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Visions and Perspectives

Histone methylation and DNA methylation: a missed *pas de deux* in invertebrates?

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Abstract

Several Authors have reported that histone methylation interacts with DNA methylation creating a self-propagating epigenetic cycle for long-term transcriptional repression of methylated genome compartments. This phenomenon, observed in plant and vertebrate genomes, does not appear to hold true in invertebrates. In particular, both structural and functional evidences suggest that, in invertebrates, DNA methylation and histone methylation do not interact, thus inhibiting the intimate *pas de deux* observed in other eukaryotes.

Key words: epigenetics; histone methylation; DNA methylation; invertebrate genome

Studies on organisms ranging from yeast to vertebrates have indicated that histones and their post-translational modifications play an evolutionary conserved pivotal role in the assembly of chromatin. In particular, the presence of different covalent modifications of histones has led to the definition of the "histone code," i.e. a code that is deciphered by chromosomal proteins in order to regulate gene expression using specific modifications of chromatin architecture. Among the plethora of modifications, histone methylation, to a greater extent than any other process (such as acetylation, phosphorylation and ubiquitination), has shown the capacity to regulate fundamental processes like gene transcription and DNA repair (Bannister and Kouzarides, 2005).

Histone methylation on either lysine or arginine residues induces alterations in chromatin architecture However, this result is not due to the methyl group itself (which does not neutralize lysine or arginine charge), but to the recruitment of silencing/regulatory proteins that bind methylated histones (Bannister and Kouzarides, 2005). Among these proteins, HP1 is an evolutionary conserved partner of histone methylase acting in synergy with histone methylation to silence chromatin. Finally, histone methylation interacts in plants and vertebrates with DNA methylation creating a self-propagating epigenetic cycle for long-term transcriptional repression of methylated genome compartments (Fuks *et al*., 2003).

Given the presence of the same histone and DNA modifications at silenced domains in different eukaryotes, several Authors have hypothesized the existence of an evolutionary conserved interaction between histone methylation and DNA methylation (Fuks *et al*., 2003, Bannister and Kouzarides, 2005). This hypothesis is strengthened by data reporting that DNA methylation is associated, in plants and vertebrates, with gene silencing or to the permanent lock of already silenced genes (Fig 1).

Viewed as a whole, the most recent findings seem to suggest that at the beginning DNA methyltransferases would add a methyl group to DNA, only on chromatin that is methylated at lysine 9 of the H3 histone, and bind HP1 (Fuks *et al*., 2003). The

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List of abbreviations:
HP1: heterochromatin protein 1; MeCP2: methyl CpG binding protein; Dnmt: DNA methyltransferase; PHD: plant homeo-domain; HDAC: histone deactylase

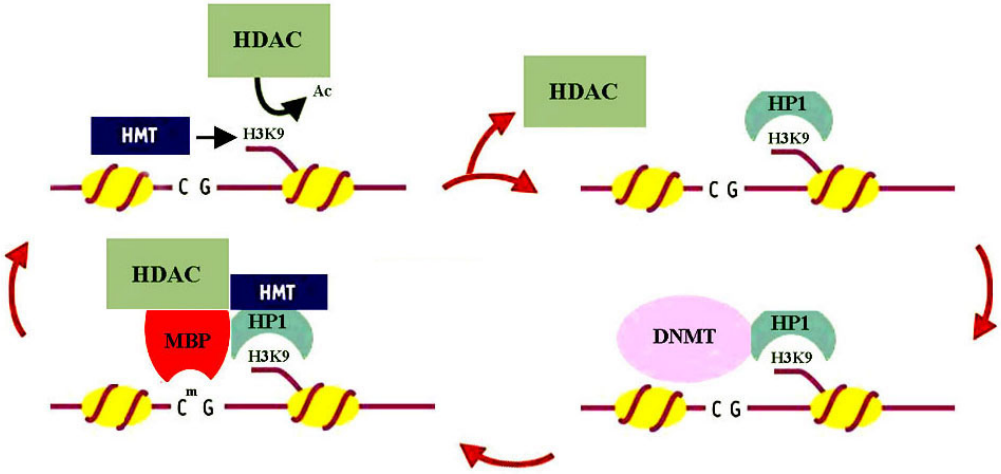

Fig 1. Recent findings in *Neurospora*, *Arabidopsis thaliana* and mammals suggested that DNA methylation and histone methylation act together in chromatin silencing. In the initial phase, the Dnmts add methyl groups to DNA only on chromatin that is methylated at lysine 9 and bind HP1. The direct physical link identified here between the Dnmts and the HMT–HP1 system would make sure that the methylation status at histone H3 directly influences DNA methylation patterns. In the second step, the generation of methylated DNA by the Dnmts would allow DNA binding of MBP, which in turn associates and favours histone methylation at lysine 9 of H3 histones. This sequential process of coupling DNA with histone methylation is attractive because it would suggest that DNA methylation may also feed back to facilitate histone methylation, thereby reinforcing the two modes of epigenetic silencing and creating a self-propagating epigenetic cycle for long-term transcriptional repression. Dnmt: DNA methyltransferase; HP1: heterochromatin protein 1; HMT: histone methyltransferase; H3K9: methylation of histone H3 at lysine 9; MBP: methyl-binding proteins.

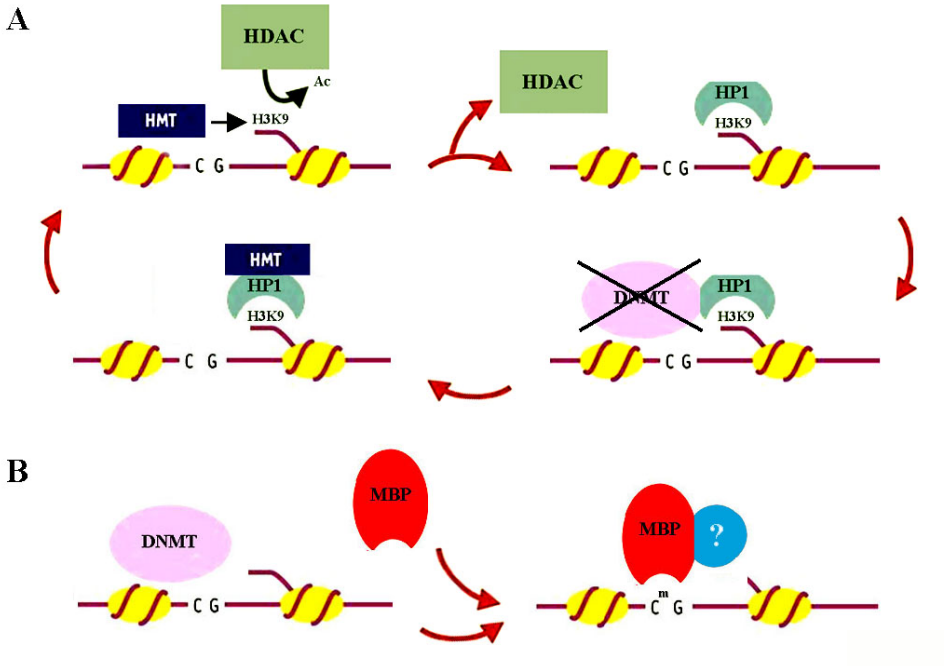

Fig 2. Insect Dnmt2 lacks the N-terminal domain, present in Dnmt1 and Dnmt3, that is responsible for DNA methyltransferase interactions with numerous other proteins (A). The absence of the N-terminal domain in Dnmt2 could prevent the coupling of Dnmt2-mediated DNA methylation with histone methylation. These two epigenetic tools could be targeted to different genome compartments where they could play entirely different roles. This hypothesis is supported by data reporting that DNA methylation was involved in gene expression instead of gene silencing at euchromatic compartments of genome (B). These data suggest, therefore, the absence of a cross talk between DNA and histone methylation in insects. Dnmt: DNA methyltransferase.

direct physical link between histone methylase and HP1 would ensure that the histone methylation status directly influences the DNA methylation pattern. In the second step, the generation of methylated CpG dinucleotides enables the binding of methyl-binding proteins (such as MeCP2), which in turn favour histone methylation so that DNA methylation may feed back to facilitate histone methylation leading to a silent state of chromatin (Fuks *et al*., 2003, Bannister and Kouzarides, 2005).

However, several data reported in invertebrates (Field *et al*., 2004; Marhold *et al*., 2004; Borsatti *et al*., 2004; Borsatti and Mandrioli, 2004) argue against an evolutionary conserved function of DNA methylation and against the interaction between histone methylation and DNA methylation. In particular, the absence of cooperation between histone methylation and DNA methylation in invertebrates can be suggested in the light of data reporting the presence of a unique DNA methyltransferase lacking the typical domain involved in such an interaction and, most of all, in view of the different functions played by DNA methylation in invertebrates in comparison with plants and vertebrates.

The insects *Drosophila melanogaster, Bombyx mori*, *Apis mellifera* and *Anopheles gambiae* possess a unique gene coding for DNA methyltransferases (and in particular for Dnmt2-like enzymes) that is responsible for DNA methylation (Marhold *et al*., 2004; Borsatti and Mandrioli, 2004). As opposed to the typical eukaryotic DNA methyltransferases, Dnmt2 lacks the N-terminal domain that is responsible for DNA methyltransferase interactions with numerous other proteins inhibiting the coupling of Dnmt2-mediated DNA methylation with histone methylation in insects. In particular, the typical eukaryotic DNA methyltransferase presents a C-terminal region, referred to as the catalytic domain, and a N-terminal region acting as a regulatory domain (Margot *et al.,* 2000). The N-terminal domain presents different structural motifs (such as the PHD domain) that are responsible for DNA methyltransferase interactions with numerous other proteins. In this connection, several Authors have assessed that Dnmt1 and Dnmt3 interact with HP1 and HDAC through their N-terminal domains (Fuks *et al*., 2003).

The hypothesis of a missed interaction of DNA methylation with other epigenetic tools is supported by the role that DNA methylation plays in insects, where experimental results have revealed that methylated genes are actively transcribed indicating the absence of the DNA methylation/gene silencing correlation (Field *et al*., 2004; Marhold *et al*., 2004; Borsatti *et al*., 2004; Borsatti and Mandrioli, 2004). In particular, in the insects *Mamestra brassicae*, *Myzus persicae* and *Planococcus citri* it has been described that several genes are actively transcribed even if methylated and that DNA methylation is essential to ensure gene expression (Field *et al*., 2004; Marhold *et al*., 2004; Borsatti *et al*., 2004; Borsatti and Mandrioli, 2004).

Interestingly, insects are not the unique biological models where methylation is not involved in gene silencing, since similar results have been obtained in other invertebrates, such as the sea urchin *Strongylocentrus purpuratus*, the sea squirt *Ciona intestinalis* and the amphioxus *Branchiostoma lanceolatum* (Tweedie *et al*., 1997; Simmen *et al*., 1999).

The present analysis indicates, therefore, that even if histone methylation and DNA methylation mechanisms are both present in vertebrates and invertebrates, only histone methylation plays an evolutionary conserved role in gene silencing, whereas DNA methylation carries out different functions in different taxa. All the evidences produced here (both at structural and functional level) suggest that, in invertebrates, DNA methylation and histone methylation are targeted to different genomic compartments, where they have different and opposite effects (Fig 2). In invertebrates we therefore observe a loss of interaction between these two epigenetic tools thus inhibiting the realization of the intimate *pas de deux* observed in other eukaryotes.

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