Abstract

The 5-methyl cytosine present in genomic DNA is one of the epigenetic modifications that determines the fate of gene expression. The methylome and transcriptome data from different organisms suggest an inverse relation between DNA methylation and gene expression. Different studies in plants and animals also indicate the interaction of a lot of histone modifying proteins with cytosine methylation. The association of DNA methylation with histone deacetylation and histone methylation is well characterized in plants and animals. A combined effect of these processes leads to chromatin condensation and gene silencing. In higher organisms, the cytosine methylation is read and interpreted by a class of proteins called methyl CpG binding domain (MBD) proteins. Another interesting phenomenon associated with genomic DNA methylation is RNA directed DNA Methylation (RdDM), a process involving RNA molecules directing the methylation of its loci in the genomic DNA. This area of regulation is poorly understood. In the present work four genes encoding MBD proteins from Arabidopsis were characterized. Transgenic plants over-expressing AtMBD4, AtMBD6, AtMBD10 and AtMBD11 were generated. Over-expression of AtMBD4 and AtMBD10 in Arabidopsis led to hypomethylation of genomic DNA which are highly methylated in wild type plants. These genes encoding MBD proteins were further characterized using mutants and knockdown lines. The down-regulation of these genes in Arabidopsis showed abnormal phenotype in root, leaf and flower. These abnormal phenotypes suggest their importance during the process of development. Transcriptome analysis identified many genes affected due to mutation in AtMBD genes. Transcriptome analysis of atmbd4 mutant suggested its physiological and molecular importance in nutrient starvation especially phosphate starvation. Genes involved in biotic stress response were up regulated in the atmbd6 mutant. Mutation in atmbd11 showed altered expression of many genes involved in protein synthesis, post transcriptional modification and protein degradation. Screening of mutants for various AtMBD genes using an assay involving silencing of gus reporter gene, identified the role of AtMBD5, AtMBD6, AtMBD9 and AtMBD13 in RNA-mediated gene silencing. Further two novel proteins which interact with AtMBD6 and might have a role in RNA-mediated gene silencing pathway were identified. These proteins contain RNA binding domain and they are predicted to bind with ssRNA and dsRNA in a sequence specific manner. The RNA molecules that bind to AtNTF2 and AtRPS2C might guide the protein complex to recognize the genomic loci complementary to it,
therefore recruiting many other proteins involved in chromatin condensation through AtMBD6. In two hybrid screening, AtMBD4 was found to interact with a ubiquitin conjugating enzyme AtUBC36. The interaction between these two proteins may be involved in ubiquitinization of histone or AtMBD4. It is possible that AtMBD4 is a substrate for AtUBC36 for ubiquitinization leading to its degradation. The AtUBC36-mediated degradation of AtMBD4 may alter expression of some genes. The over-expression of AtMBD4 gene leads to genomic DNA hypomethylation. However the mechanism of its action in affecting DNA methylation is not clear. AtMBD10 was found to interact with a Purple Acid Phosphatase (AtPAP14). This protein is a ser-thr phosphatase and is involved in protein dephosphorylation. The interaction of these two proteins suggests that the protein complex might be involved in dephosphorylation of histone. Histone dephosphorylation can lead to condensed chromatin. The protein-protein interactions found in the present work might be involved in chromatin condensation and gene silencing. Further work is needed to confirm that the chromatin condensation is due to these proteins interacting with AtMBDs. The RNA binding properties of AtRPS2C and AtNTF2 need to be confirmed. The ubiquitinization and dephosphorylation properties and targets of AtUBC36 and AtPAP14 are required to be detected. These proposed studies will help in understanding of the mechanism of gene silencing by AtMBDs.