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Methylation of hen erythrocyte DNA

R.L.P. Adams and C.L. So

Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland

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We have analysed the 5-methylcytosine content of hen erythrocyte DNA and found it to be lower than that of DNA from other chick tissues analysed. Erythrocyte DNA is also a better substrate for DNA methylase having a five-fold lower K_m than DNA from white blood cells. This is probably because it contains a large number of hemimethylated sites. Thus the inverse correlation between methylation and gene expression does not apply to the chick red blood cell.

Methylcytosine; DNA; Hemimethylated site; (Erythrocyte, Chick)

1. INTRODUCTION

By studying different genes in a variety of tissues a strong inverse correlation has been found between gene expression and DNA methylation [1]. Active genes are undermethylated, particularly in promoter regions, and inactive genes are methylated and present in DNase-resistant chromatin. Thus the inactive, embryonic globin gene is highly methylated in adult hen red blood cells and has lost the DNase sensitivity it showed in the embryo [2]. However, the adult β-globin gene is DNase-sensitive in the chromatin of embryonic chick red blood cells even though it is not yet active, i.e. it is 'preactivated' [3]. A similar, determined state is seen in uninduced mouse erythroleukaemia (MEL) cells which already show a chromatin structure around the globin gene similar to that seen in induced cells but quite different to that seen in fibroblasts [4,5].

In most experiments, inactive genes are studied in cells where other genes are active, i.e. in no cell are all the genes either active or inactive. The mature red blood cell offers a situation where no genes are active and all the chromatin is highly condensed and refractory to nucleases [6]. Despite this, it has been shown that the adult globin genes remain undermethylated at certain sites long after gene expression has ceased [7]. This undermethylation might be specific for globin genes or it might represent a situation where DNA methylase is not present or is unable to act during the final round(s) of DNA replication during the maturation of the red blood cell. In support of this, is the finding of a decreased level of global methylation in terminally differentiated MEL cells [8].

The aim of the experiments reported in this paper was to determine whether the inverse correlation between methylation and gene expression holds for the red blood cell, or whether, in a cell in which the chromatin is highly condensed, its structure plays the overriding role in switching off gene expression.

Correspondence address: R.L.P. Adams, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland

2. MATERIALS AND METHODS

DNA was prepared from various tissues of an adult hen by methods previously described [9]. Blood was collected in heparinised tubes and the red and white cells separated using Ficoll Paque (Pharmacia). Base analysis was performed as described by Adams et al. [10]. DNA methylase isolated from mouse ascites tumour cells [11] was used to methylate DNA in a standard assay in which the DNA concentration varied up to 140 μg/ml.

3. RESULTS AND DISCUSSION

Table 1 shows that, of all chick tissues analysed, red blood cell DNA has the lowest 5-methyl-

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Table 1

DNA isolated from various tissues of a hen was analysed for its methylcytosine content

Tissue	%mC
RBC	3.28 ± 0.11
Kidney	3.41, 3.43
Heart	3.47, 3.64
Liver	3.69, 3.88
WBC	3.68, 3.97
Lung	3.91
Spleen	3.97

The results are presented as mC × 100÷mC). RBC, red blood cell; WBC, white blood cell. For the RBC the result is given as the mean ± standard deviation for 7 observations

cytosine content, while DNA of liver, lung, spleen and white blood cells has a high 5-methylcytosine content. As reported by Kappler [12], DNA from kidney and heart has a lower content of 5-methylcytosine than that of liver DNA, but the value from red blood cell DNA is the lowest of all.

Table 2 compares DNA from red and white cells as a substrate for mouse DNA methylase. The DNA from red cells is a better substrate with a 5-fold lower value for K_m. The values for V_{max} are similar. The difference between the two K_m values also applies to denatured DNA but the rate of reaction with denatured DNA is much lower than that with native DNA. These results, coupled with the finding of no gross change in *Hpa*II sensitivity of red and white cell DNA (not shown), are consistent with the presence in red cell DNA of an increased number of hemimethylated CG dinucleotides. These may arise in the final round of DNA replication in the pre-erythrocyte. We propose that the amount of DNA methylase may be limiting at this time, a situation which must be aggravated by the presence of increased amounts of new chromatin proteins such as histone H5.

A chick cell has 3 pg DNA and, if we assume that the dinucleotide CG is present at only 25% of the expected frequency then each cell has about 55 million CG dinucleotides, i.e. 5.0% of cytosines are in CG dinucleotides (see also [8]). The white cell (%mC = 3.95) has 12 million (22%) of these unmethylated and the red cell (%mC = 3.28) has 20 million (36%) unmethylated. We would like to suggest that both DNAs contain about 5 million completely unmethylated CG pairs and that the white cell DNA contains 2 million hemimethylated CG

Table 2

Native or denatured DNA isolated from red or white blood cells from a hen was used as a substrate for mouse DNA methylase

DNA source	Nat/Den	K_m (μM)	V_{max} (pmol/h)
RBC	Native	1.2	1.1
WBC	Native	6.4	1.2
RBC	Denatured	0.2	0.3
WBC	Denatured	0.8	0.3

pairs and the red cell DNA 10 million. This would explain the 5-fold difference in K_m between the two DNAs and would imply that in the last round of DNA replication, maintenance methylation is repressed by 44% (10 × 100/(55 − 10)/2).

The undermethylation in erythrocyte DNA of both strands of the globin genes [7] may be typical of those genes active in the later stages of differentiation. In addition, about half the mCG dinucleotides fail to maintain their methylated status in the final round of replication. Although the chromatin is highly condensed and gene expression has ceased, the DNA is undermethylated. This undermethylation is not sufficient to activate genes and this may reflect the peculiar nature of chick red blood cell chromatin in containing histone H5 instead of H1 [13]. Indeed, it may be this peculiar chromatin structure that prevents the action of the methylase maintenance.

The presence of a large number of hemimethylated sites in chick red blood cell DNA would explain why one group found it to be a suitable substrate for vertebrate DNA methylase [14].

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