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Visions & Reflections

Epigenetic tinkering and evolution: is there any continuity in the role of cytosine methylation from invertebrates to vertebrates?

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Abstract. The function of DNA methylation has been investigated in depth in vertebrate and plant genomes, establishing that it is involved in gene silencing and transposon control. Data regarding insect methylation, even if still scanty, apparently argue against evolutionary conservation of DNA methylation functions. Cytosine methylation, therefore, proves to be an epigenetic tool repeatedly used to accomplish different functions in different taxa according to a sort of epigenetic tinkering occurring during evolution.

Key words. Epigenetics; DNA methylation; DNA methyltransferase; gene expression; cytosine methylation functions; invertebrate-vertebrate transition.

It is well established that a variable portion of cytosine residues is methylated in eukaryotic genomes. The percentage of methylated cytosines ranges from 0 to 10% in insects, 5% in mammals and birds, 10% in fish and amphibians to over 30% in plants [1].

DNA methylation has been related to numerous functions depending on the model organism and on the experimental context. In general, the presence of DNA methylation, in and around gene promoters, is associated with gene silencing [2]. On a cellular level, loss of DNA methylation can affect apoptosis in mice [3] and *Xenopus* [4], X-chromosome inactivation and chromosomal stability in mice [5], and overall chromosome organization in *Arabidopsis* [6].

DNA methylation is performed by DNA methyltransferases grouped into several distinct families [7]. Dnmt1 enzymes, which preferentially bind to hemimethylated DNA, are responsible for the maintenance of DNA methylation after each round of replication [8–9]. Dnmt2 proteins are similar to the prokaryotic methyltransferases, but their function is still somewhat enigmatic since they seem unable to methylate DNA in vitro. Moreover, loss of function mutations of the Dnmt2 gene did not produce any effect on mouse genomic methylation patterns [10]. The final methyltransferase family consists of Dnmt3a and Dnmt3b, which are the main protagonists involved in de novo methylation [11]. The third member of this family is Dnmt3L, which shares a few affinities with Dnmt3a and Dnmt3b and plays a central role in the establishment of maternal genomic imprinting, even though it is does not have any catalytic activity [12].

The analysis of methylation functions in vertebrates indicates that the primary role of methylation should be gene silencing or the permanent repression of silent promoters [2]. Our understanding of the role of DNA methylation in regulating gene expression has rapidly advanced in recent years with the identification of an increasing number of novel proteins involved in this process [2]. These data, as a whole, indicate that at least in vertebrates, actively transcribed genes are non-methylated, whereas silenced genes are methylated.

In addition, it has been suggested that cytosine methylation represents a ubiquitary defence against transposition

[13–15]. Methylation of cytosine residues can, in fact, silence the expression of transposon-encoded genes, prevent transposon-mediated DNA rearrangements and, finally, silence the read-through transcription from transposon promoters into neighbouring host genes [13–15]. Given the same role of methylation in plants and vertebrates, several authors have hypothesized the presence of evolutionarily conserved functions of methylation in eukaryotes [1, 13–15].

A uniform functional role of DNA methylation has been deduced mainly from the increasing amount of data from vertebrates and plants. In contrast, data reported in diverse insect species do not seem to be consistent with a conserved function of methylation. To date, the presence of 5-methylcytosine has been reported in several insect species belonging to various orders [16]. However, its role has not been clearly defined, and the available data demonstrate varying levels of methylation and different functions. In *Drosophila melanogaster*, for instance, methylation is present in fly embryos (0.4% of the cytosine residues) but not in adults, and it has been suggested that cytosine methylation plays an important role during the early stages of fly development [17]. Experimental results reveal that methylated genes are actively transcribed in the homopteran *Myzus persicae* [18] and *Planococcus citri* [19]. Finally, data reported in the lepidopteran *Mamestra brassicae* clearly indicate that transposons are not methylated, thus suggesting that DNA methylation is not involved in transposon control in insects [20]. The above-reported data apparently argue against a unifying and evolutionarily conserved role of cytosine methylation from invertebrates to vertebrates. In fact, it appears that the DNA methylation/gene silencing correlation is not present in insects and that the function of methylation as a genome defence against transposons appears not to hold true for insects. The presence of methylation in insects could be essential in focussing initiation of transcription on genuine promoters in order to guarantee the expression of specific (and important) genes susceptible to transcriptional interference. In view of this assumption, insects methylate genes that have to be expressed in place of silent genes.

Furthermore, a discontinuity in the functional role of methylation from invertebrates to vertebrates is sustained by the fact that the Dnmt2 proteins represent the only candidate DNA methyltransferases in *D. melanogaster*, *Drosophila pseudoobscura* and *Anopholes gambiae,* as deduced by the absence of other methyltransferase genes in their genome [21–22]. At present, the significance of this difference remains unclear, but important information could be obtained by analysis of insect DNA methyltransferase mutants.

Finally, an additional difference pertains to the fact that insect methylation is not limited to the CpG target. CpA-, CpT- and CpG-methylated doublets were, in fact, also reported in insects [17, 20, 22, 23], with the peculiarity that at least in *D. melanogaster*, methylation is confined to the non-symmetrical CpA and CpT dinucleotides [17].

Bird [24] suggested that during evolution the transition from invertebrates to vertebrates was accompanied by an increase in gene number, made possible by the use of DNA methylation to repress transcriptional background noise. This is supported by the finding that the transition from fractional to global methylation of genomes occurred close to the origin of vertebrates [25]. This statement is undoubtedly intriguing, but one should consider that the transition from invertebrates to vertebrates was accompanied not only by an increase in gene number but also by a more specialized use of DNA methylation involving the definition of precise methylation targets (that become limited to CpG targets) and the evolution of three different DNA methyltransferases with specific functions.

Therefore, invertebrates and vertebrates use DNA methylation as a common epigenetic mark that acts as a preferential substrate for specific binding proteins that recruit other proteins. The main difference seems to consist in the proteins recruitable by methylated cytosine residues. If these recruited proteins are transcriptional activators instead of repressors, the role of methylation could be the activation of specific genes (as reported in aphids) or the transcription of imprinted alleles (as reported in coccids) and not the repression of specific transposons or the silencing of methylated genes.

DNA methylation is, therefore, an extremely versatile epigenetic tool to distinguish genome compartments that need to be differently controlled. In view of its characteristics, this modification would thus have been repeatedly used during the course of evolution to accomplish different functions in different taxa according to a sort of epigenetic tinkering occurring during evolution.

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