strong conservation of the DNA methylation machinery in vertebrates and in the non-viability of mutants lacking DNA methylation.

Keywords. DNA methylation, gene silencing, transcription control, transcription initiation, eukaryote evolution.

DNA methylation is a common epigenetic modification of chromatin in eukaryotes. In particular, it is based on the addition of a methyl group to the 5′ termini of cytosine residues by specific DNA methyltransferases [1–3].

Despite its common presence, several different functions have been reported for DNA methylation and in particular numerous discrepancies have been assessed by comparing the functions that DNA methylation plays in vertebrates compared to invertebrates [3, 4]. In vertebrates, DNA methylation is limited to CpG doublets present within the CpG islands at the gene promoter and it is involved in gene silencing, transposon control, development regulation, parental imprinting and X chromosome inactivation [5].

As a general rule, in vertebrates DNA methylation is involved in the chromatin remodelling that is related to gene silencing through the recruitment of several proteins that contribute to increase chromatin condensation [6].

In contrast, experimental results have revealed that in invertebrates, DNA methylation may not be limited to the canonical CpG targets, but is also present at other doublets, as reported in *Drosophila melanogaster* [3, 4]. Moreover, methylated genes can be actively transcribed in several invertebrates, including different insects [3, 7], the sea urchin *Strongylocentrus purpuratus*, the sea squirt *Ciona intestinalis* and the marine annelid *Chaetopterus variopedatus* [8].

A possible explanation for these differences has been related to the target of DNA methylation, since in all the invertebrate genes methylated cytosine residues were located inside the coding portion of the genes, whereas in vertebrates, methyl-cytosine residues were present at promoters [4].

Within this context, Simmen et al. [8] suggested that methylation at cytosine residues located within the coding sequences could be essential to focus initiation of transcription on genuine promoters preventing risks of transcriptional interference. In view of this assumption, invertebrates seem to methylate genes that have to be expressed in place of silent ones.

Nevertheless, this peculiar pattern of methylation is not due to the absence of CpG islands, since the analysis of invertebrate genes revealed that the 5′ ends contains non-methylated CpG-rich sequences that resemble mammalian CpG islands [8]: in invertebrates, islands of non-methylated CpG occur at the promoter region of the genes and are on average 1 000 bp long [8]. Thus, CpG islands are smaller than in vertebrates, not unsurprising finding given that invertebrate and vertebrate genomes have a different GC content. The human genome, for example, covers a 30 % GC range at an average size of 50 kb [9], whereas, at the same average size, the *Drosophila* genome only covers a 10 % GC range [10].

A further difference between vertebrates and invertebrates is related to the effects of the lack of DNA methylation. Indeed, the silencing of DNA methyltransferases has a deep impact on the vertebrate genome, and it has been reported that DNA methylation is essential for proper embryonic development [11]. In contrast, silencing of *Drosophila* DNA methyltransferase 2 did not have detectable effects on embryonic development and viability [12]. Considering that the *Drosophila* genome encodes only the DNA methyltransferase 2, the conclusion must be that DNA methylation is not necessary for viable development in *Drosophila* [3]. Moreover, it has been reported that over-expression of *Drosophila* DNA methyltransferase 2 results in an extended fly life span and in the over-expression of several genes [13], suggesting that DNA methylation could enhance the expression of fly genes instead of acting as an on/off switch for gene transcription [4, 7, 13].

To explain such differences, it has been suggested that a shift in the function of DNA methylation has occurred, methylation becoming related to gene silencing only in vertebrate genomes [4]. This functional shift brought improved gene expression control and a strong functional constraint on genes coding for DNA methyltransferase, making the absence of DNA methylation not compatible with the proper functioning of vertebrate genomes [14].

However, two recently published papers have clearly suggested a revision in our understanding of the targets of DNA methylation in vertebrates and plants, indicating that two differential types of methylation could be present.

In particular, Hellman and Chess [15] assessed that (i) human active X chromosomes possess more than twice as many methylated genes as inactive X chromosomes and (ii) methylation is present at gene bodies. Therefore, in contrast to the widely held view that DNA methylation is restricted to the CpG islands on the inactive X chromosomes, Hellman and Chess [15] showed an extensive methylation affecting transcriptionally active genes, demonstrating that several active genes possess un-methylated promoters and hyper-methylated gene bodies.

At the same time, Zilberman et al. [16] showed that the genome of the plant *Arabidopsis thaliana* also possesses actively transcribed, but methylated genes [16]. Methylated genes are involved in heterogeneous molecular functions, suggesting that the methylation of gene bodies is not related to the specific gene function. At the same time, they provided evidence that DNA methylation is related to gene expression and, in particular, even if the effects of DNA methylation within the gene body have not been thoroughly studied, it can interfere with transcriptional elongation, preventing aberrant initiations of transcription from within the gene body [16].

From an evolutionary perspective, the papers of Hellman and Chess [15] and Zilberman et al. [16] are intriguing, since they are consistent with the data published for invertebrates suggesting that the most widespread and, probably, most ancestral function of DNA methylation is related to the control of genes that are susceptible to transcriptional interference.

Therefore, the function of DNA methylation as a tool for silencing gene transcription could be a successive and tinkered use of DNA methylation that occurred in plants and vertebrates.

This hypothesis is further strengthened by data reporting the absence of DNA methylation at satellite DNAs and transposons in invertebrate genomes, once again suggesting that this silencing function has been recruited successively [3–7].

In view of these additional functions played by DNA methylation in vertebrates, the transition from invertebrates to vertebrates has been accompanied by new functional constraints on DNA methylation that resulted in the strong conservation of the DNA methylation machinery in vertebrate and plant genomes and in the non-viability of mutants lacking DNA methylation.

In invertebrates, however, where all these silencing functions are absent, the silencing of the DNA

methyltransferase appears not to have detectable effects on embryonic viability, suggesting that DNA methylation is not necessary for viable development, but only for enhancing the expression of genes that, even if already active, could be incorrectly initiated from spurious promoters [7, 14]. As a consequence, in invertebrates, as clearly shown for the dipteran insects, *D. melanogaster* and *Anophles gambiae* and in the nematode *Caenorhabditis elegans*, genes coding for DNA methyltransferases have been partially (in insects) or wholly (in nematodes) lost during evolution.

As a whole, the new set of published data indicate clearly that DNA methylation plays at present both ancestral and derived functions, its role as a tool for preventing aberrant transcription being the ancestral one. During the evolution of metazoa, DNA methylation gained new functions that allowed the transition towards more complex genomes, but that impose strong constraints on the DNA methylation machinery.

In a recent research highlight, Louisa Flintoft [17] stated that the results about the presence of methylation at gene bodies of active gene 'comes as something of a surprise, prompting a rethink of how we view DNA methylation patterns'. This is only partially true, considering that gene body DNA methylation has been known for a long time, even if rarely studied, and it is not really surprising, given that this aspect of DNA methylation was already been frequently reported in various metazoans. Interestingly, therefore, the present evolving understanding of DNA methylation confirms that, as stated by Theodosius Dobzhansky [18], nothing in biology makes sense except in the light of evolution.

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