



### Detection of cytosine methyltransferase in *Drosophila melanogaster*.

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#### Summary

Methylation of DNA is a unique way of encoding information as it is a post replicative modification of DNA that is '*in cis*' which can contribute to DNA conformation and DNA protein interaction. It also confers memory and heritability to the methylation status of a particular site in the DNA (Adams *et al.*, 1990). In mammals, it has been implicated in several important processes like transcriptional repression (Razin and Riggs, 1980), parental imprinting (Feil and Khosla, 1999), and X chromosome inactivation (Colot and Rossignol, 1999)

In the case of the model organism *Drosophila melanogaster*, there have been scattered reports regarding the presence of methylation. Presence of 0.008 mol percent 5-methyl cytosine in the genomic DNA of *Drosophila melanogaster* was reported by Deobagkar (nee' Achwal) (Achwal *et al.*, 1982, 1984; Ashburner, 1989). However, earlier it was reported that 5-methyl cytosine was absent in the DNA of pupae and adults of *Drosophila melanogaster* (Urieli-Shoval *et al.*, 1982). This is an apparent contradiction to the recent reports that provided evidence for low levels of DNA methylation both in embryos and adults of *Drosophila melanogaster* (Lyko *et al.*, 2000; Gowher *et al.*, 2000). The evidence for the presence of methylation in the sequence GGCC that changes in a stage specific manner has been provided by Chatterjee *et al.* (2004). The presence of 5-methylcytosine has been reported in several insect species belonging to various orders (Field *et al.*, 2004). Deobagkar *et al.* (1997) have demonstrated the presence of methylation mediated suppression in *Drosophila*.

DNA methylation in *D. melanogaster* is characterized by several features that made it highly elusive over a long period of time (Lyko *et al.*, 2001):

- The overall methylation level is rather low with less than 1% of the cytosine residues methylated.
- The highest levels of DNA methylation are found in early embryos, which yield only limited amounts of DNA for biochemical or molecular characterization.
- Most of the 5-methylcytosine is found in the context of non-CpG dinucleotides, which rendered the traditional CpG specific assays ineffective.
- Together these characteristics established a unique profile for DNA methylation in this model organism (Field *et al.*, 2004).

DNA methylation is of two kinds: a) de novo methylation: when non-methylated double stranded DNA is methylated, and b) maintenance methylation: where the methylation of any stretch of DNA is maintained constant or after replication the daughter strand is methylated in accordance with the parent strand. The transfer of methyl groups to the 5'C atom of the cytosine residue is catalyzed by the enzymes called methyltransferases (DNMT's) using S-adenosyl Methionine as the donor for the methyl group. The enzymatic machinery for DNA methylation includes DNMT1, DNMT1b, DNMT2, DNMT3a, and DNMT3b (Liu *et al.*, 2003).

In *Drosophila melanogaster* the main candidate for the DNA methylation belongs to the DNMT2 family of DNA methyltransferases (Field *et al.*, 2004). It has been reported that over-

expression of the *Drosophila melanogaster* DNMT2 homologue caused increased DNA methylation CpA and CpT dinucleotides (Kunert *et al.*, 2003). The gene for DNMT2 has been shown to be conserved for over a period of 250 million years. This poses a strong argument for methylation to have some function in insect biology. Since DNA methylation has been implicated in a variety of biologically important functions, it would be interesting to investigate the role of DNA methylation in an organism like *Drosophila melanogaster*.

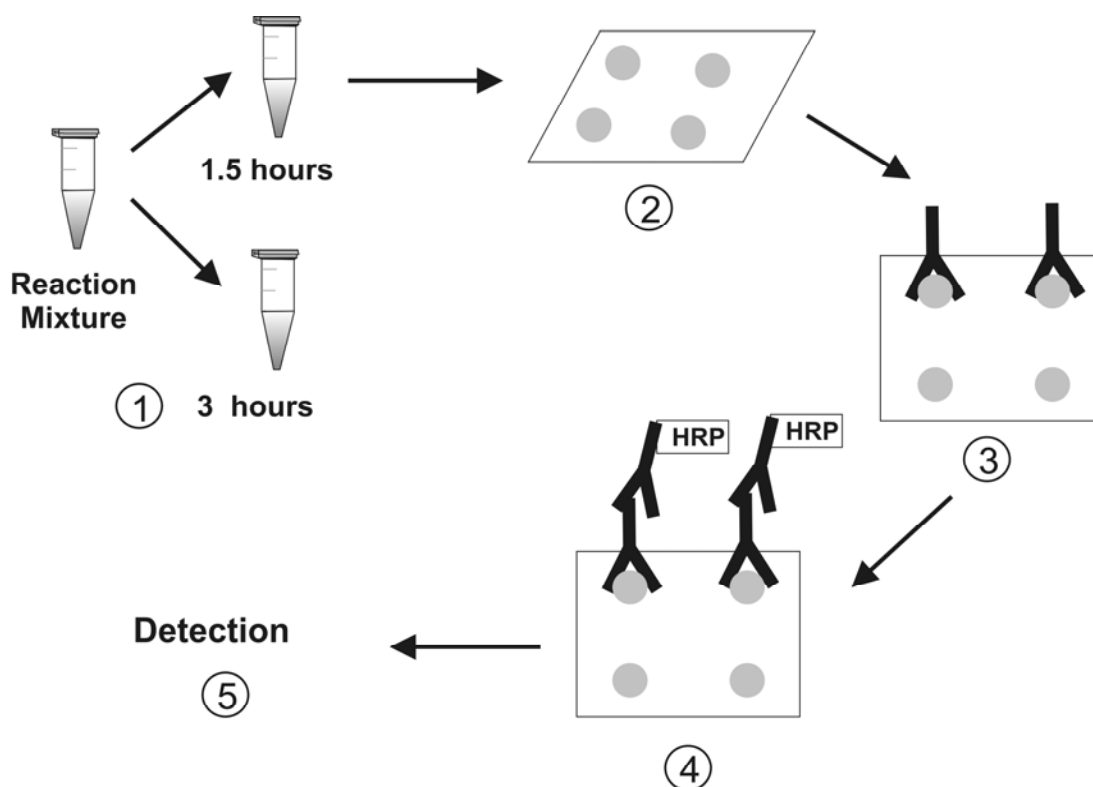


Figure 1. Diagrammatic representation of *in vitro* methyltransferase assay. 1. The reaction mixture (protein extract+SAM+unmethylated DNA+Distilled Water) was incubated at 37°C for 1.5 and 3 hrs, respectively. 2. The DNA eluted from the above reaction mixture after incubation period was spotted onto the nitrocellulose membrane and cross-linked on both sides by UV rays. 3. The membrane was then kept in 5-methycytosine (primary antibody) overnight. 4. The membrane was labelled with HRP-conjugated secondary antibody for 4 hrs. 5. They are detected by using DAB (diamino benzidine hydrochloride) and H<sub>2</sub>O<sub>2</sub> (hydrogen peroxide).

In our present study we have demonstrated the presence of De Novo cytosine methyltransferase activity in adult and pupae of *Drosophila melanogaster*. The study was carried out using an *in vitro* methyltransferase assay (Prasad *et al.*, 2005), where an unmethylated DNA substrate was prepared for the methyltransferase assay (Figure 1). For this DNA from plasmid BlueScript was PCR amplified using T3 and T7 primers that flank the multiple cloning sequence. The amplified DNA was 182bp and contained the multiple cloning sequences within. The unmethylated DNA was incubated with the reaction buffer (20mM Tris-Cl pH-7.5, 20% glycerol, 0.5mM EDTA, 100mM NaCl, 0.5mM PMSF, 0.5mM DTT), *Drosophila melanogaster* (pupa and adult) extract along with

1mM SAM incubated at 37°C for 1.5 and 3 hrs, respectively. The DNA from the reaction mixture was purified and was assayed for the presence of 5-methylcytosine in this DNA by employing an immunochemical method. DNA was spotted onto the nitrocellulose membrane (Schleicher and Schuell), dried and treated with anti-5-methylcytosine antibody. The antibody reaction was visualized by using peroxidase conjugate of anti-rabbit antibody followed by staining for peroxidase activity using diaminobenzidine tetrahydrochloride (DAB) and H<sub>2</sub>O<sub>2</sub>.

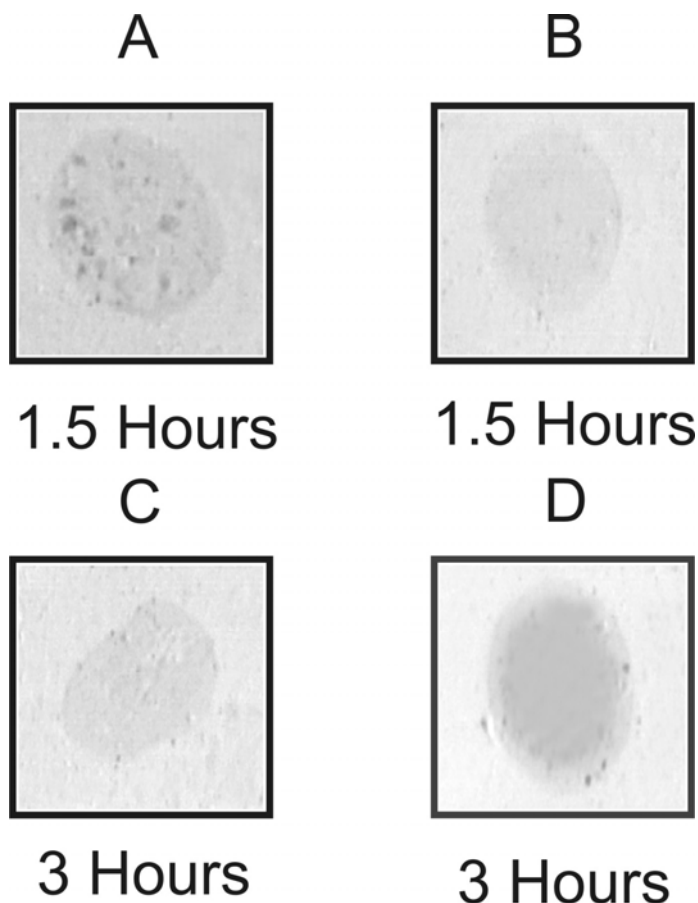


Figure 2. Immunochemical detection of cytosine 5 methyl transferase. A. cytosine 5 methyl transferase in pupa. B. cytosine 5 methyl transferase in adult.

Thus our results of the *in vitro* methyltransferase assay indicate that a DNA cytosine 5 methyltransferase activity is present in both pupae and adult of *Drosophila melanogaster* (Figure 2). The DNA cytosine methyltransferase detected has *de novo* methyltransferase activity. This opens up several interesting avenues of looking at DNA methyltransferases of *Drosophila* and elucidating their regulation. Such an analysis will provide important insight into the role of this epigenetic modification.

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