Widespread dynamic DNA methylation in response to biotic stress

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AUTHOR SUMMARY

Cytosine methylation is a heritable covalent modification of DNA that imparts a layer of regulation upon the genome. In plants, DNA methylation faithfully represses gene expression and maintains genome stability. Recent genetic studies have linked DNA methylation pathways to the plant immune response, suggesting that some immunity genes may be directly modulated by cytosine methylation (1). To investigate this further, we profiled DNA methylation levels in plants after exposure to a bacterial pathogen and identified numerous stress-responsive genes whose expression is coupled to dynamic changes in DNA methylation.

Although the mechanisms that establish and stably maintain DNA methylation between generations have been investigated thoroughly in plants, much less is known about how DNA methylation is dynamically altered. Widespread dynamic DNA methylation has only been observed in differentiated reproductive tissues, where it regulates the expression of target genes, including transposable elements (TEs) (2–5). TEs are mobile DNAs that can propagate throughout the genome and modify the expression of nearby genes. Transposon repression requires the accumulation of small RNA (smRNA) molecules that direct methylation of cytosines within the sequence of the TE site (4). It remains unclear if the smRNA and DNA methylation landscapes change in response to stress in a manner analogous to that observed in reproductive tissues.

Using Arabidopsis thaliana as a well-established system to study DNA methylation, we investigated whether cytosine methylation is required for stress responses, specifically defense against the bacterial pathogen Pseudomonas syringae pv. tomato DCC3000 (Pst). We found that mutants that are globally depleted of cytosine methylation (met1 and ddc) were more resistant to Pst infection and failed to develop typical disease symptoms (Fig. P1A). Furthermore, numerous pathogen-responsive genes were misregulated in these mutants, suggesting that DNA methylation directly controls the expression of some plant defense genes. This suggests that some of these genes might be stably repressed by DNA methylation normally, but, upon infection, the methylation state may be dynamically altered, thereby resulting in gene expression changes.

Therefore, we performed a genome-wide analysis of DNA methylation in plants infected with Pst to resolve single-nucleotide methylation changes. We discovered that Pst exposure induces changes in cytosine methylation throughout the genome relative to untreated plants. We developed a method to identify statistically significant differentially methylated regions (DMRs) and found that they tend to be small and occur in gene-rich regions, suggesting that they may participate in gene regulation. The DMRs were enriched directly upstream and downstream of protein-coding genes and within TEs, consistent with the dynamic methylation changes observed in reproductive development (2, 3). Although these DMRs were significant, the levels of methylation change were relatively modest, indicating that only a subpopulation of cells may respond to the pathogen stress.

To determine if different stress conditions trigger unique patterns of differential methylation, we profiled the DNA methyomes of plants exposed to nonpathogenic bacteria or salicylic acid (SA), a chemical that...
triggers plant defense responses. We observed that many DMRs were similarly targeted under different stress conditions, suggesting that these regions are general regulatory hubs for stress-responsive genes. To determine if DMRs regulate stress response genes, we performed a genome-wide gene expression analysis. We first assigned each DMR to the most proximal gene (i.e., DMR-associated gene). An analysis of all DMR-associated genes revealed that these genes tend to function in plant defense pathways and that their demethylation correlated with increased gene expression (Fig. P1B, Upper). Last, we investigated whether these genes were misregulated in mutants that globally lack DNA methylation and discovered that they were highly misexpressed (Fig. P1B, Lower). Together, these results suggest that DNA methylation normally reduces expression of these genes, but, upon stress, this inhibition is relieved by active demethylation of proximal regulatory regions.

Because many DMRs were positioned within TEs, we investigated whether the levels of smRNAs produced from these elements were also altered. Thus, we performed a genome-wide analysis of smRNAs in SA-treated plants and found that the levels of a specific class of smRNAs, those 21 nt in length, were elevated at the DMR-associated TEs. Transcriptional analysis of these DMR-associated TEs revealed that SA-induced demethylation, along with up-regulation of 21-nt smRNAs, correlated with increased transcription of TEs, and, in some cases, up-regulation of proximal protein-coding genes (Fig. P1B, Lower). These results indicate that dynamic changes in smRNA and methylation levels at TEs may effect the expression of neighboring genes.

Although published work indicates that DNA methylation is capable of being altered upon stress exposure, these studies are limited by low-resolution, nonquantitative, or noncomprehensive approaches (1). Our unbiased, genome-wide approaches have revealed unique aspects of dynamic DNA methylation, including a correlation between stress-induced differential methylation, induction of specific smRNAs, and transcriptional changes at TEs and proximal genes. Moreover, the response to stress does not result in methylation changes at a few target genes, but rather consists of a multitude of genome-wide changes that together alter transcriptional programs. Our study provides the framework for future mechanistic analysis aimed at dissecting how dynamic methylation changes are executed, and suggests that targeted manipulation of DNA methylation at some loci could serve as a strategy to engineer pathogen-resistant crop strains.