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Cell. Mol. Life Sci. 63 (2006) 1933–1936
1420-682X/06/171933-4
DOI 10.1007/s00018-006-6039-1
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Cellular and Molecular Life Sciences

Visions & Reflections

DNA methylation of fly genes and transposons

M. Mandrioli* and F. Borsatti

Dipartimento di Biologia Animale, Università di Modena e Reggio Emilia, Via Campi 213/d, 41100 Modena (Italy), Fax: +39 59 2055548, e-mail: mandrioli.mauro@unimore.it

Received 26 January 2006; received after revision 8 May 2006; accepted 2 June 2006
Online First 17 July 2006

Abstract. The use of anti-5-methylcytosine antibodies in affinity columns allowed the identification of methylated sequences in the genome of *Drosophila melanogaster* adults. In view of the presence of transposable elements amongst the identified sequences, it has been suggested that DNA methylation is involved in transposon control in the fly genome. On the contrary, a reanalysis of these data furnishes several intriguing elements that could raise new questions about the role that DNA methylation plays in the fly genome. The aim of the present paper is to discuss some features that emerge from the analysis of the identified methylated sequences.

Keywords. DNA methylation, gene expression, transposon silencing, development, cytosine methylation function, *Drosophila melanogaster*.

For a long time, authors reported the absence of detectable methylated cytosine residues in the *Drosophila melanogaster* genome, suggesting that it does not undergo DNA methylation [1]. This contention was subsequently revised since different papers claimed not only the presence of 5-methylcytosine in *D. melanogaster* [2–4] but also that the fly genome encodes a DNA methyltransferase 2-like enzyme and a methyl-binding protein [5]. Moreover, Lyko and colleagues showed that DNA methylation is present at a higher level in fly embryos (0.4% of the cytosine residues) and absent in adults, therefore proposing a role of cytosine methylation during the early stages of fly development [3–4].
Salzberg and colleagues identified in the adult fly genome 27 sequences presenting methylation at cytosine residues by using an anti-5-methylcytosine antibody in an affinity column [6]. In particular, they pointed out 5 sequences consisting of repetitive DNAs and 4 of transposable elements, and suggested that DNA methylation is involved in the silencing of transposons and repetitive elements [6]. The remaining 18 DNA fragments corresponded to unique sequences. Concerning these genes, the authors suggested that the presence of methylation was due to their GC content [6]. However, a further analysis of these data furnishes several intriguing elements that could open new questions about the real role of DNA methylation in the fly genome.
Salzberg and colleagues [6] identified the following sequences as methylated single-copy genes: CG5383, CG12106, G7724, CG18024, CG13363, M84606, CG11094, X95241, CG2822, CG3706, CG11642, CG14186 and CG5205. The first nine sequences correspond to well- or partially studied genes, so it is possible to verify the existence of a relationship between the reported methylation status and the expression profile of each sequence (Fig. 1). The remaining four sequences correspond to genes identified during the fly genome project, but lack any molecular or functional characterization. It is impossible to analyse them in detail, even though it is possible to perform the analysis of the distribution of methylated cytosine residues within the genes (Fig. 2).
Salzberg and colleagues [6] identified a further five unique sequences possessing DNA methylation. But they are part of uncharacterized genome portions, and it is thus impossible to verify the relation between gene

* Corresponding author.

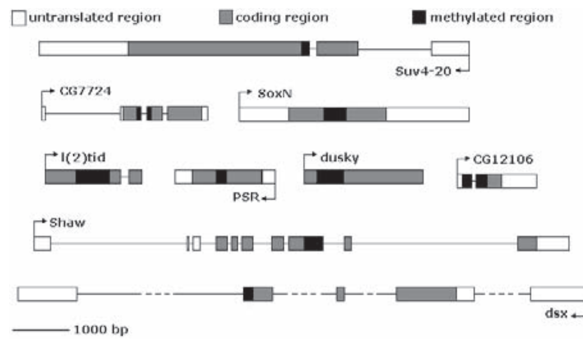

Figure 1. Schematic representation of the structure of the nine well-studied genes reported as methylated by Salberg et al. [6]. For these genes, the relationship between methylation status and expression pattern has been reported.

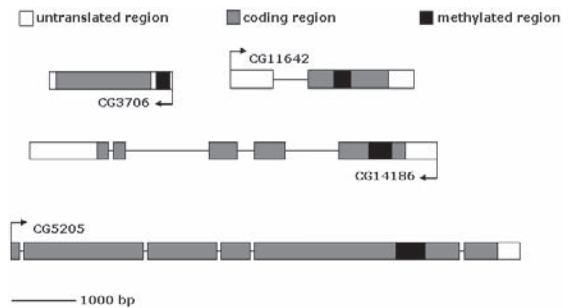

Figure 2. Schematic representation of the structure of the four partially studied genes reported as methylated by Salberg et al. [6]. For these genes, it has been possible to analyse the distribution of DNA methylation only, in view of the absence of data regarding their expression patterns.

structure and the presence of DNA methylation for these fragments.

Even when coding for unrelated proteins, all the genes identified using the anti-5-methylcytosine antibodies turned out to be methylated at cytosine residues located within the coding portion of the genes (Table 1). This result is peculiar, since in plant and vertebrate genomes DNA methylation generally occurs in the promoter region of genes.

However, the location of the methylated cytosine residues in fly genes is consistent with previous data reported in several other invertebrate species (including the sea urchin *Strongylocentrus purpuratus*, the sea squirt *Ciona intestinalis*, the aphid *Myzus persicae*, the moth *Mamestra brassicae*, the marine anellid *Chaetopterus variopedatus* and the amphioxus *Branchiostoma lanceolatum*) [14]. *Drosophila* methylated genes confirm, therefore, the presence of DNA methylation predominantly inside

Table 1. Analysis of the distribution of methylated cytosine residues in the DNA sequences isolated in fly genome by using the anti-5-methylcytosine antibody affinity column.

Gene name/ID	Methylated region	Methylation in the coding portion	Expression profile
Phosphatidylserine receptor *PSR* (CG5383)	850–1231	yes	expression restricted to the first stage of embryo development, silent in larvae, pupae and adults [7]
Oxidoreductase CG7724	299–417	yes	expression restricted to the last stages of embryo development, silent in the larvae, pupae and adults [7]
CG12106	1–467	yes	expression restricted to the last stages of embryo development and to the last phases of larval development, silent in the first stages of embryo development and during pupae and adult stages [7]
SoxNeuro (CG18024)	1493–1899	yes	expression from the early steps of development onwards [8]
Dusky (M84606)	919–1385	yes	expression at two discrete periods during the life cycle: (i) during embryonic development and early larval instars; (ii) during adult development in coincidence with wing differentiation [9]
Potassium channel *Shaker cognate w* (Shaw) (CG2822)	1478–1803	yes	expression is observed mostly in the central nervous system, in the peripheral nervous system and in the visceral mesoderm of the late developmental embryo stages [10]
Lethal tumorous imaginal discs (X95241)	3706–3136	yes	expression in the the imaginal discs and at puparium formation [11]
Doublesex (CG11094)	2801–2972	yes	expression starts in the late stage of fly embryo development and continues in male adults [12]
Histone lysine N-methyltransferase *Suv4-20* (CG13363)	1391–1522	yes	expression is reported in adult flies [13]
CG14186	622–949	yes	unknown
CG5205	5188–5596	yes	unknown
CG11642	751–1004	yes	unknown
CG3706	13–243	yes	unknown

the coding portion of the genes in insects and in other invertebrates. This location of methylated cytosine residues is not due to the absence of CG-rich sequences at promoters, since the analysis of invertebrate genes revealed that the 5′ ends can contain non-methylated CpG-rich sequences that resemble mammalian CpG islands [15–16]. In particular, islands of non-methylated CpG occur at the promoter region of the genes and are on average 1000 bp [15–16]. This result indicates that CpG islands are smaller in invertebrates than in vertebrates. But these data are not surprising considering that invertebrate and vertebrate genomes have different GC content [17–18]. The human genome, for instance, covers a 30% GC range at an average size of 50 kb [18], whereas, at the same average size, the *Drosophila* genome only covers a 10% GC range [17].

The presence of methylated cytosine residues within the coding portion of the genes is very intriguing, since the methylation could be involved in gene expression instead of silencing. The idea that methylation within genes can be positively correlated with transcription has been suggested by Simmen et al. [16], who proposed that methylation within coding regions can prevent the production of incorrectly initiated transcripts by silencing expression from spurious promoters. Indeed, the presence of methylation in insects could be essential to focus initiation of transcription on genuine promoters in order to guarantee the expression of specific genes susceptible to transcriptional interference [14].

The hypothesis about the absence of a positive relationship between the presence of DNA methylation and gene silencing in *Drosophila* is confirmed by the expression pattern of the identified genes. In particular, the analysis of the expression profiles reported in the *Drosophila* Developmental Database [7] clearly indicated that, even when most of the identified genes are silent in adult tissues, *doublesex* and *suv4-20* are expressed in adults despite the reported presence of methylation inside their coding regions.

A further element of interest that emerges from the sequences identified by Salzberg and colleagues [6] is related to the fact that six of the identified genes present a strictly developmentally regulated expression pattern, suggesting that DNA methylation could be involved in regulation of *Drosophila* development [3–4]. In this regard, it has been reported that silencing of the *Drosophila* DNA methyltransferase 2 gene by RNA interference appears not to have detectable effects on the embryonic viability, suggesting that DNA methylation does not seem necessary for viable development in *Drosophila* [4]. However, at the same time, it has been also observed that overexpression of *Drosophila* DNA methyltransferase 2 results in an extended fly life span and in overexpression of several genes [19]. These results, as a whole, suggest that, instead of acting as an on/off switch for gene transcription, DNA methylation could enhance or improve the expression of fly genes that, even if already active, could be incorrectly initiated by spurious promoters.

Interestingly, 4 of the 27 methylated sequences were due to transposons. However, if the primary function of DNA methylation is the silencing of mobile DNAs, sequences belonging to transposable elements should be overrepresented in the methylated portion of the fly genome with respect to single-copy genes. Considering that about 15–20% of the *D. melanogaster* genome consists of transposable and retrotransposable elements, it is surprising that only 14% of identified methylated sequences are due to transposable elements since it seems that DNA methylation is spread in the genome without any preferential targeting on transposons and retrotransposons.

A final element of interest is related to the presence of the R1Dm retrotransposon among the methylated mobile DNAs. This result is interesting since R1 elements are passively transcribed in view of their presence inside the genes coding for the 28S ribosomal RNA (rRNA) that are actively transcribed despite the presence of the retrotransposon [20].

These data, as a whole, seem to argue against a role of DNA methylation in transposon control in the fly genome. In particular, in *Drosophila* (and more generally in insects) the main control of transposons could be at a posttranscriptional level and could involve mechanisms of RNA interference that are responsible for the degradation of transposon RNAs. This hypothesis is intriguing, considering that the RNA interference machinery has been already described in *Drosophila* genome, in particular its involvement in fly heterochromatin silencing [21–22].

Acknowledgements. This work has been subsidized by an 'F. A. R.' grant from the University of Modena and Reggio Emilia (M. M.). We are greatly indebted to the two anonymous referees, whose suggestions allowed us to improve the discussion of the data reported in the paper.

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