

# Epigenetics: Beyond genes

A Fossey

*Forestry, Natural Resources and the Environment,  
CSIR, P.O. Box 17001, Congella, Durban 4013, South Africa  
Email: [afossey@csir.co.za](mailto:afossey@csir.co.za)*

## **Abstract**

Gene regulatory processes lead to differential gene expression and are referred to as epigenetic phenomena; ubiquitous processes in the biological world. These reversible heritable changes concern DNA and RNA, their interactions, and chromatin-mediated and RNA-mediated mechanisms. DNA compaction is associated with gene inactivation in which chromatin is rendered either transcriptionally active or transcriptionally repressed. A “histone code hypothesis” proposes that each histone combination defines different epigenetic states. RNA also participates in diverse regulatory activities. Three RNA silencing pathways have been identified in plants.

Prolonged juvenility in trees greatly limits tree domestication and thus is of considerable interest to tree breeders. However, for production forestry delayed flowering is desirable. Transition to maturity has been associated with changes in the degree of DNA methylation and polyamine concentration. Tree aging is associated with the loss of morphogenic potentials, and has been shown to be associated with epigenetic control mechanisms. Meiotic imprinting renders maternal and paternal epigenomes particularly in the endosperm and thus displays differential gene manipulations. Interspecific hybridization and polyploid formation are practiced in many forestry species. When the specific endosperm balance number of a species is manipulated by changing the ploidy imprinting differences are exhibited; also demonstrated in interspecific hybrids. With the continued growth in knowledge of epigenetic phenomena, it is expected to play a major role in forestry breeding.

## **Keywords**

Gene regulation; chromatin; histone code hypothesis; RNA silencing; post transcriptional gene silencing; forestry.

## **Introduction to epigenetic phenomena**

Most living organisms share a vast amount of genetic information. Proteins are very similar, if not the same, across widely divergent species. What separates humans from the amoebas lies in how genes are expressed; the difference lies in the regulation of their genes. These

regulatory processes are referred to today as epigenetic phenomena, which lead to differential gene expression that are crucial for cell differentiation and development (Reyes *et al.*, 2002). Researchers are unravelling what other biological information, besides DNA, parents pass to their offspring, and cells pass on to each other when they divide.

Epigenetics describes the study of reversible heritable changes in genome function that occur without a change in DNA sequence, and may have morphological, physiological, and ecological consequences (Rapp and Wendel, 2005). Epigenetic phenomena pervade all aspects of cell proliferation and plant development and are often in conflict with Mendelian models of genetics (Grant-Downton and Dickinson, 2005). A key element in many epigenetic effects concerns the recognition of nucleic acid sequence homologies at both the DNA and RNA levels; DNA-DNA and RNA-RNA interactions, and the regulation of gene silencing through chromatin-mediated and RNA-mediated mechanisms.

### ***Chromatin mediated epigenetic systems***

In eukaryotes, linear DNA is associated with histones to form a tightly compacted nucleoprotein complex, chromatin. The lowest level of compaction of chromatin is the 10-nm fibre, which is made up of repeated units of nucleosomes linked with linker DNA, giving DNA the appearance of "beads-on-a-string" (Dolinoy *et al.*, 2007). Higher levels of coiling are attained through further looping and coiling. Interphase chromatin is present mostly in the form of 10-nm fibre, coiled parts of 30-nm fibre, with some regions folded into looped domains of higher compaction. Interphase chromatin comprises domains of heterochromatin that are highly condensed and predominantly associated with transcriptionally inactive, gene-poor sequences, and, domains of less compacted euchromatin containing actively expressed genes (Brzeski and Jerzmanowski, 2004). Some heterochromatin domains remain permanently inactive, referred to as constitutive heterochromatin, while facultative heterochromatin with a similar appearance to constitutive heterochromatin may be transcriptionally inactive in certain cell lineages or developmental

stages, while active in others (Brzeski and Jerzmanowski, 2004). A heterochromatic state is capable of spreading to neighbouring euchromatic genes, known as position effect variegation and can be transmitted through mitosis, forming the basis of epigenetic inheritance from one cell generation to another; creating cellular memory (Brzeski and Jerzmanowski, 2004).

Chromatin not only ensures the compaction necessary for DNA packaging inside the cell nucleus, but also provides the framework for gene-regulated expression (Prunell, 1998). The fluctuation between decondensed and a more condensed state of chromatin plays a central role in cellular differentiation and is referred to as “chromatin remodeling”. Chromatin remodeling alters the basal state of chromatin by rendering it either as transcriptionally active, an “open state”, or transcriptionally repressed, a “closed state” (Reyes *et al.*, 2002). A “histone code hypothesis” has been proposed that suggests that each histone combination with specific sets of proteins define different epigenetic states (Li *et al.*, 2002; Baulcombe, 2004).

The regulation of chromatin structure has a key role in the epigenetic control of gene expression. A central mechanism whereby chromatin can be modulated is by recognition by chromatin remodeling proteins of post-translational modifications of highly charged and flexible histone tails, which protrude from the nucleosomes. The core histones can be modified (sometimes reversibly) by acetylation, methylation, phosphorylation, ubiquitination or ADP-ribosylation (Chen and Tian, 2007). Such dynamic and reversible modifications considerably extend the information potential of DNA and provide heritable (although often reversible) mechanisms for the epigenetic control of gene expression during development. (Bowler *et al.*, 2004)

The epigenetic marking of chromatin represents a fundamental regulatory mechanism that impacts on most, if not all, chromatin-templated processes (Dolinoy *et al.*, 2007). The discovery of reorganising chromatin into accessible and inaccessible configurations extends the information of the gene codes to generate many “epigenomes” (Baulcombe, 2004).

**Figure 1** provides a summary of the known chromatin-related factors and processes.

### ***RNA mediated epigenetic systems***

Over the past few years, we have come to appreciate that RNA has many more roles to play than was first thought. RNA participates in diverse roles of RNA silencing, ranging from defense against viruses to the regulation of gene expression and changes in chromosome structure. At least three RNA silencing pathways have been identified in plants. One of these pathways is a post transcriptional gene silencing (PTGS) pathway, known as co-suppression or sense suppression in plants, quelling in *Neurospora* and RNA-mediated genetic interference in *Caenorhabditis elegans*; today it is generally referred to as RNA interference (RNAi) (Baulcomb, 2004). Another PTGS pathway involves RNA-directed RNA polymerase (RdRP). This pathway plays a central role in plants in sequence specificity of RNA degradation. The third pathway of transcriptional gene silencing (TGS) RNA silencing in plants is associated with DNA methylation and suppression of transcription. RNAs produced in the cytoplasm have been shown to be able to feed back and induce epigenetic changes on DNA in plants causing nuclear genes to become methylated (Wolffe and Matzke, 1999). Green plants are unusual in that they have retained the capacity for all three types of silencing, whereas other organisms may have lost one or more of these pathways.

RNAi regulates gene expression through gene silencing using ribonucleoprotein complexes (Vaughn and Martienssen, 2005). In the cytoplasm the RNAi silencing mechanism acts as a natural defence mechanism against viral infection, also known as Virus-induced gene silencing (VIGS). RNAi controls the levels of gene activity through small fragments of RNA that cause degradation of mRNA before it is translated into protein (Baulcomb, 2004). Unlike DNA, RNA normally exists only in the single-stranded form in a cell, thus because the genetic material of some viruses is double stranded RNA (dsRNA), cells treat the presence of dsRNA as a sign of

viral infection. Other dsRNAs that are also interpreted as of viral origin are viral replication intermediates or secondary-structure features of single-stranded viral RNA (Baulcombe, 2004). Post transcriptional gene silencing works by producing short small interfering dsRNA molecules (siRNAs) by cleaving dsDNA, which in turn destroys mRNA with the same sequence of bases preventing multiplication of the invading virus. Another common, although not universal, protein of the RNAi machinery is RNA-dependent RNA polymerase (RdRP), which synthesises dsRNA from ssRNA templates to initiate or amplify the RNAi reaction (Matzke and Birchler, 2005).

### ***Epigenetic systems of importance to forestry***

Epigenetic phenomena are ubiquitous in the biological world. Equivalent processes in different species follow equivalent epigenetic regulation using systems and proteins belonging to equivalent groups. Some of the known processes that occur in trees are highlighted.

Higher plants are characterised by a complex life cycle consisting of alternating haploid and diploid generations. The sporophyte, the diploid life form, supports meiosis that produces haploid male and female spores and initiates the gametophytic generation. Gametogenesis and subsequent fertilisation take place when gametophytic and sporophytic structures interact. The product of fertilisation, the seed, contains the zygote that develops into the embryo and is supported by the endosperm, also a product of fertilization. Finally, the embryo grows into a mature plant. During the entire life cycle of a plant, a wide variety of developmental decisions are carried out by selective activation or repression of different sets of genes. Major plant developmental events are connected by epigenetic actions involved with the alteration of gene expression by the modification of chromatin organisation and the activities of small RNA molecules (Li *et al.*, 2002).

Regulation of forest tree flowering is of considerable interest to tree breeders. Prolonged juvenility of trees has greatly limited tree domestication (Brunner *et al.*, 2004). Most tree

breeding programmes are only in their fourth breeding cycle. Decreasing the juvenile period could advance breeding programmes faster. In contrast, for production plantations the prevention or delay of flowering is highly desirable (Strauss *et al.*, 1995).

During the extended life spans of trees to attain great size and complexity, they exhibit a complex array of developmental phases. The most obvious phase is the change from juvenility to maturity when trees reach the age of floral onset. During the period of early development vegetative growth occurs involving the growth of the embryo and maturation of the plant. This period is controlled by the *EARLY FLOWERING (EMF)* genes that repress flower formation (Sung *et al.*, 1992; Kinoshita *et al.*, 2001). Plants that are defective in the *EMF1* or *EMF2* gene skip vegetative growth and flower upon germination (Sung *et al.*, 1992). The *DAL1* gene, a MADS-box gene, has been identified as a candidate gene for the regulation of the transition of juvenility to maturity in Norway spruce (Carsbecker *et al.*, 2004). Transition to maturity has also been associated with changes in the degree of DNA methylation and polyamine concentration (Fraga *et al.*, 2002). DNA methylation is higher in adult meristems than in juvenile and juvenile-like meristems (Bitonti *et al.*, 2002). In *Pinus radiata* juvenile individuals (without reproductive ability) were characterised by a degree of DNA methylation of 30–35%, while mature trees (with reproductive ability) had 60% 5-methylcytosine (Fraga *et al.*, 2002). The increase in DNA methylation levels takes place just after the phase change (after 5–8 years of growth) (Fraga *et al.*, 2002). Reinvigoration can be induced by serial grafting or pruning and a reduction of 35% in 5-methylcytosine content can be reached after the fourth serial graft (Valledor *et al.*, 2007).

Most tree characteristics are only expressed definitively once trees become mature. Ageing always implies the loss of morphogenic potentials of some characteristics such as rooting ability, which is dependent upon a juvenile-specific ability. This loss in ability drastically reduces the potential for clonal multiplication for production plantations. In *Pinus radiata* the accumulation of

an AAA-ATPase gene transcript has been confirmed with tree ageing and may play a role in the loss of morphogenic potential (Diego *et al.*, 2004).

Some of the chromatin changes, referred to as imprinting, occur during meiosis producing an egg and polar nuclei with maternal (m) epigender and sperm with paternal (p) epigender. The maternal and paternal quality of a genome is determined by these epigenetic marks on its imprinted alleles, but the extent to which the genome's epigender is reflected in a gene expression pattern or a developmental phenotype further depends on the cellular environment (Spielman *et al.*, 2001). Angiosperm zygotes, which include many of our forestry species, are apparently insensitive to imprinting; however the endosperm is sensitive to the balance of parental genomes. Endosperm imprinting is usually due to the differences in epigenetic marks, DNA and histone methylation, on alleles in the central and sperm cells (Huh *et al.*, 2008).

Both interspecific hybridization and polyploid formation are practiced in various tree breeding programmes. Epigenetic changes have been shown to occur following both these processes (Brunner *et al.*, 2004). Each species has an endosperm balance number (EBN) that reflects effective ploidy and it is EBNs rather than chromosome sets *per se* that must be in a 2m:1p ratio for successful endosperm development (Johnson *et al.*, 1980). The EBN system reflects the existence of imprinted genes that either promote or inhibit the acquisition of resources, thus a species' EBN reflects how effective the paternally transmitted genome can extract resources for offspring and the maternally transmitted genome can inhibit resource transfer. When EBN ratios are manipulated by changing the ploidy of polar nuclei or sperm, incompatible species would set seed (Johnston and Hanneman, 1982) permitting crosses between species with otherwise incompatible EBNs (Spielman *et al.*, 2001). The impact of epigender is illustrated by interspecific crosses that behave as though they have different levels of ploidy (Spielman *et al.*, 2001). In new allopolyploids, imbalances in the interaction between different genomes could lead to altered gene expression. One of the best-studied epigenetic phenomena in polyploids is

nucleolar dominance: the transcriptional silencing of one set of progenitor rRNA genes in interspecific hybrids (Osborn *et al*, 2003).

## Discussion

It is suggested that the ramifications of the ability of plants to recognise and interpret environmental cues and adjust gene expression accordingly is still largely unknown and unappreciated. Whereas DNA sequences are studied with relative ease, epigenetic processes pose many problems. However, it has been recognised that epigenetic molecular phenomena are capable of altering the temporal, spatial, and abundance patterns of gene expression, which may have morphological, physiological, and ecological consequences, and are heritable across generations, suggesting they are important in evolution. Direct evidence of the adaptive role of epigenetic responses is still scarce and our knowledge of the evolutionary implications at different levels within individuals, or across generations, is limited.

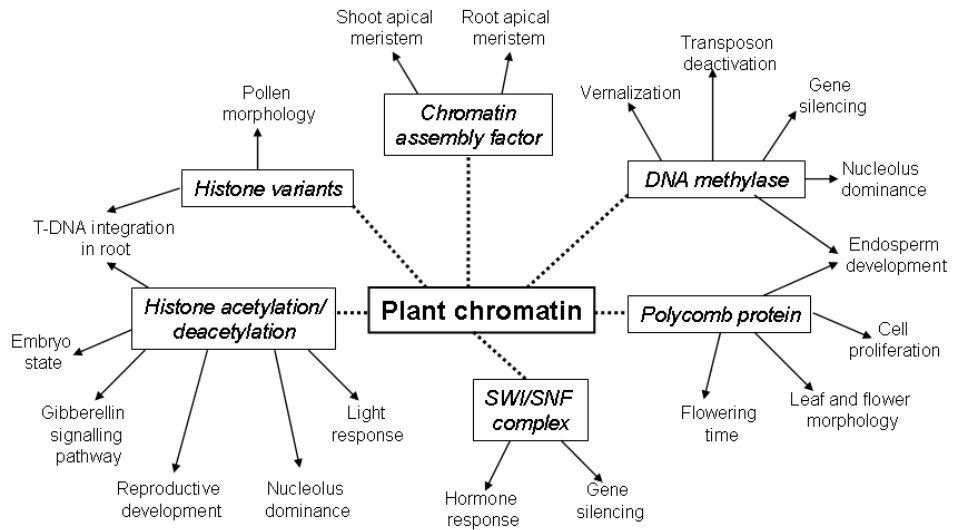
It is expected that a better understanding of these processes will play a major role in breeding practices. In forestry, where clonal propagation, interspecific hybridization and polyploidy formation are practiced, an understanding of genetic switches could open doors to a variety of new applications of biotechnology.

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**Figure 1** Flow diagram of plant chromatin-related factors and processes. Developmental processes are unboxed; Chromatin related factors are boxed and in italics and solid arrows connect chromatin-related factors with their respective developmental processes (Extracted from Li *et al.*, 2002).