

Review

Chromatin and *Arabidopsis* root development

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ABSTRACT

During development cells transit through different states as they pass from stem cell to terminally differentiated cell. There is evidence that the transition from one state to another can be accompanied by changes in epigenetic state of genes, which is embodied in chromatin state. Here we give an overview of the changes in chromatin that accompany the regulation of expression and review the evidence for the involvement of such changes during epidermal root development and discuss the roles that these changes play in the differentiation of the cell types involved.

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1. Introduction

Epigenetics has been defined as the “mitotic and meiotic variations in gene expression that cannot be explained by changes in DNA sequence” [1]. In researching the role of these variations in gene expression during development, there are advantages in using a system in which the epigenetic states of the products of mitosis can be assessed. Then changes in chromatin state can be observed as they occur during defined stages in development. The regular cell division patterns and fate map of the *Arabidopsis* root make this organ an ideal system in which to monitor epigenetic changes during development. Not only can changes in chromatin state be imaged at different developmental stages, but the reprogramming

of these epigenetic states can be observed as cells alter their fates, a phenomenon which occurs infrequently but regularly during plant ontogeny. Furthermore the developmental defects that result from the modification of epigenetic state in mutant backgrounds can identify the genes that are involved in epigenetic regulation and precisely define their roles in development.

2. A general model of development

2.1. Cell state changes occur during development

Cells transit from one state to another during development. For example, a stem cell may give rise to a cell in a “non-stem cell state”. In *Arabidopsis* roots the quiescent centre is surrounded by a population of initial cells that act as stem cells for each of the different tissue systems of the root [2]. For example, a ring of 16 stem cells divides to give rise to the epidermis and lateral root cap. Daughter

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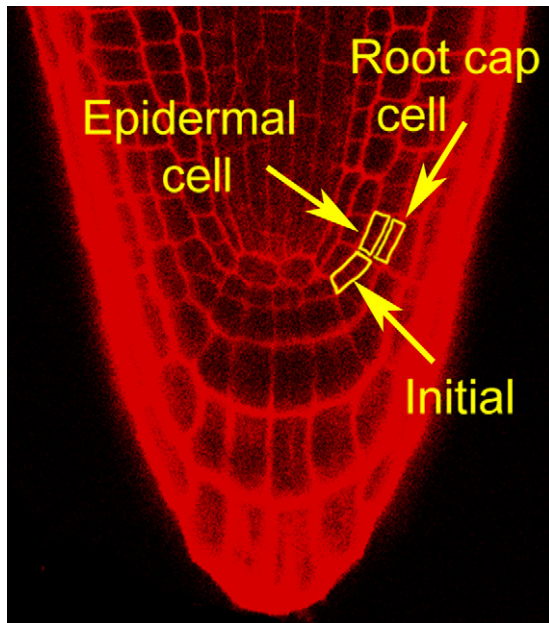


Fig. 1. Epidermal stem cells in the root. The stem cell (initial cell) divides to produce two daughter cells. The daughter cells on the inside develop as an epidermal cell and the outside daughter cell develops as a root cap cell.

cells resulting from the division of an epidermal stem cell assume either epidermal or root cap cell fate, depending on their relative position—cells on the outside assume root cap identity and those on the inside develop as epidermal cells (Fig. 1). The change from stem cell to non-stem cell involves a change in cell state since the precise state will by definition be different in epidermal and root cap cells. Since different genes are expressed in these different states, the change will be accompanied by changes in gene expression. For example, there will be genes that are expressed in epidermal stem cells that are not expressed in either epidermal or root cap descendants.

2.2. Gene expression changes when cells change fate during development

Given that gene expression is different in different differentiating cell types, if a cell changes from one cell type to another during development then its gene expression state will change. For example, most cells in the H position develop as root-hair bearing cells [3] (Fig. 2). Occasionally a daughter resulting from the division of an H cell will not be in the H position and it will change fate accordingly and differentiate as a hairless cell (N cell) [4] (Fig. 2A and B). This change in fate is accompanied by a change in gene expression that is in part responsible for moving a cell from one state (H state) to another (N state).

2.3. Changes in gene expression during the changes in cell state are accompanied by changes in epigenetic regulation

The progression through the cell states that constitute a developmental pathway requires changes in gene expression. Since epigenetic changes control gene expression this suggests that the changes in epigenetic state are required for the change from one state to another. There is now good evidence that epigenetic regulation of gene expression, and thus change in chromatin state, is required for root development. The nature of the changes in chromatin state are likely to range from very localised 'cis' changes, such as binding of transcription factors, activators and

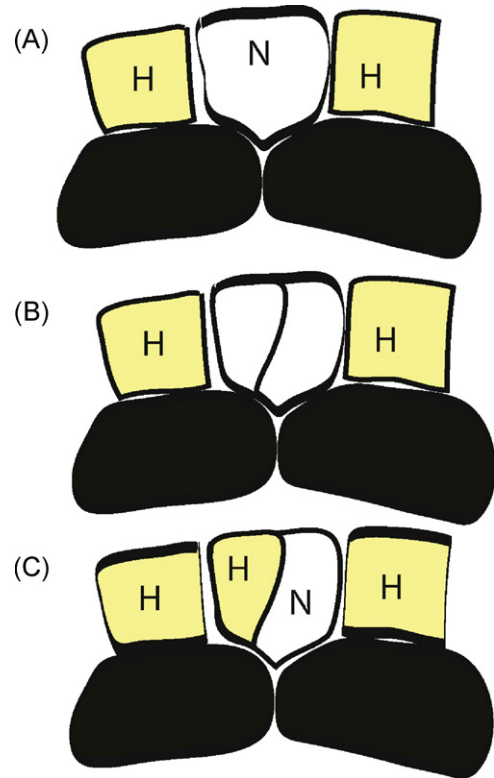


Fig. 2. Organization of cells in the root. The schematic shows a slice through the root. The epidermis is composed of hair forming cells (H) in yellow and hairless epidermal cells (N) in white. Epidermal cells are located outside cortical cells (black). H cells (yellow) are located in the junction between two cortical cells (black). (A) Cellular organization in the root. (B) A cell division occurs in the H cell (white) results in two daughters, one contacts a single cortical cell file (black) and the other contacts a single cortical cell file. (C) After division the daughter cell that contacts two underlying cortical cells develops as an H cell (yellow) and the cell that contacts a single cortical cell file develops as an N cell (white).

repressors, changes in DNA methylation patterns and changes in post-translational modifications of histones and other chromatin proteins, to higher order changes, such as in chromatin packing (heterochromatinization and decondensation), large-scale reorganization of chromosome territories both internally and with respect to one another, and relocation of specific chromatin loops to regions either favourable or unfavourable to transcription. There is evidence for all these different aspects being involved in the regulation of gene expression in various systems, plant, animal and fungal, but so far only some aspects have been studied in detail in plants. In the following section, we give an overview of the involvement chromatin organization in gene expression, and then in the subsequent sections we will focus on what is currently known about epigenetics/chromatin organization in *Arabidopsis* root epidermal development.

3. Chromatin state and gene expression

In the interphase nucleus, chromatin is dynamic, decondensing where genes are active and condensing where they are inactive. Measurements of mobility of specific chromatin regions using GFP markers to specific DNA segments shows generally random constrained movements within a radius of $\sim 0.5\text{--}1.0\ \mu\text{m}$ [5,6]. In eukaryotes, DNA is complexed with histones, 146 bp of DNA being wound around an octamer of the core histones H2A, H2B, H3 and H4, forming a nucleosome with 1.75 turns of DNA [7]. Nucleosomes with interspersed linking DNA form the first level of DNA organi-

zation above the DNA double helix, and have been visualised in EM spread preparations as beads-on-a-string, often described as a 10-nm fibre [8]. Naked B-DNA has a length of 2.9 kb/ μm , and becomes ~ 7 -fold compacted in the 10-nm fibre. Thus a typical gene would be 1–2 μm in length if in the extended B-DNA form, and $\sim 0.2 \mu\text{m}$ if present as a 10-nm fibre. In fact current data, although very limited, suggests that very active genes may be less compacted than the 10-nm fibre (e.g. 3.6-fold compacted for the 75S gene in Balbiani rings and a similar value for active rDNA) [9].

However most chromatin is much more highly compacted than this. Successive nucleosomes interact, mediated by linker (H1) histones and other chromatin proteins such as heterochromatin protein 1 (closest homologue is LHP1/TFL2 in *Arabidopsis*), to form a hierarchy of higher order structures, ranging from a proposed 30-nm fibre, which current evidence suggests is a two-start helical arrangement [10], up through thicker fibres assumed to be formed by long range interactions of the thinner fibres, with compaction up to 500-fold or more. For the most part the details of higher order models are speculative.

Histones are modified post-translationally in a variety of ways, principally in their conformationally flexible N-terminal tails, which are directed out from the nucleosome and are thus the most accessible parts of the proteins. These modifications include phosphorylation, ubiquitination, acetylation, and mono-, di-, and tri-methylation, principally of lysine and arginine residues. The number of possible combinations of the various modifications is extremely large and has been proposed to constitute a histone code for conferring different degrees of transcribability on different chromatin regions [11].

The most easily observable aspect of chromatin state is seen in the distinction between heterochromatin and euchromatin. Heterochromatin was defined by Heitz [12] as chromatin that was easily observable by cytological methods, and this is still the fundamental property used, nowadays by fluorescence optical microscopy using DNA dyes such as DAPI. Heterochromatin is assumed to consist of regular nucleosomal arrays, containing a high proportion of repetitive sequences interspersed with relatively few genes [13]. Heterochromatin has been traditionally assumed to be mostly transcriptionally silent; however emerging evidence is suggesting that even these repetitive sequences are transcribed to some extent, and that resulting small RNAs contribute to heterochromatinization and continuing transcriptional repression [14,15]. In organisms with large genomes, such as cereals, the majority of the genome is in the form of heterochromatin, and this gives a characteristic reticulate appearance to the interphase nuclei when stained with DAPI. *Arabidopsis*, with a very small genome, has comparatively little heterochromatin, and almost all of it is located at or near the centromeres of the chromosomes. These concentrations of condensed chromatin are often called chromocentres. Fransz et al. [16] have estimated that the heterochromatic knob on chromosome 4 of *Arabidopsis* shows a condensation of about 350-fold (1 Mb/ μm), compared to adjacent euchromatin with a condensation of about 60-fold (180 kb/ μm). Euchromatin is thought to contain irregular nucleosome spacing and is relatively gene-rich. Although heterochromatin/euchromatin has been traditionally observed at large-scale (cytological) levels, similar considerations about relative condensation of different regions of chromatin apply right down to the level of single genes or even different parts of an individual locus. Epigenetic marks of silent chromatin are histone hypoacetylation, histone H3 methylation at lysine 9 (H3K9) and cytosine methylation [17]. Euchromatin tends to be enriched in histone hyperacetylation and methylation of lysine 4 in histone H3 (H3K4). *Arabidopsis* shows some differences in the distribution of the various levels of methylation compared to species in other domains [18]. Although H3K4me1,2,3 are associated with

euchromatin in *Arabidopsis* as in many other species, H3K9me3, H3K27me3 and H4K20me2,3 are also associated with euchromatin in *Arabidopsis*, whereas H3K9me1,2 are marks for heterochromatin. *Arabidopsis* heterochromatin has also been found to be enriched in H3K9me1,2, H3K27me1,2 and H4K20me1 [18].

At the highest level, chromatin is partitioned into a number of distinct chromosomes. This is very obvious when chromatin is in its most condensed form during mitosis, and has been known for many years. But even during interphase, each chromosome occupies a distinct region of the nucleus, usually called an interphase chromosome territory. In some species, for example, yeast, *Drosophila* and wheat, chromosomes span the width of the nucleus in rod-like structures with the two arms of each chromosome close to each other and centromeres and telomeres located at opposite poles of the nucleus in a Rabl configuration [19,20]. In *Arabidopsis*, the chromosomes are in a less well-defined organization, with the telomeres located at the nucleolar periphery and the centromeric heterochromatin located at the nuclear periphery. In mammals, the territories usually have a radial distribution, with chromosomes, either centrally or peripherally located in the nucleus, having an irregular territory shape [21]. Mammalian chromosomes carrying more active genes tend to be peripheral, whereas chromosomes with fewer active genes tend to be more central. There is no evidence for any specific arrangement of chromosome territories with respect to each other in *Arabidopsis*, except for the NOR-bearing chromosomes, which are associated with the nucleolus and thus have a tendency to be nearer to each other [22]. The extent to which adjacent interphase chromosome territories overlap is still the subject of active debate. Earlier data suggested that adjacent territories do not overlap. More recent data suggests that there may be significant intermingling of adjacent territories [23,24].

Transcriptional activity has been visualised by the incorporation of BrUTP into nascent transcripts or by immunofluorescence labelling of active RNA polymerases. These experiments in both plants and animals consistently show from a few tens to a few hundreds of bright foci in the nucleoplasm, which are interpreted as transcription sites. Data from wheat [25] and from human culture cells [26] suggests that transcription sites are found throughout the chromosome territories, rather than being located at the periphery of the territories as suggested earlier [27,28]. There are many fewer such sites than the number of genes presumed to be transcribed, and this has led to suggestions that each such site represents a transcription 'factory' where several genes are transcribed [29]. On the other hand, it is difficult to know exactly what the bright sites represent; they may simply be the most active genes, with many other less actively transcribed genes being dispersed through the nucleoplasm but below the detection level of the technique. The BrUTP labelling may also include the transcription of non-genic regions, producing large numbers of rapidly degraded transcripts.

If the labelled transcription sites do indeed represent multi-gene transcription factories, this implies that an active gene must be translocated to a suitable site for transcription. Testing this idea is technically very challenging and involves either high resolution *in situ* labelling, or, better, *in vivo* imaging of specific genes and transcripts. Over the past few years a number of studies have indeed shown specific changes in location of individual genes according to their transcriptional activation. From what is known so far, specific 3D locations of individual genes are correlated with transcriptional repression, rather than transcriptional activation. This has been studied in detail in yeast telomeric silencing [30,31]. Another example is the location of the *Brown* locus in *Drosophila*, where insertion of heterochromatin caused the association with centromeric heterochromatin at a particular developmental stage [32]. Ikaros is a protein necessary for human lymphocyte development, and is located in bodies containing centromeric heterochromatin; *in situ*

labelling has shown that a subset of genes that undergo heritable developmental silencing is specifically recruited to these heterochromatin bodies via *ikaros* in lymphocytes but not in other cell types [33,34]. In plants, it was shown that multiple unlinked transgene copies in a series of transgenic wheat lines were colocalised in interphase cells. However these copies were partially silenced; relieving the transcriptional repression by drug treatment caused the copies to disassociate, suggesting that the colocalising was a consequence of silencing [25,35]. In another study using transgenic wheat, Wegel et al. [36] showed that multiple tandem copies of a transgene (endosperm-expressed glutenin) dispersed into extended loops on developmental activation in the endosperm. The physical extent of the loops was surprisingly large, amounting to several micrometres for an estimated 20 copies making a total of about 100 kb in size; large-scale rearrangements in the genome clearly happen on transcriptional activation.

Thus there is evidence for the involvement of a number of different levels of chromatin organization in the regulation of gene expression, from localised changes in histone properties, to larger scale opening or condensation of chromatin, to specific changes in 3D organization of extended chromatin regions.

4. CAF1 shows a link between chromatin, transcriptional gene silencing and development in *Arabidopsis*

If chromatin state is directly involved in developmental decisions, we should expect factors involved in setting up and modifying chromatin to have phenotypic effects on development. This is indeed the case. One example of this was found in the *fasciata* mutants, *fas1* and *fas2*, which show a fasciated stem, abnormal phyllotaxy and short roots [37]. The gene products have been shown to be constituents of the *Arabidopsis* counterpart of the chromatin assembly factor 1 (CAF1) complex. This complex is known from animal and yeast studies to be involved in assembling histones, in particular H3 and H4, into replicating chromatin, and also in DNA repair; yeast CAF1 mutants are hypersensitive to UV irradiation [38]. CAF1 interacts with replication coupling assembly factor (RCAF), a homologue of *Drosophila* anti-silencing function-1 protein (ASF1). CAF1 has been purified as a complex of three subunits (p150, p60 and p48 in animals [39]; FAS1, FAS2 and MSI1 respectively in *Arabidopsis*). p48/MSI1 is found in a number of different complexes affecting chromatin [40], and its *Arabidopsis* homologue MSI1 is found in complexes with MEDEA (MEA), and with CURLY LEAF in a polycomb group complex [41]. p60 interacts with a histone chaperone [42], and p150 interacts with heterochromatin protein 1 (HP1). FAS1 and FAS2 are necessary for normal development of both the shoot and root apical meristems (SAM and RAM) [43], and are also expressed at a high level in actively dividing cells of all tissues [44]. In the *fas* mutants the expression of *SCARECROW* (*SCR*) in roots and *WUSCHEL* (*WUS*) in shoots is not maintained in the correct spatial pattern, which leads to abnormalities in meristems and the succeeding development patterning. Recent data has shown that CAF1 is necessary to maintain silent, transcriptionally repressed chromatin in *Arabidopsis* [45]. In *fas* mutants, normally silenced loci, such as endogenous transposons and a hypothetical gene located in a heterochromatic knob, as well as a silenced GUS transgene, were derepressed randomly. Transcriptional gene silencing was released in a stochastic way from cell to cell and plant to plant. This suggests that CAF-1 is necessary to ensure the stable propagation of epigenetic states during development. This may involve defective de novo nucleosome assembly during replication, defective segregation of the parental nucleosomes or defective recruitment of silencing factors onto the chromatin.

5. Other links between gene silencing, heterochromatinization and development

Other genes have been identified that show a link between DNA repair, the stability of heterochromatin and development. In two parallel searches for *Arabidopsis* mutants affecting DNA damage repair, a number of mutant alleles of *BRU1* were identified that showed increased sensitivity to genotoxic stress [46]. These mutants showed similar phenotypes to *CAF1* mutants, including stem fasciation and other developmental abnormalities, and were also compromised in the inheritance of transcriptional gene silencing. The mutants also showed increased intrachromosomal homologous recombination. Although genome-wide DNA methylation appeared unaffected, there were clear differences in pericentromeric heterochromatin organization. *BRU1* encodes a novel nuclear protein, with no related genes in the *Arabidopsis* genome, nor any clear similarity to any other known protein, but contains two domains probably involved in protein-protein interactions (one containing tetratricopeptide repeats, the other, leucine-rich repeats), as well as a putative coiled coil domain and a leucine zipper motif, which may interact with DNA [46].

Mutants of *MRE11* also show similar developmental defects to *CAF1* and *BRU1* mutants [47]. *MRE11* is part of a complex with Rad50 and Xrs2/Nbs1 in yeast, which functions in DNA repair. In the *Arabidopsis* *MRE11* mutants telomere length was increased, showing a role in telomere control. Another example of similar developmental defects is provided by *NRP1* and *NRP2*. These genes are distantly related to *NAP1* in yeast and animals, and encode putative histone H2A and H2B chaperones [48]. An *nrp1/nrp2* double mutant showed impaired root growth and disorganized cell patterning, which were attributed to defects in the maintenance of epigenetic chromatin states [48]. The expression of about 100 genes, including *GL2*, was altered. Finally reduction in the level of the *Arabidopsis* orthologue of the SMC2 subunit of the condensin complex also had a similar phenotypic effect on root development. In *Arabidopsis* there are two homologous *SMC2* genes, *AtCAP-E1* and *AtCAP-E2*, which show functional redundancy. Double knockouts of these two genes are embryo-lethal, but RNAi knockdown of both leads to plants showing meristem disorganization and fasciation [49]. Condensin is required in mitotic chromatin condensation and sister chromatid resolution.

6. Chromatin state at *GLABRA2* is important in the trichoblast/atrichoblast cell fate decision

Thus there is a clear link between factors which affect chromatin organization, replication and repair and development. A few studies have examined this using a very clearly defined cell fate choice in the *Arabidopsis* root epidermis—that of trichoblast versus atrichoblast. These two cell types are easily observable in primary *Arabidopsis* roots. As described above and in Fig. 2, the choice between the two fates is determined by the number of underlying cortical cell files; trichoblasts or root-hair forming cells overlay two cortical cell files, whereas atrichoblasts or non-root-hair forming cells overlay only one cortical cell file. Thus there is a single cell fate choice that is made on the basis of a simple positional cue. A receptor-like kinase, *SCRAMBLED* (*SCM*), has recently been identified which is genetically upstream of all the other known controlling factors needed for this fate decision, and may be involved in sensing the positional cues [50]. The transcription factor *GLABRA2* (*GL2*) is known to be a crucial controlling factor in this developmental choice, and negatively regulates root-hair formation; thus *GL2* expression is high in atrichoblasts and low in trichoblasts. Current evidence suggests that a complex containing TTG1, *GLABRA3*

(GL3), enhancer of GL3 (EGL3) and WEREWOLF (WER) binds to and activates the *GL2* promoter in atrichoblasts [51]. CAPRICE (CPC) negatively regulates *GL2*, probably by inactivating WER function, and *CPC* expression is also controlled by this complex [52,53].

Costa and Shaw [54] used 3D fluorescence *in situ* hybridisation in well-preserved root tissue to examine the genomic region of *GL2* directly, using as a probe a BAC that spanned *GL2*. Somewhat surprisingly, the result of this experiment was that the BAC probe labelled strongly in atrichoblasts, but significantly more weakly in trichoblasts. The difference in labelling was restricted to this region, as probes from flanking BACs upstream or downstream labelled both cell types equally. The simplest interpretation of this result is that the chromatin region around *GL2* is more easily accessible to the probe in the atrichoblasts, where *GL2* is expressed, than in the trichoblasts, where it is not. *CPC* negatively regulates *GL2*; thus in *cpc* mutants, *GL2* is expressed in all epidermal cells and all epidermal cells develop into non-hair cells. In a *cpc* mutant, *in situ* labelling with the *GL2* BAC probe indeed labelled all the cells, as would be predicted if an accessible chromatin state was associated with *GL2* expression and a less accessible state with *GL2* repression. On the other hand WER positively regulates *GL2*. In a *wer* mutant, *GL2* is not expressed, and all epidermal cells develop into hair cells. *In situ* labelling with the *GL2* BAC probe in a *wer* mutant, however showed equally strong labelling in all cell files. This shows that accessibility and by implication chromatin state in this region is not simply a consequence of transcriptional activation, but may be set up before transcription is initiated, and remain so when transcription cannot take place because of lack of an essential factor. One interpretation of these results is that WER and *CPC* are required for the inaccessible *GL2* chromatin state. In the *CAF1* mutant, *fas2*, specification of the alternate fates is lost, leading to the random loss of position-dependent *GL2* expression. In parallel with this, the difference between the different epidermal cell files in labelling intensity with the *GL2 in situ* probe is randomly lost, some cells labelling and others not. This, again, strongly suggests a link between chromatin state and this developmental cell fate choice.

In 'T-clones', in which an unusual transverse division creates two cell files from a single file, the two files must have different positional cues from the underlying cortical cell files. Berger et al. [4] showed that in these T-clones the cells adopt the fate specified by the positional cues, rather than the fate inherited from the ancestral cells. Costa and Shaw [54] showed that, in agreement with this, the chromatin state as judged by *in situ* labelling is altered within a single cell cycle. Interestingly, they showed that all cells, whether trichoblast or atrichoblast, label strongly during mitosis, and that the differential labelling is re-established during G1. One interpretation of this is that the chromatin state is reset during each cell cycle in response to the cell's position, and that the epigenetic 'memory' of the previous state may be erased each cell cycle.

What could the different chromatin states at *GL2* involve? Clearly any or all of the factors in chromatin organization described above may be involved. At the lowest level, there is much data now about changes in histone post-translational modifications in gene expression. Histone acetylation is often associated with chromatin opening and up-regulation of expression. Xu et al. [55] tested the idea that histone acetylation might be involved in the regulation of root development. They initially showed that treatment of growing *Arabidopsis* roots with trichostatin A (TSA), an inhibitor of histone deacetylases, caused a striking increase in the number of root hairs in *Arabidopsis* roots. This effect was reversible and concentration dependent, and altered the expression levels and cellular patterning of the key controlling genes *GL2*, *CPC* and *WER*. The authors then screened all the available mutants for the 16 known or putative histone deacetylase genes, and showed that mutants in 5 genes had an effect on root-hair numbers. Of these, three had a clear effect

on root-hair patterning (as opposed to a general increase in root-hair numbers): *HDA17*, *HDA18* and *HDT3*, although only *HDA18* was further characterised. This study clearly shows that changes in histone acetylation are an important factor in the alternate chromatin states.

Other histone modifications that may be involved in chromatin state include histone methylation. In a recent paper Caro et al. [56] identified a novel protein, GEM that is involved in interaction with the DNA replication machinery and in the trichoblast/atrichoblast cell fate determination. The authors observed that *Arabidopsis* plants overexpressing *CDT1*, a component of the complex that controls initiation of replication, have increased expression of *GL2*. They then carried a yeast two-hybrid screen to identify *GEM* (*GL2* expression modulator) as an interacting factor of *CDT1*. Plants either reduced in *GEM* expression (tDNA insertion mutants) or overexpressing *GEM* showed that the spatial patterning of *GL2* expression and trichoblasts/atrichoblasts was altered. The frequency of longitudinal, anti-clinal cell divisions was increased, giving a high frequency of T-clones. *CPC* expression was altered in a similar way to *GL2* being greatly increased in a *gem* mutant, but *TTG1*, *GL3* and *EGL3* were not consistently affected. *GEM* interacted with *TTG1* by yeast two-hybrid assay, and ChIP analysis showed that *GEM* is specifically bound to the *GL2* and *CPC* promoters. Both promoters contained increased levels of H3K9me3, a mark of euchromatin in *Arabidopsis*, in *gem-1* mutant plants, but decreased levels of H3K9me2, a mark of silent or heterochromatin in *Arabidopsis*. Caro et al. [56] concluded that *GEM* is part of a complex containing *TTG1* that specifically represses *GL2* and *CPC* to give rise to trichoblast cell fate, and that part of this action is to regulate histone H3K9 methylation at the *GL2* and *CPC* promoters. Caro et al. [56] also showed that in synchronised cells, the methylation patterns were altered during the cell cycle. This supports the conclusion from the observations of Costa and Shaw [54] that the cell fate is reset each cell cycle.

7. Conclusion

There is now much evidence that epigenetic changes in chromatin state are important in the regulation of gene expression that controls development. This evidence has been obtained in a variety of different organisms including *Arabidopsis*, but as yet we lack a complete understanding of the mechanism of epigenetic regulation in any single developmental system, and current speculative models are largely based on analogy. As we gain more knowledge of the correlation between epigenetic and organizational changes in chromatin and gene expression, we are faced with the problem of how to interpret this data; to what extent are the observed changes in chromatin state a cause of changes in expression and to what extent a consequence? This type of question is extremely hard to answer, since gene expression is certainly the result of many interacting factors, including biochemical interactions of transcription and enhancer factors, biochemical alterations in chromatin proteins and in DNA itself, as well as conformational and organizational changes in chromatin; changing any of these factors will in turn feed back to change the others. Therefore identifying which, if any, constitute 'master regulators' may turn out to be a meaningless question in the context of the complex interacting system. A detailed interpretation of the functional consequences of chromatin state changes will also require a better understanding of how gene expression itself is organized. For example, in the context of the still controversial idea that genes are transcribed in transcription factories [57,58], many of the factors that are now regarded as transcription factors and enhancers, would be interpreted as factors that modulate the location of their target genes,

helping their recruitment to the correct part of the nucleus for transcription.

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