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Review

# Epigenetics in plants—vernalisation and hybrid vigour ☆

Michael Groszmann [a,b], Ian K. Greaves [a,c], Nicolas Albert [a], Ryo Fujimoto [a], Chris A. Helliwell [a], Elizabeth S. Dennis [a,b], W. James Peacock [a,*]

[a] Commonwealth Scientific and Industrial Research Organisation, Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia
[b] NSW Agricultural Genomics Centre, PMB, Wagga Wagga, NSW 2650, Australia
[c] Department of Genome Biology, John Curtin School of Medical Research, Australian National University, ACT 0200, Australia

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ABSTRACT

In this review we have analysed two major biological systems involving epigenetic control of gene activity. In the first system we demonstrate the interplay between genetic and epigenetic controls over the transcriptional activity of FLC, a major repressor of flowering in Arabidopsis. FLC is down-regulated by low temperature treatment (vernalisation) releasing the repressor effect on flowering. We discuss the mechanisms of the reduced transcription and the memory of the vernalisation treatment through vegetative development. We also discuss the resetting of the repressed activity level of the FLC gene, following vernalisation, to the default high activity level and show it occurs during both male and female gametogenesis but with different timing in each.

In the second part of the review discussed the complex multigenic system which is responsible for the patterns of gene activity which bring about hybrid vigour in crosses between genetically similar but epigenetically distinct parents. The epigenetic systems that we have identified as contributing to the heterotic phenotype are the 24nt siRNAs and their effects on RNA dependent DNA methylation (RdDM) at the target loci leading to changed expression levels.

We conclude that it is likely that epigenetic controls are involved in expression systems in many aspects of plant development and plant function.



## 1. Introduction

Thirty years ago, in probing the molecular basis for control of gene activity, emphasis was on the coded sequences in the DNA molecule. In both plants and animals, a number of sequence motifs important for transcriptional activation were identified. More recently, it has been realised that it is important to look at both the DNA and the proteins with which it is associated in chromatin. We now know the histone proteins of chromatin have modifications such as acetylation and methylation of particular amino acid residues which are associated with the control of gene activity. Methylation of cytosine residues in DNA can also influence the transcriptional activity of a gene. The makeup of the suite of these different epigenetic marks for any gene is critical in determining the transcriptional state of that gene, probably through effects on localised chromatin architecture. Protein complexes which bind to chromatin are responsible for the modification of histones and small RNA molecules also influence transcriptional states directly through targeted effects on DNA methylation, or on the production and/or destruction of transcripts from the gene. All these components of chromatin contribute to epigenetic controls of gene activity.

## 2. Chromatin packaging

The primary unit of chromatin packaging is the nucleosome. The nucleosome core particle is comprised of an octamer of histones H2A, H2B, H3 and H4 with an associated 147 bp of DNA. Chromatin can undergo modifications to either DNA or histones that can have consequences for gene expression by affecting access of RNA polymerases or the interaction of transcription factors with specific DNA sequences.

Until recently, determining the location of nucleosomes in the genome was a laborious process, however the development of high throughput sequencing technologies has made genome-wide mapping of nucleosome occupancy straightforward [1]. Some general trends have emerged from these studies, most notably that eukaryotic genes, including those of Arabidopsis, generally have a nucleosome-free region at the beginning and end of the transcription unit which could facilitate access by RNA polymerase although is not sufficient for high transcriptional activity as this feature is present at many genes that are expressed at a low level.

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* Corresponding author. CSIRO Plant Industry (Black Mountain Laboratories) Acton, ACT 2601, Australia. Tel.: +61 2 6246 5250.
*E-mail address:* jim.peacock@csiro.au (W.J. Peacock).

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The extent to which the position of a nucleosome is determined by the underlying DNA sequence appears to be somewhat variable. Alignment of nucleosome core DNA sequences shows a 10 bp periodicity of AT and GC rich sequences. Nucleosomes are often aligned to the same position, particularly the nucleosome closest to the transcription start site. Other nucleosomes do not have as well defined positions. Data from comparisons of expression states suggest that positions of key nucleosomes near transcription starts can change, but that the bulk of nucleosomes do not show expression-related differences in position.

Nucleosomes are enriched in coding regions and often mark intron-exon boundaries. This suggests that the nucleosome landscape may have an important role in intron splicing events as well as in transcription. The nucleosomes also appear to be sites of DNA methylation, with a strong correlation between DNA methylation and the presence of nucleosomes [2].

## 3. Epigenetic marks on chromatin

Epigenetic controls are ubiquitous in flowering plants and are operative not only for the control of processes involved in development but also for the plant's responses in its growth environment to ensure its survival and function. The interaction of epigenetic mechanisms with environmental cues is important in many gene activity responses.

In this review we discuss the role of epigenetic controls in two key biological activities of plants. One is the initiation of flowering following exposure to extended periods of cold temperature—vernalisation. In the second part we examine the evidence that epigenetic controls are significant in the patterns and levels of gene expression which give rise to hybrid vigour or heterosis in many groups of plants. Arabidopsis and the knowledge of its genome sequence and the many tools that exist for detailed analysis of particular genomic regions and their functions have contributed to an understanding of the balance of genetics and epigenetics in the control of gene activity in biological processes.

## 4. DNA methylation and gene regulation

In the mid 1970s two key papers were published on the occurrence of methylation of cytosine residues in DNA in animals [3,4]. This work focussed on the methylation of cytosine residues in CG dinucleotides. The suggestion was that this epigenetic modification to the genomic DNA was likely to be of importance in modifying the transcriptional activity of genes. This was noted to occur both at a whole chromosomal level as evidenced by the X inactivation phenomenon in mammals and for individual genes operating in many metabolic pathways in cells.

In plants, DNA methylation is found in three different contexts, CG, CHG and CHH (H = A, C or T). The methyl groups are added by three different methyltransferase enzymes; *METHYLTRANSFERASE 1* (*MET1*) methylates CG sites, *CHROMOMETHYLASE 3* (*CMT3*) methylates CHG sites and *DOMAINS REARRANGED METHYLTRANSFERASE 2* (*DRM2*) establishes *de novo* methylation of all three contexts and maintains CHH sites [5].

The RNA-dependent DNA methylation (RdDM) pathway uses a series of plant-specific polymerases and processing enzymes to generate short interfering RNAs (siRNAs). These siRNAs are then used as targeting sequences for gene specific transcriptional silencing through establishment of *de novo* DNA methylation of all three contexts and maintenance of CHH methylation [5,6].

## 5. Distribution of DNA methylation across the genome

DNA methylation is predominately found in transposons and repetitive sequences and in promoter regions, intergenic regions, pseudogenes and intragenic regions [7,8]. Approximately one third of Arabidopsis genes contain CG methylation in their gene body [7,8]. These are predominantly constitutively expressed genes. Promoter methylation appears important for conferring specific tissue expression [7]. The higher frequency of CHG and CHH methylation in the flanking regions of genes correlates with siRNA distribution across genic regions, consistent with RdDM establishing and maintaining these two methylation contexts.

Recently an important contribution was made by Lister et al. [9] comparing the methylomes in human DNA samples extracted from embryonic stem cells and from foetal fibroblast cells. They found that CG methylation is present in both cell types but differed in its distribution across the genomes. Significantly, other contexts of cytosine methylation (CHH and CHG) were present in the embryonic stem cells and absent from the fibroblast cells implying that these other contexts of cytosine methylation are important in determining the pluripotent nature of the embryonic stem cells. It is likely that in the early paths of cell differentiation there are different patterns of alteration of the methyl cytosine residues in the genome. The CG context is the only context to survive into fully differentiated cells.

CG methylation in the genome has some important properties. It is inherited through cell divisions and is closely associated with DNA replication. Changes in the methyl C residue distribution can occur in different tissues through development but ultimately the original parental embryonic pattern is re-established (in animals) after meiosis in the new embryo. In flowering plants, methylation of DNA is common. The first indication that methylation is associated with regulation of gene activity came from studies on transposable elements, particularly the Ac and Spm systems in maize [10,11]. The correlation that was evident was that the presence of methylated cytosine residues was associated with non-active transposons and the absence of methylation characterised active transposons, particularly in their transcriptional activity and genome mobility.

## 6. Vernalisation—an epigenetic control system

In plants the biological phenomenon of cold temperature-induced induction of flowering, vernalisation, has properties paralleling DNA methylation. Vernalisation-induced states of gene activity instigated in the germinating seed are maintained through vegetative development of the plant, to finally operate in the transition of the growing apex from a vegetative meristem to a reproductive meristem. Each generation of plants requires the exposure to a low temperature period in order to flower.

Treatment of germinating seeds of both Arabidopsis and wheat with 5-azacytidine, which prevents DNA methylation when incorporated into DNA, caused early flowering without vernalisation. The results mimicked the accelerated initiation of flowering by vernalisation [12,13]. In Arabidopsis the reduction of CG methylation by inhibiting the activity of the methyl transferase 1 (MET1) enzyme responsible for CG methylation also accelerated flowering [14].

Genetic analysis of the vernalisation response identified two genes which had major effects in the response, *FLOWERING LOCUS C* (*FLC*) and *FRIGIDA* (*FRI*) [15–17]. *FRI* ensures a high level activity of *FLC* through a promotive transcription complex. FLC is a repressor of flowering and the vernalisation treatment reduces *FLC* transcriptional activity. When the *FLC* gene was cloned we were able to determine that there was only one methyl C in intron one and no other methyl Cs in the gene body or in the flanking promoter region [18,19]. We found there was no change in the methylation pattern following vernalisation and concluded that the reduction of CG methylation across the genome must result in reduction of *FLC* activity not through a direct action on the *FLC* gene but through interactions with products from at least one other gene which had changed activity levels as a result of the demethylation treatment.

The vernalisation treatment represses the transcription of *FLC* and poses the question as to how the down regulation of *FLC* occurs. A