Epigenetic Control of Genome Activity in Plants

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Introduction

Our group was established in 2002 with the aim of investigating epigenetic processes through genomic approaches in the model plant Arabidopsis. Plants are indeed characterized by a plethora of epigenetic phenomena, many of which have subsequently been re-discovered in animals. With its compact and nearly fully sequenced genome, extensive collections of insertion mutant lines, and a long tradition of genetic analysis, Arabidopsis provides an ideal system with which to explore self-perpetuating chromatin modifications, such as DNA methylation, and their contribution to somatically and meiotically transmissible changes of genome activity. In this context, we are particularly interested in determining whether epigenetic processes contribute significantly in nature to the generation of heritable phenotypic variation.

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A genomic tiling microarray for epigenomic mapping

We have constructed, in collaboration with the group of R. Martienssen at CSHL (USA), a genomic-tiling microarray that allows us to profile gene expression, DNA methylation, histone modifications and protein binding across the entire known sequence (19 Mb) of *Arabidopsis* chromosome 4, with a ~1kb resolution. This chromosome was chosen as a model for the whole genome, which is 130-160Mb long and divided into five chromosomes. Starting with a pilot array that covers a 0.5Mb region of interstitial heterochromatin (knob) and 1Mb of flanking euchromatin on the short arm of chromosome 4, we have constructed the first detailed epigenomic map of a large chromosomal region in plants. Microarrays were hybridized with labelled complementary DNA, or with labelled genomic DNA that had been depleted of methylated sequences, or enriched for sequences recovered by chromatin immunoprecipitation (ChIP) with antibodies raised against euchromatin or heterochromatin-associated

modifications of histone H3 (Gendrel et al., 2002). Control hybridizations with total genomic DNA allowed DNA methylation and histone H3 methylation to be quantified for each feature on the array. This analysis revealed that transposable elements and related repeats are silent and associated with heterochromatic hallmarks (H3K9 dimethylation and DNA methylation) whether located in cytologically visible heterochromatin or not. Genes on the other hand are expressed and associated with euchromatic marks, even when located within interstitial heterochromatin. From this work, we conclude that TEs and related repeats define heterochromatin. Furthermore the RNAi machinery is likely to be involved in this process, as small interfering RNA (siRNAs) are abundant in *Arabidopsis* and correspond predominantly to these sequences (Lippman et al., 2004).

Epigenetic control of gene expression

Using the pilot microarray, we have shown that a mutation in the gene *DDM1*, which encodes a chromatin remodeling ATPase also found in yeast and mammals, leads to a massive increase in steady-state messenger RNA levels of all types of transposable elements, with no detectable effect on most genes. Moreover, these changes are transmitted to the progeny, independent of the *ddm1* mutation. These results suggest therefore a genome-wide role for DDM1 in controlling mobile sequences epigenetically and we have subsequently shown that by run-on experiments that this control occurs at the transcriptional level (Y. Deveaux, A. Evrard and V. Colot, manuscript in preparation). Although our study reveals that genes are usually insulated from the silencing of nearby transposable elements, it also demonstrates that such elements can control gene expression epigenetically when inserted within or very near genes (Lippman, 2004). We are now interested in evaluating along the entire chromosome 4 the prevalence of this type of control, and its impact on development and natural variation.

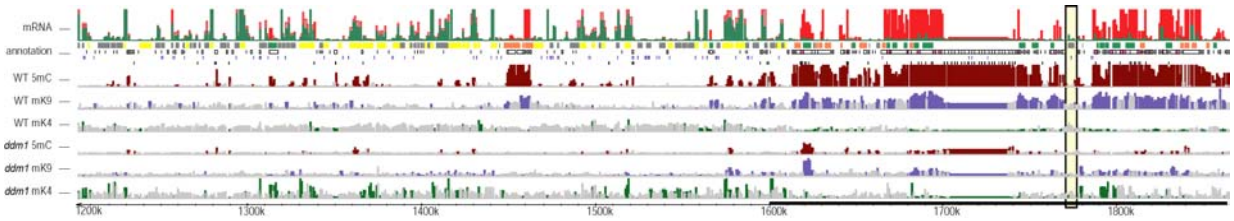

Figure 1. An epigenomic map of part of chromosome 4, with expression, DNA methylation and histone modification (H3mK4 and H3mK9) data. Note the contrast between euchromatin (left) and heterochromatin (right, except for one gene island, boxed).

To this end, we are pursuing several approaches:

(i) We wish to study the ability of epigenetic processes to generate genetic variability that is potentially adaptive and is independent of any DNA polymorphism. The *ddm1* mutation was used to induce numerous epigenetic alterations across the genome, that were then fixed independently of the inducing *ddm1* mutation through two successive backcrosses and six generations of selfing. A total of 500 *DDM1/DDM1* recombinant inbred lines (RILs), each theoretically retaining - in the “homozygous” state - 25% of the transmissible changes originally induced by *ddm1,* will be phenotyped for several traits related to fitness. On the basis of this study, 100 extreme lines will be analyzed by means of the genomic tiling array to profile chromatin along chromosome 4. QTL detection will be performed to link phenotypic variation to possible alterations in chromatin compared to wild type. Candidate regions will be explored through transcription profiling in order to identify potential epimutations. This project will further our knowledge on the importance of epigenetic phenomena in generating quantitative phenotypic variation (Génoplante project).

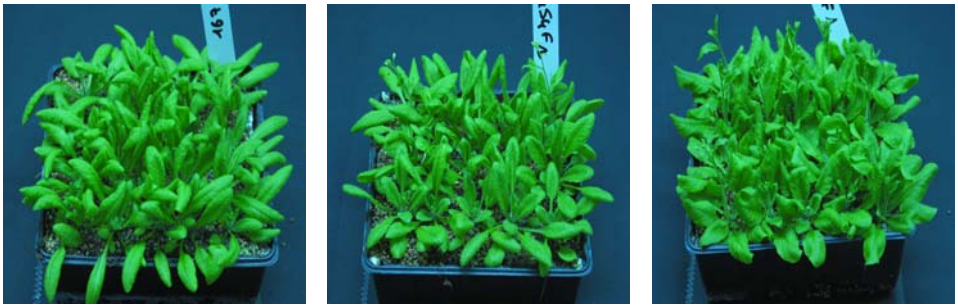

Figure 2. Examples of isogenic "epiRILs" with distinct phenotypes.

(ii) Anecdotal evidence suggests that *ddm1*-induced reactivation is remarkably stable. The molecular study performed in (i) should provide a comprehensive analysis of this issue. In parallel, we have developed a crossing scheme involving a balancer chromosome that allows us to follow through successive backcrosses and in individual plants the activity and chromatin state of the region that normally forms interstitial heterochromatin on the short arm of chromosome 4. We wish to determine when sequences that are normally silent in this region can become re-inactivated, and whether or not re-silencing occurs in a concerted manner for all sequences. Results of this study should provide important information on heterochromatin formation.

(iii) Using a core collection of Arabidopsis strains collected from the wild and that maximizes known natural genetic and phenotypic diversity (http://dbsgap.versailles.inra.fr/publiclines/), we wish to determine the fraction of phenotypic variation observed in nature that can be attributed to epigenetic differences. Hybridization of the genomic tiling array will be performed first with genomic DNA from each strain to identify DNA fragments present (not always on chromosome 4) in all 48 strains. Chromatin profiling will be restricted to these sequences, and potential "epi-alleles" will be further characterized using RILs already derived from several pairs of strains.

Selected Publications

Gendrel AV, Colot V (2005) Arabidopsis epigenetics: when RNA meets chromatin. Curr Opin Plant Biol 8: 142-147

Gendrel AV, Lippman Z, Martienssen R, Colot V (2005) Profiling histone modification patterns in plants using genomic tiling microarrays. Nat Methods 2: 213-218

Lippman Z, Gendrel AV, Colot V, Martienssen R (2005) Profiling DNA methylation patterns using genomic tiling microarrays. Nat Methods 2: 219-224

Martienssen RA, Doerge RW, Colot V (2005) Epigenomic mapping in Arabidopsis using tiling microarrays. Chromosome Res 13: 299-308

Prouteau M, Colot V (2005) Epigenetic control, development and natural genetic variation in plants. Med Sci (Paris) 21: 422-427

Lippman, Z., Gendrel, A.-V., Black, M., Vaughn, M., Dedhia N., McCombie W.R., Lavine, K., Mittal V., May B., Kasschau, K.D., Carrington J., Doerge, R., Colot, V.# and Martienssen R.A.# (2004) Role of transposable elements in heterochromatin and epigenetic control. **Nature** *430*, 471-476

Mathieu, O., Jasencakova, Z., Vaillant, I., Gendrel, A. V., Colot, V., Schubert, I., and Tourmente, S. (2003). Changes in 5S rDNA Chromatin organization and transcription during heterochromatin establishment in Arabidopsis. **Plant Cell** *15*, 2929-2939.

Gendrel, A.-V., Lippman, Z., Yordan, C., Colot, V. , and Martienssen, R. (2002). Heterochromatic histone H3 methylation patterns depend on the *Arabidopsis* gene *DDM1*. **Science**, 297, 1871-1873.