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DNA methylation effects on expression of a chromatin-regulated gene in maize

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Packaging of DNA into chromatin can affect gene expression by controlling accessibility of the gene to transcriptional machinery needed for gene expression. When loosely packaged, chromatin allows active gene expression, whereas tightly packaged chromatin renders genes inactive. Chromatin-level regulation has been studied extensively in yeast and mammals, but little is known about chromatin control of gene expression in plants. To expand out understanding, we are taking a functional genomics approach, applying transgenic technology to knock down expression of maize genes thought to be involved in chromatin-level regulation, to assess any phenotypic changes. This project focused on silencing expression of maize genes corresponding to two classes of proteins that play roles in gene silencing in other organisms. DNA methyltransferases (DMT) methylate cytosines in DNA; high levels of cytosine methylation are correlated to low gene expression. Methylcytosine binding domain (MBD) proteins bind methylated DNA; MBDs function in multi-protein complexes containing other proteins responsible for tightening DNA packaging. To examine the effect of knocking down DMT and MBD expression, we crossed transgenic lines to a reporter line containing a chromatin-regulated gene called PI-Blotched. PI-Blotched regulates synthesis of purple anthocyanin pigments in plant tissues. PI-Blotched is unusual because it contains tightly packaged chromatin and a variegated, rather than uniformly purple, pigmentation. If tight packaging of PI-Blotched chromatin is mediated by DMT or MBD proteins, then we predict that knocking down expression the genes for these factors will loosen PI-Blotched chromatin, resulting in more gene expression and more pigment. To test this idea, we have measured pigment levels in plants with reduced DMT or MBD expression and compared the pigmentation to sibling plants with normal expression. The results of our studies should help us better understand the genes that regulate PI-Blotched and also help us to define the roles of DMT and MBD proteins.