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Spotlight

The 1001 *Arabidopsis* DNA Methylomes: An Important Resource for Studying Natural Genetic, Epigenetic, and Phenotypic Variation

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Intraspecific phenotypic diversity is controlled by natural genetic and epigenetic variation.

Kawakatsu *et al.* recently sequenced the DNA methylomes of a global collection of over 1000 *Arabidopsis* accessions, and have thereby provided a comprehensive resource for studying natural genetic and epigenetic variation as well as the association of such variation with phenotypic diversity.

DNA methylation (5-methyl-cytosine) is an ancient epigenetic mark typically associated with inactive transcription in many higher eukaryotes [1]. DNA methylation influences genome structure and function and is involved in gene imprinting, genome stability, and other important biological processes. Over the past two decades, the mechanisms and functions of DNA methylation have been extensively studied. DNA methylation in plants occurs in three sequence contexts, CG, CHG, and CHH (H = A, T, or C). The most common methylation in genic regions is CG methylation (referred to as gene-body methylation or gbM), the function of which is still unclear. By contrast, DNA methylation in transposable elements (TE) and some genic regions occurs in all sequence contexts (referred to as TE-like methylation or teM) and is associated with gene silencing. Genomic DNA methylation patterns change during development and in response to environmental stimuli. Aberrations in DNA methylation patterns have been linked to aging, cancers, and many other diseases in humans. How DNA methylation patterns are established, maintained, and pruned, as well as how DNA methylation regulates gene expression and influences organismal phenotypes, has been studied extensively by using mutants in epigenetic regulators. However, how DNA methylation patterns are shaped during evolution and how natural epigenetic variation may contribute to phenotypic diversity is still poorly understood.

Although other models are on the rise, *Arabidopsis thaliana* is still one of the most important model organisms for plant

biology and is also a prominent model for epigenetic studies in eukaryotes. *Arabidopsis* had the first fully sequenced and annotated plant genome in 2000, and also had the first sequenced DNA methylome in all eukaryotes in 2008. Genome-wide association studies (GWAS) are a powerful tool for discovering the genomic basis of phenotypic variation. A prerequisite for GWAS is the availability of genetic markers in the studied population. As of 2015, about ~1300 worldwide *A. thaliana* accessions had been genotyped using 250k single-nucleotide polymorphism (SNP) arrays, and three medium-sized collections of *A. thaliana* inbred lines had been sequenced [2–5]. In these studies, however, genetic variations could not be fully explored because of the technical limitations of the 250k SNP array, which omits many SNPs and other variations, or because of the relatively small sizes of the sequenced populations, in which minor-allele counts were too low to be used in the analysis of rare genetic variants. A more recent study by the 1001 Genome Consortium resequenced the genomes of >1000 worldwide *A. thaliana* inbred lines, capturing all SNPs and other variations, thus making it possible to study rare variants in the population [6]. In all, ~2000 natural accessions that have been resequenced or that were covered by the 250k SNP arrays can potentially be used for high-resolution GWAS analysis in *Arabidopsis*.

Phenotypic diversity is shaped not only by genetic change but also by epigenetic variation (Figure 1). Before 2016, the DNA methylomes of two medium-sized sets of *Arabidopsis* accessions had been sequenced [4,7]. In 2016, Kawakatsu *et al.* [8] generated the DNA methylomes of >1000 worldwide *A. thaliana* inbred lines and thereby provided the information required for studying the association between natural epigenetic variation and phenotypic diversity. Some DNA methylation variation is linked with or controlled by genetic variation, such as gene rearrangement, transposon insertion, or

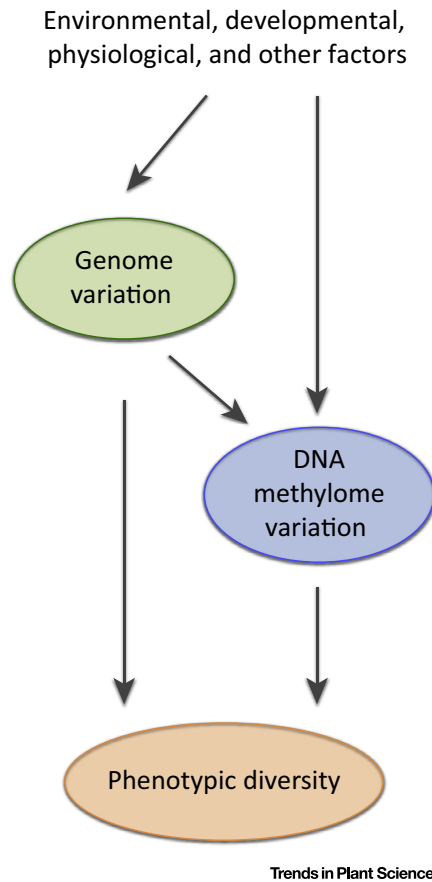


Figure 1. Diagram Showing that Environmental, Developmental, Physiological, and Other Factors Influence both the Genomic and Epigenomic Variations that Contribute to Phenotypic Diversity.

functional mutations in genes of DNA methylation pathways [9]. Kawakatsu *et al.* generated physical genome maps for nine selected accessions and found that over half of the differentially methylated genes among these nine accessions are located in regions with structural variations, suggesting that structural variation is important in shaping DNA methylomes. Further studies may determine whether accessions with similar structural variations always have similar methylation or expression levels of the differentially methylated genes. Some DNA methylation variations are independent of genetic variations, especially in terms of SNPs [4]. Environmental, developmental, physiological, and other factors may cause not only genetic changes but also directly cause epigenetic changes (Figure 1). For epigenetic variations that are independent

of genetic variations, the SNP-based GWAS analysis may not be able to identify associations between epigenetic variations and phenotypic diversity. The basis and adaptive significance of this type of epigenetic variation warrants additional investigation.

Both Kawakatsu *et al.* and the authors of previous studies showed that variation in teM but not in gbM is associated with transcriptome differences [4,8]. Consistent with previous notions that DNA methylation plays a role in plant immunity and plant responses to environmental stimuli such as heat [10,11], Kawakatsu *et al.* found that natural teM variations are associated with plant disease resistance genes and genes involved in plant responses to the environment. These results suggested that the DNA methylation pathways responsible

for teM help plants to adapt to the environment and help to determine transcriptome diversity in natural accessions.

While gbM is largely affected by the mCG maintenance DNA methyltransferase MET1, teM mainly depends on two pathways, in other words the chromomethylase 2 (CMT2)-mediated CHG and CHH methylation pathway, and the RNA-directed DNA methylation (RdDM) pathway. In the RdDM pathway, siRNAs guide the DNA methyltransferase DRM2 to generate mCG, mCHG, and mCHH. By using the methylation levels of different groups of genomic regions as a phenotype for GWAS analysis, Kawakatsu *et al.* determined that linked SNPs were located near genes encoding several known factors involved in teM, including CMT2, and several RdDM pathway components. Future GWAS analysis using DNA methylation levels as phenotypes should be applied to obtain new information about known epigenetic regulators and also to discover as yet unknown epigenetic regulators. Kawakatsu *et al.* also analyzed associations between gene expression levels, SNPs, and DNA methylation variations, and thereby identified methylation-dependent and -independent expression quantitative trait loci (eQTLs). Furthermore, their comparisons of the eQTLs with data on genome-wide transcription factor binding sites suggested that genome and methylome variations interact to regulate gene expression by influencing the binding of distinct sets of transcription factors. The current genome, DNA methylome, cistrome, and mRNA transcriptome data will prove even more useful by adding other chromatin-related omics data, such as the genome-wide profiles of small RNAs, long non-coding RNAs, histone variants, and histone modifications.

In conclusion, the high-resolution methylomes of >1000 *Arabidopsis* accessions represent an extraordinary foundation for studying epigenome evolution and the

association between natural DNA methylation variation and phenotypic diversity. The genome, DNA methylome, and transcriptome resources together make it possible to systematically investigate the significance of natural genomic and epigenomic variations in plant environmental adaptation and evolution (Figure 1).

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