Epigenetic control of expression of the human L- and M- pigment genes

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Differential DNA methylation is an essential epigenetic signal for gene regulation without changing the DNA sequence. Epigenetic signals include methylation of cytosine bases at CpG sites in DNA resulting in reduction of gene expression. DNA methylation has been shown to play important roles in cell differentiation, genomic imprinting, X chromosome inactivation, and genome stability. We compared the CpG methylation patterns of the promoter of the L-pigment gene (*OPN1LW*) plus an upstream DNase 1 hypersensitive (DHS) site between the human retinoblastoma cell line WERI, which expresses the L and M pigment genes when treated with thyroid hormone (T3), and lymphoblastoid cell lines that do not express these genes. DNA was isolated from these cells and then treated with bisulfite to convert the unmethylated cytosines (Cs) to thymines (Ts). The targeted regions were PCR-amplified, cloned and sequenced to determine the position and ratio of methylated to unmethylated Cs at each site in at least 35 clones of each cell line.

The great majority of the 14 CpGs located within the proximal 200 bp of the promoter, plus 20 bp of the 5'-untranslated region were unmethylated in WERI cells whether or not treated with T3, but almost totally methylated in the lymphoblastoid cell line. Three of the CpGs located just upstream of the 200 bp of the promoter were methylated in both WERI and lymphoblastoid cells. Significant differential methylation was also observed within the DHS region located about 6 kb upstream of *OPN1LW* gene. This DHS region contains a highly conserved sequence "insulator" motif that has been shown to regulate gene expression. In conclusion, methylation patterns contribute to epigenetic regulation of expression of the L- and M-pigment genes and potentially to differentiation of L and M cones and determination of the retinal L:M cone ratio.

Supported by NIH grant EY008395