Chromatin-Remodeling and Memory Factors. New Regulators of Plant Development

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The establishment and heritable maintenance of specific epigenetic states that lead to differential gene expression are crucial for cell differentiation and development. Over the past few years, it has become apparent that epigenetic control of transcription is mediated through specific states of the chromatin structure. Therefore, changes in the chromatin structure associated with activation and silencing of gene expression are of paramount importance during development. Here, we discuss recent findings on plant proteins involved in modifying, remodeling, or maintenance of chromatin structures. Many of the proteins affect normal development when their function is lost.

Cell differentiation and development are controlled through temporal and spatial activation and silencing of specific genes. Once established, cell type-specific pattern of gene expression must be stable over many cell generations and long after inductive developmental signals have disappeared. Although chromatin assembly is still largely unexplored, genetic and biochemical studies in yeast (*Saccharomyces cerevisiae*), fruitfly (*Drosophila melanogaster*), and mammals have already revealed that changes in expression patterns require remodeling of the chromatin structure at promoters and other regulatory regions of DNA (for recent reviews, see Kingston and Narlikar, 1999; Aalfs and Kingston, 2000; Urnov and Wolffe, 2001). Thus, packaging of DNA into nucleosomes and higher order structures represents an obstacle to regulatory DNA-binding proteins (e.g. see Niu et al., 1996) and RNA polymerases (Williamson and Felsenfeld, 1978), thereby perhaps imposing a default state in which genetic information is repressed. Chromatin remodeling alters this basal state by promoting either an "open" (activation of transcription) or a "closed" (repression of transcription) chromatin configuration. The propagation of specific transcriptional states to daughter cells through mitosis or even meiosis invokes the stable inheritance of chromatin structures.

Association of specific chromosomal proteins (such as the Polycomb group [PcG] proteins), posttranslational histone modifications, and DNA methylation (Wolffe and Matzke, 1999) are good candidates for epigenetic mechanisms that control the memory of chromatin states. Mutations in the function of "chromatin memory factors" strongly impair animal development and cell differentiation (Muller and Leutz, 2001). Plant developmental mutants for genes encoding chromatin factors have been identified only recently, perhaps as a consequence of their more complex phenotypes. In this *Update* we will discuss the potential roles of chromatin assembly, remodeling, and memory factors in controlling development and differentiation (for an overview of characterized plant chromatin modifiers, see Table I and Fig. 1). Several plant epigenetic processes also related to chromatin function such as paramutations (Chandler et al., 2000), nucleolar dominance (Pikaard, 2000a, 2000b), and transcriptional transgene silencing (Meyer, 2000; Mittelsten Scheid and Paszkowski, 2000) have been reviewed elsewhere and will not be considered here.

CHROMATIN ASSEMBLY PROTEINS

During DNA replication, new nucleosomes must be assembled onto the daughter DNA strands to propagate or modify chromatin configurations. Several proteins facilitate packaging of DNA into chromatin, but only chromatin assembly factor 1 (CAF-1) has been analyzed in more detail in yeast, animals, and plants (Smith and Stillman, 1989; Kaya et al., 2001). CAF-1 is a trimeric complex consisting of chromatin assembly complex (CAC) 1, CAC2, and CAC3 in yeast; p150, p60, and p48 in humans; and FAS1, FAS2, and MSI1 in Arabidopsis (Smith and Stillman, 1989; Kaya et al., 2001; Fig. 2). The two larger subunits appear to be specific for the CAF-1 complex and are not well conserved between mammals, Arabidopsis, and fruitfly. They are encoded by single genes in Arabidopsis. In contrast, CAC3/p48/MSI1 belongs to a family of closely related WD40 proteins in many eukaryotes. The Arabidopsis genome contains five genes for CAC3-like proteins, designated *AtMSI1* to *5*

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Table I. *Chromatin modifiers involved in Arabidopsis development*

Process Factors	Proposed Function	Reference
Chromatin assembly		
FAS1, FAS2	Organization of shoot and root apical meristems during postembryonic development	Kaya et al. (2001)
Histone deacetylase		
HDA1	*HDA1* antisense provoke several developmental abnormalities, including homeotic changes, male and female sterility, and delay of flowering	Tian and Chen (2001)
HDA3	Seed development	Wu et al. (2000b)
ATP-dependent chromatin remodeling		
PKL	Repression of meristematic- and embryonic-specific genes	Ogas et al. (1999); Eshed et al. (1999)
DDM1	Maintenance of DNA methylation	Vongs et al. (1993)
SYD	LFY-dependent repressor of the transition from inflorescence to flower meristem	Wagner and Meyerowitz (2002)
Histone methyltransferase		
KYP	KYP mutation affect DNA methylation at CpNpGp sites; SET-domain protein	Jackson et al. (2002)
Polycomb		
CLF	Repressor of *AGAMOUS*; SET-domain protein	Goodrich et al. (1997)
MEA	Repressor of endosperm development before fertilization; SET-domain protein	Grossniklaus et al. (1998)
EMF2	Repression of *AGAMOUS* in vegetative tissues	Yoshida et al. (2001)
FIS2	Repressor of endosperm development before fertilization	Luo et al. (1999)
FIE	Repressor of endosperm development before fertilization	Ohad et al. (1999)
VRN2	Maintaining stable repression of flowering locus C (*FLC*)	Gendall et al. (2001)
Other coactivators or corepressors		
LUG	Repression of AGAMOUS expression in whorls 1 and 2	Conner and Liu (2000)
Additional components		
LHP1	Silencing genes involved in the transition to flowering and other developmental processes	Gaudin et al. (2001)

(Ach et al., 1997; Kenzior and Folk, 1998). Homologs have also been reported from other plant species (Ach et al., 1997; Delichere et al., 1999; Rossi et al., 2001). CAC3/p48/MSI1 proteins can bind histones and associate with several other proteins, including histone deacetylases (HDACs), retinoblastoma (Rb)-like proteins and ATP-dependent chromatin-remodeling machines (CRMs; Ridgway and Almouzni, 2000), although these interactions are not well understood. CAF-1 is associated with newly synthesized histones H3 and H4 acetylated at specific sites (deposition-related acetylation sites), which is distinct from histone acetylation associated with gene expression in euchromatin (Sobel et al., 1995). The complex facilitates deposition of nucleosomes on newly synthesized DNA during replication and nucleotide excision repair in vitro (Smith and Stillman, 1989; Gaillard et al., 1996), but the mechanisms are still unknown. It is possible that CAF-1 is recruited to DNA replication forks by proliferating cell nuclear antigen, a homotrimeric protein that forms a sliding clamp around DNA in the proximity of DNA polymerase (Shibahara and Stillman, 1999; Moggs et al., 2000).

In the Arabidopsis fasciated mutants *fas1* and *fas2* (Reinholz, 1966; Leyser and Furner, 1992), the corresponding *CAC1* and *CAC2* genes are disrupted (Kaya et al., 2001). Both mutants show a spectrum of developmental changes caused by the enlarged shoot apical meristem (SAM), including altered phyllotaxy, leaf shape, root growth, and flower organ number. The domain of *WUSCHEL* expression, usually confined to the SAM quiescent center, is expanded in *fas1* and *fas2*. The expression of *SCARECROW*, which directs radial patterning in roots and stems, is also affected (Kaya et al., 2001). Thus, CAF-1 appears to be important for the maintenance of plant developmental gene expression patterns. A likely role of CAF-1 in development has also been demonstrated in *Xenopus laevis* oocytes (Quivy et al., 2001) and mammalian cells (Tchenio et al., 2001). In yeast, CAF-1 is required for stable inheritance of gene silencing (Kaufman et al., 1997; Monson et al., 1997), suggesting that the role of CAF-1 in the epigenetic control of gene expression has been conserved between yeast and mammals.

CHROMATIN REMODELING BY HISTONE ACETYLATION. AN OLD MODIFICATION REVISITED

Early studies in plants and animals demonstrated the existence of posttranslational modifications (mainly acetylation, methylation, and phosphorylation) of the amino-terminal tails of the histones. However, their role in chromatin organization and function has remained elusive until recently. In plants, histone H4 can be acetylated at Lys 5, 8, 12, 16, and 20, whereas histone H3 is acetylated at positions 9, 14, 18, and 23 (Waterborg, 1990, 1992). Histone

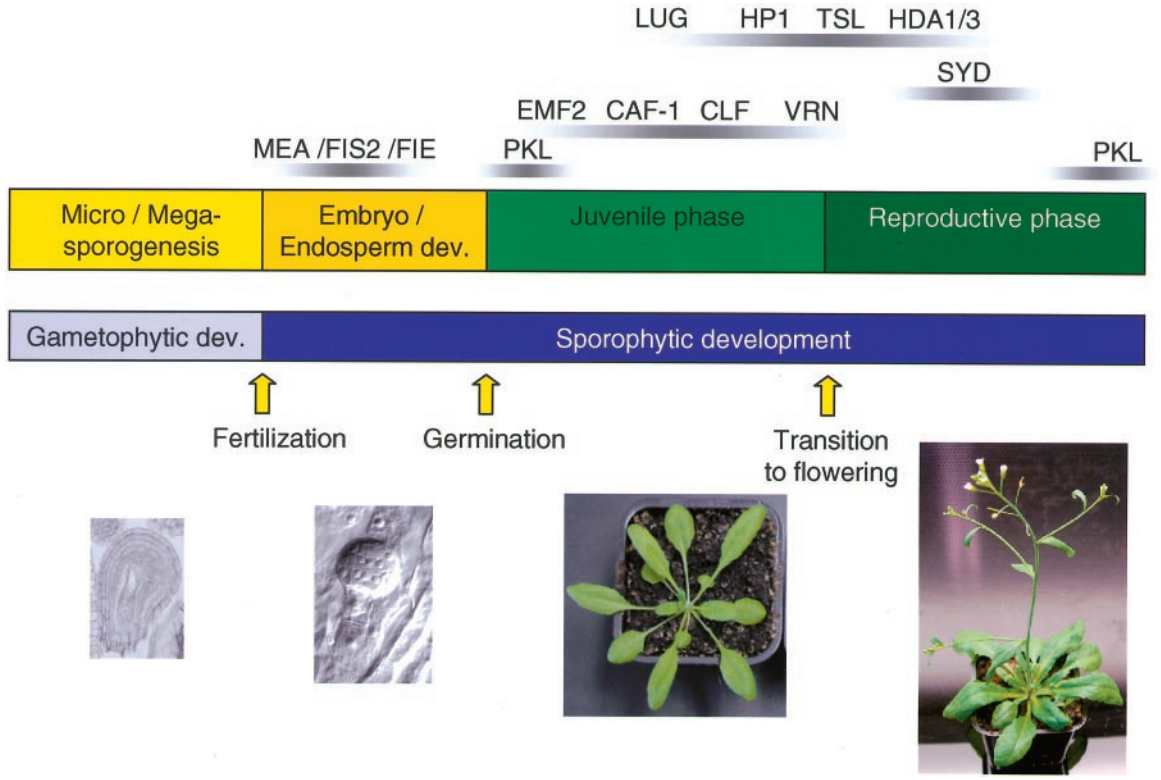

Figure 1. Summary of Arabidopsis chromatin components that control development. Time windows of known functions are schematically represented below the protein names (for details, see text).

H2A and H2B are also acetylated but to a lower extent. Animal and yeast histone acetyltransferases (HAT) interact with transcription activators, indicating a positive role in transcription for this posttranslational modification (Roth et al., 2001). HDACs conversely interact with transcriptional repressors, suggesting that deacetylation is involved in repression and silencing (Courey and Jia, 2001). The interplay between HATs and HDACs results in a dynamic equilibrium between acetylation and deacetylation at promoters and regulatory regions that affect chromatin structure and transcription. Thus, promoter-specific modulation in core histone acetylation concomitant with changes in transcription activity has been demonstrated for several yeast and human genes and in one plant gene (e.g. Kuo et al., 1998; Krebs et al., 1999; Chua et al., 2001). How does histone acetylation or deacetylation change chromatin structure to promote transcription activation or repression? The histone H4 N-terminal domain can make inter-nucleosome contacts between adjacent octamers (Luger et al., 1997), consistent with the observation that chromatin fibers reconstituted with hypoacetylated histones are more compact than those reconstituted with hyperacetylated histones (Tse et al., 1998; Fig. 3). On the other hand, acetylation together with other posttranslational histone modifications (see below) may constitute a "histone code" that can be interpreted by chromatin-remodeling machines and transcription factors (Strahl and Allis, 2000; Turner, 2000; Jenuwein and Allis, 2001). Thus, several chromatin modifiers contain protein-protein recognition domains involved in interactions with acetylated or methylated histones.

How are histone acetylation patterns inherited during development? During DNA replication, histone octamers of the parental DNA molecule segregate approximately equally on the daughter DNA molecules (Fig. 3). New octamers required for chromatin packaging are assembled from de novo synthesized histones. Histones with specific acetylation patterns could therefore be maintained throughout mitosis (Perry et al., 1993) and may serve as templates for HATs and HADCs to modify the newly assembled octamers accordingly. This would explain why alteration of acetylation status induced by trychostatin A, an inhibitor of HDACs, can be epigenetically propagated after the drug has been removed (Ekwall et al., 1997). It should be noted, however that, at present, no evidence exists for epigenetic inheritance of induced changes in acetylation patterns in plants.

HATS AND HDACS ARE MODIFIERS OF HISTONE ACETYLATION AND TRANSCRIPTION ACTIVITY

HATs have been classified into two categories depending on their subcellular distribution (Roth et al.,

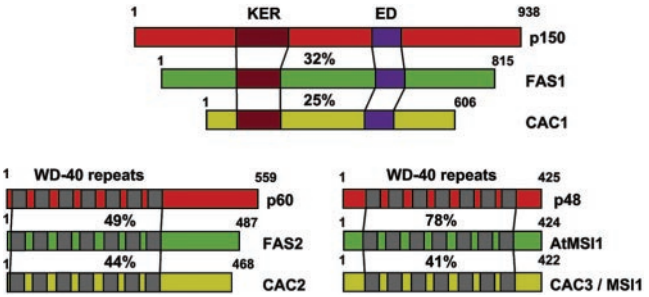

Figure 2. CAF-1 subunit proteins are conserved in different organisms. CAF-1 (CAC) subunits are shown from humans (red), Arabidopsis (green), and yeast (yellow). Numbers represent amino acid positions and percentage of similarity between human and Arabidopsis, and Arabidopsis and yeast, respectively. CAF-1 subunits were termed CAC1, CAC2, and CAC3 in yeast. The corresponding subunits in human and Arabidopsis are p150/FAS1, p60/FAS2, and p48/AtMSI1. CAC1/p150/FAS1 contains a highly charged KER domain, which consists mainly of Lys, Glu, and Arg residues, and an ED region, which consists of Glu and Asp residues. The KER domain is believed to form a coiled-coil structure, and the acidic ED region could interact with basic histones (Kaya et al., 2001). CAC2/p60/FAS2 and CAC3/p48/AtMSI1 contain seven WD40 repeats, which are likely to mediate protein-protein interactions. For MSI-like proteins, direct interaction with histones has been shown (e.g. Rossi et al., 2001).

2001). Type B HATs are cytoplasmic complexes involved in acetylation of histone H4 at positions 5 and 12 before its incorporation into nucleosomes. Maize (*Zea mays*) HAT type B is a heterodimeric complex (Lusser et al., 1999). The gene for the 50-kD enzymatic subunit is homologous to yeast *HAT1*. The 45-kD subunit is immunologically related to the mammalian Rb-associated protein (RbAp) and yeast/plant MSI1 proteins (Ach et al., 1997), which are also found in other chromatin-remodeling complexes (see above).

HAT type A are nuclear proteins that fall into four classes with different specificities: the GCN5, CBP/p300, TAFII 250, and MYST family of proteins (Marmorstein, 2001). Open reading frames with homology to members of all of these families are present in the Arabidopsis genome. HAT activity has been demonstrated for Arabidopsis p300 and GCN5 homologs, but their functions are still unknown (Bordoli et al., 2001; Stockinger et al., 2001). HATs of the CBP/p300 and GCN5 families are recruited to promoters by specific transcription factors in animals and yeast. Interestingly, Arabidopsis CBF1, a transcription activator involved in cold-regulated gene expression, can interact with GCN5 in vitro (Stockinger et al., 2001), suggesting that similar recruitment of HATs to promoter regions also occurs in plants.

The situation for HDACs is equally complex. Four enzyme types have been identified in eukaryotes. Classes I and II compose proteins that are homologous to yeast Rpd3 and HDA1, respectively. Class III proteins share similarity with yeast Sir2 (Khochbin et al., 2001), which unlike class I and II enzymes has NAD^+-dependent HDAC activity and ADP-ribosyltransferase activity in vitro. Members of these three classes are present in all eukaryotes including plants. A fourth class of HDACs, whose founding member is the maize HD2 protein (Lusser et al., 1997), has been identified only in plants.

At least five class I HDACs are present in the Arabidopsis genome, but functional information is available for only two of them. HDA19 (following the nomenclature of the Chromo database at http://www.chromdb.org; also termed AtHD1 or AtRPD3A) is expressed at high levels in leaves, stems, flowers, and young siliques. An HDA19 fusion protein can repress transcription when tethered to a promoter through a DNA-binding domain (Wu et al., 2000a), providing direct evidence that HDACs are involved in transcriptional repression in plants. Histone H4 is hyperacetylated in Arabidopsis *HDA19* antisense RNA mutants, and plants have developmental abnormalities, including early senescence, suppression of apical dominance, homeotic changes, male and female sterility, and delay of flowering (Tian and Chen, 2001). In contrast, mutations in the *HDA6* gene (also called *AtRPD3B*) increase expression of *GUS* and *HPH* transgenes without affecting Arabidopsis development, suggesting that HDA6 plays a more specific role in gene silencing (Murfett et al., 2001).

Maize HD2 is a plant-specific HDAC not related to other known HDAC enzymes (Lusser et al., 1997). The maize protein is localized in the nucleolus, suggesting a possible role in the regulation of rRNA genes. Four Arabidopsis gene products (HDA4, HDA3, HDA11, and HDA13) share high sequence similarity with the maize HD2 protein (Wu et al., 2000b). Antisense HDA3 transgenic plants resulted in plants with stunted siliques that contain a high number of aborted seeds, suggesting that HDA3 is involved in embryo development.

HDACs are recruited to specific loci by interacting directly or indirectly with DNA-binding proteins that regulate development. For example, *Drosophila melanogaster* Groucho is a transcriptional corepressor that recruits Rpd3 (Chen et al., 1999) and interacts with transcription factors such as Engrailed or Dorsal. Groucho has three functionally distinct domains: a Gln-rich region, a Gly/Pro-rich region, and six WD40-repeats at the C terminus. Yeast TUP1 repressor (Wu et al., 2001) and LEUNIG (LUG), a repressor of AGAMOUS (AG) expression in Arabidopsis, have striking similarity with Groucho (Conner and Liu, 2000). *AG* is a class C floral homeotic MADS box gene, which is expressed in whorls 3 and 4 of developing flowers (Yanofsky et al., 1990). *AG* mRNA is ectopically expressed in *lug* mutants, resulting in homeotic transformations of floral organ identity. Other mutant phenotypes are independent of *AG*, suggesting that LUG is controlling several target genes during Arabidopsis development. Whether LUG interacts with HDACs is currently unknown.

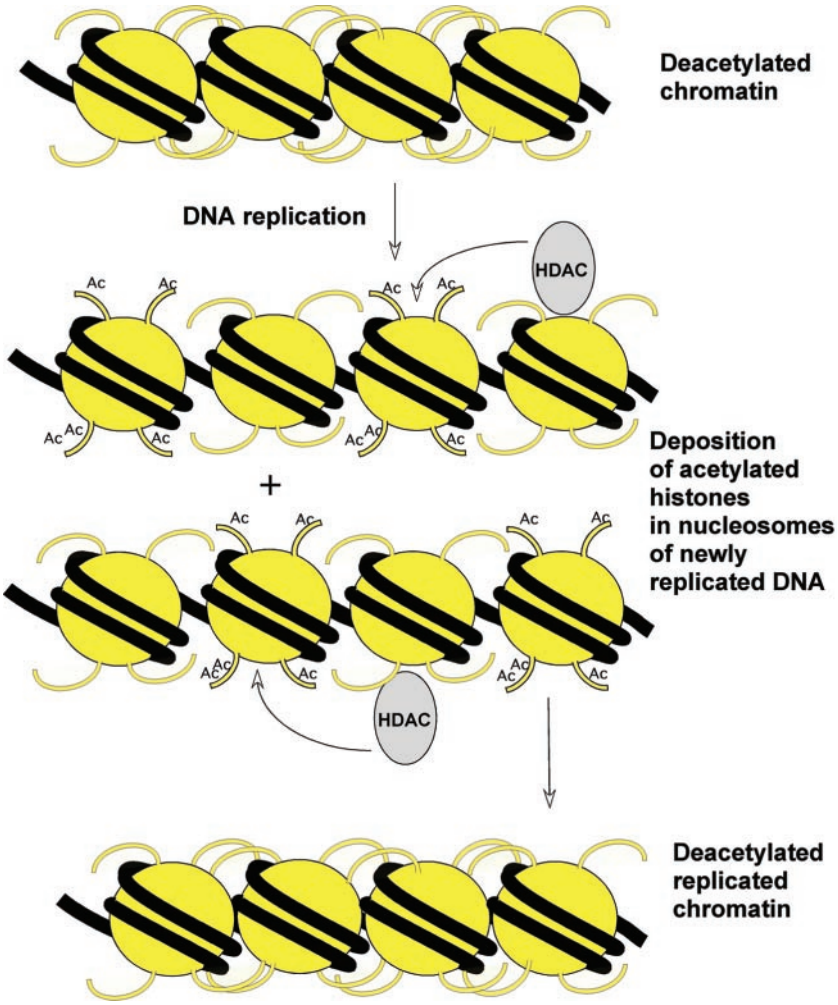

Figure 3. Heredity of histone acetylation patterns during DNA replication. When a genomic region containing deacetylated histones and a condensed chromatin structure is replicated, chromatin structure is transiently altered as a consequence of the incorporation of de novo synthesized histones, which have been acetylated in the cytoplasm by class B HATs and lack any epigenetic information. In contrast, preexisting histone octamers are segregated equally onto the two daughter DNA molecules, providing information about the degree of acetylation that can be interpreted by HDACs and other protein complexes.

HISTONE METHYLATION MAY BE REQUIRED FOR HETEROCHROMATIN FORMATION AND GENE SILENCING

In addition to acetylation, methylation is another specific histone posttranslational modification in plants (Waterborg, 1990) and other organisms. But the function of histone methylation was not known until *Su*(*var*)*3-9*, a fruitfly heterochromatin-associated protein involved in position effect variegation, was identified as a histone methyltransferase (Rea et al., 2000). *Su*(*var*)*3-9* and its yeast and human counterparts contain a SET-domain (first identified in *Su(var)3-9*, *E(z)*, and *trithorax*), which is necessary for catalysis and specific methylation of K9 in histone H3. Methylation of this Lys appears to be required during heterochromatin formation, because *Su*(*var*)*3-9* mutants are impaired in heterochromatin-mediated gene silencing (Nakayama et al., 2001). Interestingly, H3 is also methylated at K9 in Arabidopsis heterochromatin (Z. Jasencakova, W. Soppe, P. Fransz, A. Houben, and I. Schubert, unpublished data; see "Note Added in Proof"). Of the more than 30 SET-domain proteins present in the Arabidopsis genome (Baumbusch et al., 2001), nine are homologous to Su(var)3-9. One of them, KRYPTONITE (KYP), is the only plant SET-domain protein for which histone methyltransferase activity has been demonstrated (Jackson et al., 2002). Similar to human SUV39h1, KYP methylates histone H3 at Lys 9. The *kyp* mutant was isolated as a suppressor of gene silencing at the Arabidopsis SUPERMAN locus. Interestingly, *kyp* plants show a strong decrease of cytosine methylation at CpNpGp sites, indicating a connection between histone methylation and DNA methylation. The *kyp* mutants do not show morphological defects in a wild-type background, suggesting that KYP is not directly involved in developmental control. However, given the large number of genes for SET-domain proteins identified in the Arabidopsis genome, it is tempting to speculate that histone methylation will be a regulatory factor in different aspects of gene expression (see below), including control of developmental genes.

The role of histone methylation may be better understood through the analysis of proteins that recognize this modification. Heterochromatin protein 1

(HP1), a conserved heterochromatin-associated protein present in animals, fission yeast (*Schizosaccharomyces pombe*), and plants, contains a chromo domain and chromo-shadow domain. The HP1 chromo domain interacts specifically with K9-methylated H3, which explains the association of these proteins with heterochromatin (Bannister et al., 2001; Lachner et al., 2001). Subsequent oligomerization of HP1 via the chromo-shadow domain may then propagate and maintain heterochromatin structures and gene silencing (Jenuwein, 2001). The Arabidopsis HP1 homolog LHP1 (LIKE HP1) can also interact with histone H3 methylated at Lys 9 (Jackson et al., 2002). Mutations in LHP1 cause early flowering, a general reduction in plant and leaf epidermal cell size, and several other phenotypes (Gaudin et al., 2001). Thus, LHP1 may be required for silencing genes involved in the transition to flowering and other developmental processes. Expression of *CONSTANS*, a gene regulating time-to-flowering, is increased in *lhp1*, although it is currently unknown if *CONSTANS* is a direct target of LHP1. LHP1 has been shown to interact with CHROMOMETHYLASE3, a DNA methyltransferase specific for CpXpG trinucleotides (Lindroth et al., 2001). *cmt3* mutants show no obvious developmental defects but exhibit decreased CpXpG methylation of the SUP gene and other sequences throughout the genome, suggesting that LHP1 may be involved in silencing of methylated loci. The different phenotypes of *cmt3* and *lhp1* plants also indicate that LHP1 can function independently of CMT3. Further characterization of *lhp1* will clarify the role of LHP1 for regulation of heterochromatin structure and function, which could also affect silent transposons or genomic stability in the mutant.

HISTONE PHOSPHORYLATION. A ROLE IN CHROMATIN CONDENSATION?

Among histone modifications, phosphorylation of histone H3 is required for chromosome condensation during mitosis and in transcription activation in animals. Plant H3 phosphorylation of Ser 10 at mitosis begins in the pericentromeric chromatin at late prophase and ends at telophase, however, whether this is correlated with chromosome condensation is currently under debate (Houben et al., 1999; Kaszas and Cande, 2000). In addition, it may also have an important role in sister chromatin cohesion during meiosis (Kaszas and Cande, 2000; Manzanero et al., 2000), but a role in transcriptional regulation of plant genes has not been demonstrated to date.

Histone H3 kinases have not yet been identified in plants, but a role of the human Tousled-like kinase in histone H3 phosphorylation was recently reported (Li et al., 2001). TOUSLED was first identified in Arabidopsis as nuclear Ser/Thr protein kinase involved in flower and leaf development (Roe et al., 1993). Interestingly, human Tousled can also phosphorylate two human homologs of the fruitfly chromatin assembly factor anti-silencing function 1 (Sillje and Nigg, 2001), but its regulatory significance is currently unknown.

ATP-DEPENDENT CHROMATIN-REMODELING FACTORS SHARE SNF2-LIKE PROTEINS

If modification of histones and DNA organize chromatin structure and function, then how are modification patterns recognized and interpreted by regulatory proteins? This task is accomplished by at least some of the ATP-dependent chromatin remodeling machines (CRMs), which are multisubunit complexes that alter DNA-histone interactions using ATP hydrolysis (for review, see Varga-Weisz, 2001). CRMs are multisubunit complexes that destabilize nucleosome structure by introducing superhelical torsion into DNA (Havas et al., 2000), although the mechanism is not well understood. Activity of CRMs in vitro can change nucleosome position or spacing (sliding) or accessibility to nucleosomal DNA, or provoke histone eviction, resulting in all of the cases in an increase of chromatin fluidity (Fig. 4). CRMs share the presence of a DNA-dependent ATPase of the SNF2 family of proteins. SNF2 is the ATPase of the yeast SWI/SNF complex, the first CRM characterized (Peterson and Herskowitz, 1992). In addition to the ATPase domain, SNF2-like proteins have very different N- and C-terminal domains for their interactions with other proteins in the CRM complexes or with specific chromatin-associated proteins (Eisen et al., 1995). On the basis of these domains, SNF2-like pro-

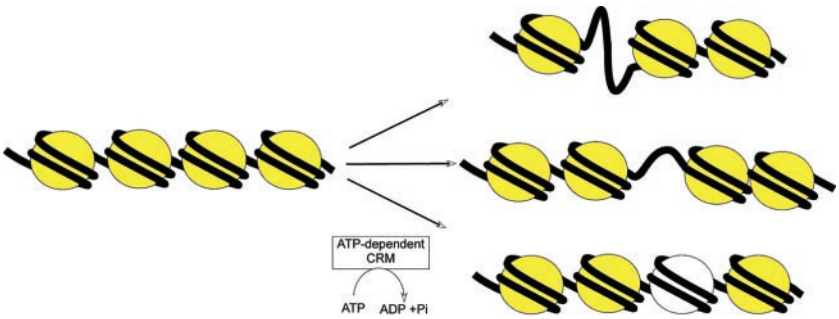

Figure 4. In vitro activities displayed by CRM. The activity of CRMs can be tested in vitro by several biochemical assays. High concentration of CRM (a 1:1 CRM to nucleosome ratio) can facilitate histone octamer transfer to other DNA molecule and histone eviction (top). At catalytic concentrations, CRMs facilitate changes in the position of the histone octamer (sliding), resulting in altered nucleosome spacing (middle). In other cases, changes in the accessibility of DNase I or restriction enzymes without apparent change in the position of the histone octamer have been also reported (bottom).

teins fall into several subfamilies, such as SWI2/SNF2, ISWI, and CHD, or lymphoid-specific helicase (LSH)/DDM1.

LOSS OF SNF2-LIKE FUNCTIONS AFFECT TRANSCRIPTIONAL REPRESSION AND DEVELOPMENT

The Arabidopsis genome encodes more than 40 SNF2-like proteins, but functions are known only for PICKLE (PKL), DDM1, MOM, and SPLAYED (SYD). Loss of PKL function affects root development and repression of *LEAFY COTYLEDON1* (*LEC1*), which controls embryonic identity (Ogas et al., 1997, 1999). As a result, *pkl* roots express seed storage proteins. Another PKL mutant allele, *gymnos*, enhances the defect of mutants that affect carpel abaxial-adaxial polarity. However, single *gymnos* mutants do not display polarity defects but instead are delayed in the differentiation of several carpel cell types. PKL is therefore not specifically involved in organ or cell polarity but more likely in cell differentiation. This, together with the pattern of PKL expression, suggests a more general role for PKL in undifferentiated dividing cells (Eshed et al., 1999). PKL is most similar to human Mi-2, which belongs to the CHD subfamily. CHD proteins contain a *c*hromo domain, a helicase-like ATPase domain, and a DNA-binding domain. Mi-2 associates with HDACs (HDAC1 and HDAC2) in the 2-MD NuRD complex (for nucleosome remodeling and histone deacetylation) that regulates repression of transcription (Ahringer, 2000). In addition, the MSI1-related proteins RbAp46 and RbAp48, a methyl-cytosine-binding protein and two zinc finger proteins (MTA1 and MTA2) are found in the NuRD complex. But whether PKL is part of a multisubunit complex in Arabidopsis is currently unknown. In fruitfly, dMi-2 interacts with Hunchback, which together with Polycomb proteins establishes a repressive state of homeotic genes that is epigenetically inherited during development (Kehle et al., 1998). Thus, it is possible that PKL represses meristem- or embryonic-specific genes in nondifferentiated cells to promote the transition to differentiation. Because PKL is mostly expressed in meristems, the repressive state would be maintained by epigenetic imprints in differentiated cells after PKL is not longer present. But how can PKL establish epigenetic imprints? One possibility is through interaction with one or several HDACs. The identification of proteins that interact with PKL will soon provide further insights into this mechanism.

DDM1 is also a SNF2-like protein, but it is more closely related to the mammalian LSH protein (Jeddeloh et al., 1999). LSH is expressed in proliferating tissues at the onset of S phase and appears to be required for genome-wide methylation (Dennis et al., 2001). Loss of DDM1 function similarly decreases genome-wide cytosine methylation of heavily methylated repetitive DNA and heterochromatic regions in particular (Vongs et al., 1993). Methylation of low-copy DNA sequences is progressively decreased after a few generations of inbreeding, suggesting that DDM1 is required for methylation of hemimethylated sequences after DNA replication. As a result, transcriptional gene silencing is impaired in *ddm1* plants (Jeddeloh et al., 1998). The accumulation of developmental abnormalities after inbreeding is the consequence of two kinds of events. Some alterations are caused by insertion of transposons, which are activated in the *ddm1* plants but silenced in the wild-type background (Miura et al., 2001; Singer et al., 2001). In addition, *ddm1* plants also accumulate epigenetically inherited changes of transcription provoked by alterations of methylation patterns. For example, hypermethylation and silencing of *superman* (*sup*) and *ag* or hypomethylation and ectopic gene expression of *fwa* have been reported (Jacobsen et al., 2000; Soppe et al., 2000). Interestingly, this phenomenon of specific hyper-methylation in a hypomethylated background is also found in certain mammalian tumor cell lines. How then does DDM1 affect DNA methylation? It is possible that *ddm1* plants are impaired in methyltransferases or in *S*-adenosyl-Met metabolism, but this is not the case. The most attractive hypothesis is that the remodeling activity of DDM1 increases the accessibility of hemimethylated DNA in newly replicated chromatin to methyltransferases (see "Note Added in Proof"). This view is consistent with the stronger effect of *ddm1* on DNA methylation in highly compact heterochromatin relative to euchromatic regions.

Analysis of other mutants suggests that DNA methylation acts as a signal but is not required per se for transcriptional repression, because silenced transgenes can be activated without changing the hypermethylation pattern of the loci. The best characterized of these mutants is *mom* (Amedeo et al., 2000). Although MOM belongs to the SNF2 family, it lacks the first part of the helicase domain. Thus, it is currently unknown whether MOM functions in ATP-dependent remodeling. The fact that MOM has a transmembrane region and homology to cytoskeletal proteins might suggest that MOM recruits methylated loci to transcriptionally inactive regions within the nuclear matrix, such as the periphery of the nucleus.

SWI/SNF COMPLEXES IN PLANTS?

The paradigm for transcriptional activation by CRMs is the SWI/SNF complex itself (Peterson and Workman, 2000; Varga-Weisz, 2001). For example, Brahma, the DNA-dependent ATPase of the fruitfly SWI/SNF complex, was initially identified as an activator of homeotic genes (Tamkun et al., 1992). SWI/SNF complexes in yeast, fruitfly, and human contain 10 to 12 subunits. One of these subunits, SNF5 in

yeast (and its human and fruitfly homologs INI1/BAF47 and SNR1, respectively), interacts with different transcription factors, perhaps for targeting of the complex to specific loci (Cheng et al., 1999). The Arabidopsis SNF5 homolog BSH (for bushy growth exhibited by mutants deficient in *BSH* mRNA) suggests that SWI/SNF-like complexes also exist in plants. Antisense *BSH* plants show reduced apical dominance and flowers that were unable to produce seeds (Brzeski et al., 1999). BSH is associated with a multisubunit complex of 1.7-MD in Arabidopsis, which is perhaps the equivalent of the SWI/SNF complex (A. Jerzmanowski, personal communication). This suggests that a plant SWI/SNF-like complex also functions in development control and cell proliferation, as in the case of the fruitfly and mammalian SWI/SNF complexes.

Amino acid alignment and phylogenetic analysis of the more than 40 putative SNF2-like members identified in Arabidopsis reveals the existence of four proteins closely related to Brahma (Verbsky and Richards, 2001). One of them is SYD. *syd* mutations enhance the floral phenotype of weak *leafy* (*lfy*) alleles, suggesting that SYD is a coactivator of LFY. However, and interestingly, SYD also behaves as a LFY-dependent repressor of the transition from inflorescence to flower meristem (Wagner and Meyerowitz, 2002). In addition, genetic analysis also suggests that SYD is a positive regulator of YABBY genes (J. Bowman, personal communication). YABBY are transcription factors expressed on the abaxial side of lateral organ primordia and promote abaxial cell fate (Bowman, 2000). These data indicate that SYD plays multiple roles in Arabidopsis development.

POLYCOMB PROTEINS: FIXING CHROMATIN STATES

Regulatory decisions must be maintained during development. This is especially important for the expression of homeotic genes that control cell fate and patterning. Genes of the Polycomb Group (PcG) in fruitfly and vertebrates mediate repression of homeotic genes in cells where they must remain inactive (Schumacher and Magnuson, 1997). Homeotic genes encode transcriptional activators that belong to the HOX class in animals and predominantly to the MADS-box class in plants (McGinnis et al., 1984; Yanofsky et al., 1990). Nevertheless, both animals and plants seems to recruit PcG proteins to control expression of homeotic genes. Recent work is now revealing the molecular mechanism of PcG action: sequence-specific DNA-binding factors recruit PcG proteins, which are subunits of larger complexes, to their target promoters (Franke et al., 1992; Shao et al., 1999; Brock and van Lohuizen, 2001). In some cases, transcriptional repression seems to be mediated by an RPD3-like HDAC associated with the PcG complex (van der Vlag and Otte, 1999; Tie et al., 2001; Fig. 5). In addition, the PcG protein *Enhancer of Zeste* [*E*(*z*)] contains a SET domain. As discussed above, a subset of SET domain proteins, including *Su*(*var*)*3-9*, have histone methyltransferase activity (Rea et al., 2000). Thus, transcriptional repression could be initiated by histone deacetylation followed by methylation of the same Lys residues, thereby preventing re-acetylation and reactivation of the repressed state. Association of *Su*(*var*)*3-9* with HP1, a methyl-histone-binding heterochromatin protein, could then facilitate spreading of repressive chromatin structures (Bannister et al., 2001).

Two main PcG complexes have been described in fruitfly: PRC1 and the extra sex combs (ESC)-E(z) complex. Although experimental data indicate the presence of ESC-E(z)-like complexes in plants, no evidence for the existence of PRC1 has been reported. *MEDEA* (*MEA*) and *FERTILIZATION INDEPENDENT ENDOSPERM DEVELOPMENT* (*FIE*) are Arabidopsis homologs of the PcG genes *E*(*z*) and *esc*, respectively (Grossniklaus et al., 1998; Kiyosue et al., 1999; Ohad et al., 1999). FERTILIZATION INDEPENDENT SEED DEVELOPMENT2 (*FIS*2) is a homolog of the recently identified fruitfly PcG gene *Su*(*z*)*12* (Luo et al., 1999; Birve et al., 2001). Mutants of the *FIS* class (presently including *fis1*/*mea*, *fis2*, and *fis3*/*fie*) are disrupted in normal endosperm and embryo development. The common phenotype suggests that MEA and FIE may function in the same complex, and their interaction has been confirmed (Luo et al., 2000; Spillane et al., 2000) (Fig. 5).

Interestingly, the phenotype of *fis* mutants is under maternal control. This is consistent with evidence of genomic imprinting at the *MEA* locus, which maintains the paternal allele in a silenced state during early embryo and endosperm development (Kinoshita et al., 1999; Vielle-Calzada et al., 1999). The silenced paternal allele can be activated later in development, a modifying effect that appears to be ecotype specific (Grossniklaus et al., 2001). The paternal alleles of *FIS2* and *FIE* appear to be silenced during early seed development as well but are consistently activated later in development (Luo et al., 2000).

In addition to MEA, CURLY LEAF (CLF) is one of several SET domain proteins in Arabidopsis (Goodrich et al., 1997). CLF most likely controls the expression of *AG*. In *clf*, AG is expressed in leaves and other organs, resulting in phenotypes also observed in transgenic plants that ectopically express *AG* (Goodrich et al., 1997). *EMBRYONIC FLOWER2* (*EMF2*), a homolog of *FIS2*, is also required for repression of *AG* in vegetative tissues (Chen et al., 1997; Birve et al., 2001; Yoshida et al., 2001). Finally, *VRN2*, another Arabidopsis homolog of *Su*(*z*)*12*, is required for maintaining stable repression of *FLOWERING LOCUS* C to delay flowering (Sheldon et al., 2000; Birve et al., 2001; Gendall et al., 2001). Together, the function of PcG gene products in transcriptional repression of homeotic genes and perhaps chromatin

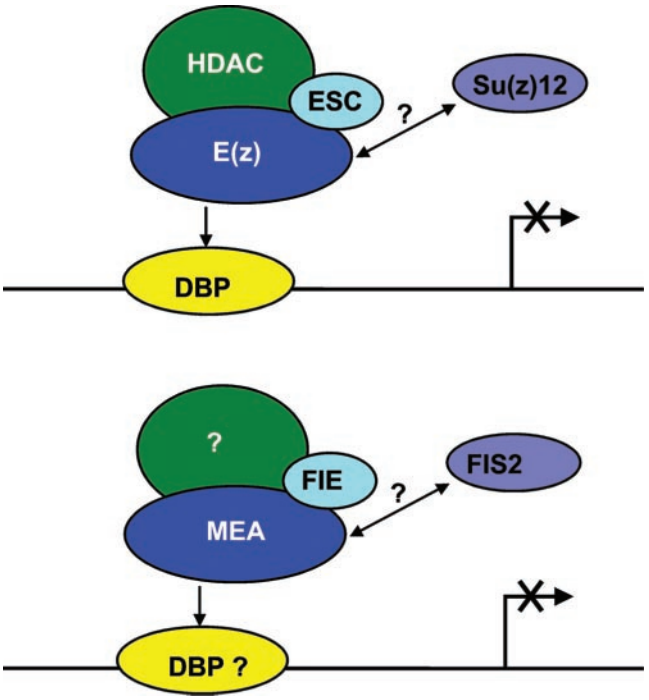

Figure 5. Proteins involved in PcG-mediated transcriptional repression. A fruitfly PcG complex is shown at the top, and a putative Arabidopsis PcG complex is shown at the bottom. Sequence-specific DNA-binding proteins (DBP) could target specific genomic locations and recruit HDAC complexes, PcG proteins (MEA/E(z), FIE/ESC), and possibly additional factors such as Su(z)12 and FIS2. Subsequently established changes in chromatin conformation would lead to transcriptional repression. In addition to MEA, CLF and Eza1 are also E(z) homologs in Arabidopsis. VRN2 and EMF2 are also Su(z)12 homologs (for details, see text).

condensation during mitosis (Brock and van Lohuizen, 2001) has been conserved in animals and plants.

Certain PcG proteins interact with Rb and Rb-related (RBR) proteins, which are central regulators of the cell cycle in animals and plants (Weinberg, 1995; Durfee et al., 2000; Gutierrez, 2000). When recruited to promoters by transcription factors in animals, PcG-Rb complexes can function as transcriptional corepressors or coactivators through interactions with the Sin3-HDAC-complex, the BRG1/hBrm SWI/SNF-type complexes, the histone-methyltransferase SUV39H1, HP1, and others (Trouche et al., 1997; Brehm et al., 1998; Magnaghi-Jaulin et al., 1998; Nielsen et al., 2001; Vandel et al., 2001). These observations suggest that Rb/RBR proteins can perhaps also control proteins that direct local modifications of chromatin structure. Maize RBR1 and human pRb can interact with Arabidopsis CLF, suggesting that RBR1 may also function in the transcriptional repression of *AG* (Williams and Grafi, 2000).

CHROMATIN AND PLANT DEVELOPMENT. A NEW FRONTIER

During the last few years, an increasing number of developmental mutants has been identified in which genes encoding chromatin factors are affected. Thus, focus in plant development research is in a transition from direct transcriptional control to higher-order mechanisms that control chromatin structure and function. In retrospect, it is interesting to ask why developmental genetics approaches are only now uncovering more genes for chromatin-related functions but fewer in the past. Mutations in specific transcription factors generally result in well-defined phenotypes that can be easily scored, whereas mutations that affect chromatin factors often display subtle or more complex and pleiotropic phenotypes, which are more difficult to interpret. On the other hand, several mutants affected in chromatin factors (*spy*, *gymnos* allele of PKL, *lug*, and *vrn*) have been isolated in genetic screens designed to find second-site enhancer or suppressor mutations.

The number of identified target genes directly controlled by chromatin factors is still small. But even early research already suggests that chromatin factors often control the expression of specific developmental transcription factors. Thus, chromatin factors can be regarded as the next level in the regulatory hierarchy of factors controlling plant development. But surprisingly and at first sight contrasting this view, many mutants affected in plant chromatin factors are not lethal. Plant chromatin factors have perhaps only an accessory role or a high degree of functional redundancy.

The transition to flowering in Arabidopsis is probably the most critical decision the SAM has to make during development. The discussion above shows that the transition from vegetative to reproductive development (floral transition) is clearly controlled by chromatin-remodeling factors. Reduced levels of the HDAC HDA1 and mutations in the SNF2 homolog *SPY*, in polycomb genes such as *VRN*, *CLF*, and *EMF2*, or in the heterochromatin protein gene *LHP1* cause alterations in flowering time. Furthermore, genome-wide demethylation, induced by treatment with 5-azacytidine or by a *METHYLTRANSFERASEI* antisense construct, promotes flowering by decreasing the level of flowering locus C (Finnegan et al., 1998). Genetic data suggest that the default program during Arabidopsis development is the transition to the reproductive phase. It is therefore tempting to speculate that plants have evolved chromatin-based repression mechanisms to finely control the phase transition.

In contrast to animal development, plant development is plastic and strongly influenced by biotic and abiotic factors. Plants therefore require specific cross-talk between developmental programs and signaling pathways from external stimuli that must be coordinated at the level of chromatin organization, a mechanism that is still poorly understood. But the fact that plant cells are generally totipotent, as opposed to the weak de-differentiation capacity of non-transformed animal cells, is perhaps telling us that silenced chro-

matin states can be easily reactivated in plants. Reversibility of silenced chromatin states is of broad interest at present because it has implications for cancer biology and transgenic technologies. Finally, the late divergence between the somatic and the germinal lineage in plants allows the epigenetic inheritance of specific chromatin structures acquired during vegetative development. How these epimutations and paramutations are inherited through meiosis and what their implications are for plant evolution will be a rich research field and a new frontier.

Note Added in Proof

R. Martienssen, V. Colot, and coworkers have recently demonstrated using chromatin immunoprecipitations that transposons and silent genes localized in the heterochromatic knob of Arabidopsis chromosome IV are associated with histone H3 methylated at K9 (A.V. Gendrel, Z. Lippman, C. Yordan, V. Colot, R. Martienssen [2002] Science, in press). Interestingly, in *ddm1* mutant heterochromatin, not only DNA methylation is lost, but H3 K9 methylation is largely replaced by methylation of K4, demonstrating again the interconnection between DNA methylation and histone methylation. This opens new possibilities about how DDM1 affects DNA methylation. The cited paper is available online in Science-xpress ahead of print.

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