The plant E2F–Rb pathway and epigenetic control

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Plants and animals use the E2F–Rb pathway as a major mechanism of control in the decision to continue or stop cell division. The E2F–Rb pathway controls the G1-to-S-phase transition by the timely activation of genes involved in DNA synthesis and cell-cycle control. Recent findings reveal that the E2F–Rb pathway communicates with chromatin-remodelling factors in the control of transcription and cell-cycle progression. This article highlights the fast-moving advances in the molecular and functional characterization of plant E2F proteins, and in our understanding of how the E2F–Rb pathway is activated and repressed.

The coordinated function of cell-cycle genes is essential for cell division and differentiation in all eukaryotes, including higher plants [1–3]. Among the various cell-cycle regulators, the E2F (see Glossary) transcription factor and the RETINOBLASTOMA (Rb) repressor are the key players; their deregulation causes tumour formation in mammals [4,5].

E2F controls the transcription of a wide range of genes, including genes involved in cell-cycle progression and DNA synthesis, replication and repair [6,7].

It is well established that E2F plays a crucial role in the regulation of G1-to-S-phase transition. In growth-arrested cells (G0) and during early G1 phase, the E2F activity is repressed by Rb. Upon growth stimulation, Rb is phosphorylated during late G1 phase by CYCLIN-DEPENDENT KINASES (CDKs) and consequently loses its affinity for E2F. The release of Rb triggers the activation of E2F-target genes, which irreversibly commits cells to undergoing DNA replication (S phase). Rb represses E2F activity not only by physically masking the E2F transactivation domain but also by actively recruiting chromatin-remodelling factors, linking the E2F–Rb pathway to epigenetic control [4,5]. Recent advances reveal that both the E2F–Rb pathway and its epigenetic connection are conserved and essential for coordinated cell division and development in plants.

E2F-family transcription factors

E2F represents a family of related proteins, which in humans comprises six E2F (E2F1 to E2F6) and two distantly related DP (DP1 and DP2) members [5]. Both E2F and DP proteins have been identified in several plant species including wheat, tobacco, carrot, *Arabidopsis* and rice [8–16]. *Arabidopsis* contains eight proteins that can be classified by sequence homology into the E2F, DP and DEL (DP- and E2F-like) groups (Fig. 1a). This probably represents the complete E2F family in *Arabidopsis* because searches of the complete nuclear genome sequence have not detected any additional *E2F* or *DP* genes [17].

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The AtE2Fa–AtE2Fc proteins and the characterized E2F proteins of other plant species exhibit an overall domain organization similar to the animal E2F1–E2F5 proteins, including a highly conserved DNA-binding domain, a moderately conserved leucine-zipper dimerization domain and a C-terminal transactivation domain embracing a conserved Rb-binding site. The plant DP proteins, with two members in *Arabidopsis* (AtDPa, AtDPb), contain conserved DNA-binding and leucine-zipper dimerization domains, like their animal counterparts. However, the AtDEL1–AtDEL3 proteins form a group with unique features that have not been described in animals. Each contains two DNA-binding domains of high homology to both E2F and DP proteins, but lack the other conserved regions

The transcript levels of the plant E2F-family genes are generally low. Nevertheless, tissue-specific expression has been demonstrated for some *Arabidopsis* genes. Reverse-transcription polymerase-chain-reaction analyses revealed that *AtE2Fa*, *AtDPa* and *AtDEL1–AtDEL3* are expressed more abundantly in organs containing proliferating tissues [11,14]. Analyses by *in situ* hybridization showed that *AtE2Fa* and *AtDPa* are strongly expressed in the actively dividing regions (e.g. the root tip, the shoot apical meristem, young leaf primordia) and the vascular tissues of leaves and roots [18]. The *AtE2Fa* and *AtDPa* transcripts are also abundant in tissues undergoing extensive endoreduplication (e.g. the epidermis and cortex of the hypocotyl) [18]. Overall, these expression data strongly support a role for E2F-family genes in regulating the mitotic cell cycle and the endocycle during plant development.

In partially synchronized suspension cells, the plant *E2F* genes show an increased level of transcripts at the G1-to-S-phase transition [8–11,13,16]. Although the expression of *AtDPa* remains constant during different phases of the cell cycle, the expression of *AtDPb* increases considerably on entry into S phase [16]. Conversely, *AtDEL1* and *AtDEL3* (which are likely antagonists of the *E2F* genes) are barely expressed during S phase [16]. These findings are consistent with a requirement for E2F activity during the G1-to-S-phase transition.

Although AtDEL1–AtDEL3 are located in the nucleus, AtDPa and AtDPb are found in both cytoplasm and nucleus, and AtE2Fa–AtE2Fc can be in the cytoplasm and/or the nucleus in different cells [14,19]. For the AtE2F proteins, however, it is still unclear

 1360-1385/02/$ – see front matter PII: S1360-1385(02)02351-8

Glossary

Cyclin-dependent kinases
Cyclin-dependent kinases (CDKs) are a class of highly conserved serine/threonine kinases found in all eukaryotes. They are composed of a catalytic and a regulatory subunit. Animal and plants contain multiple types of catalytic subunits (CDK1–CDK8 in animals and CDKA–CDKE in plants) as well as multiple classes of regulatory subunits (cyclins) named CycA–CycH. Each CDK–cyclin complex acts at a precise time and drives the progression of the cell cycle by phosphorylating downstream target proteins. One of the best-characterized targets of CDKs is the E2F–Rb pathway.

DNA methyltransferase
DNA methyltransferase (DNMT) catalyses the methylation of cytosines in both CG and CNG (where N can be any nucleotide) symmetric sequence contexts.

DP
A group of proteins distantly related to the E2F family that can dimerize with E2F members. E2F–DP dimers bind specific DNA sequences, with a consensus of TTTC(G)C(G)CGC, called the E2F-binding site.

E2F
Originally discovered as a cellular factor required for transcriptional activation of the adenovirus E2 promoter, E2F is currently recognized as representing a crucial family of transcription factors of cellular genes that are essential for the G1-to-S-phase transition in animals and plants. Humans contain six E2F proteins. E2F1–E2F3 are potent transcriptional activators, whereas E2F4–E2F6 are primarily transcriptional repressors.

Histone acetyltransferases
Histone acetyltransferases (HATs) catalyse the acetylation of specific lysine residues of histones using acetyl-CoA as substrate.

Histone deacetylases
Histone deacetylases (HDACs) catalyse the remove of acetyl groups from acetylated histones.

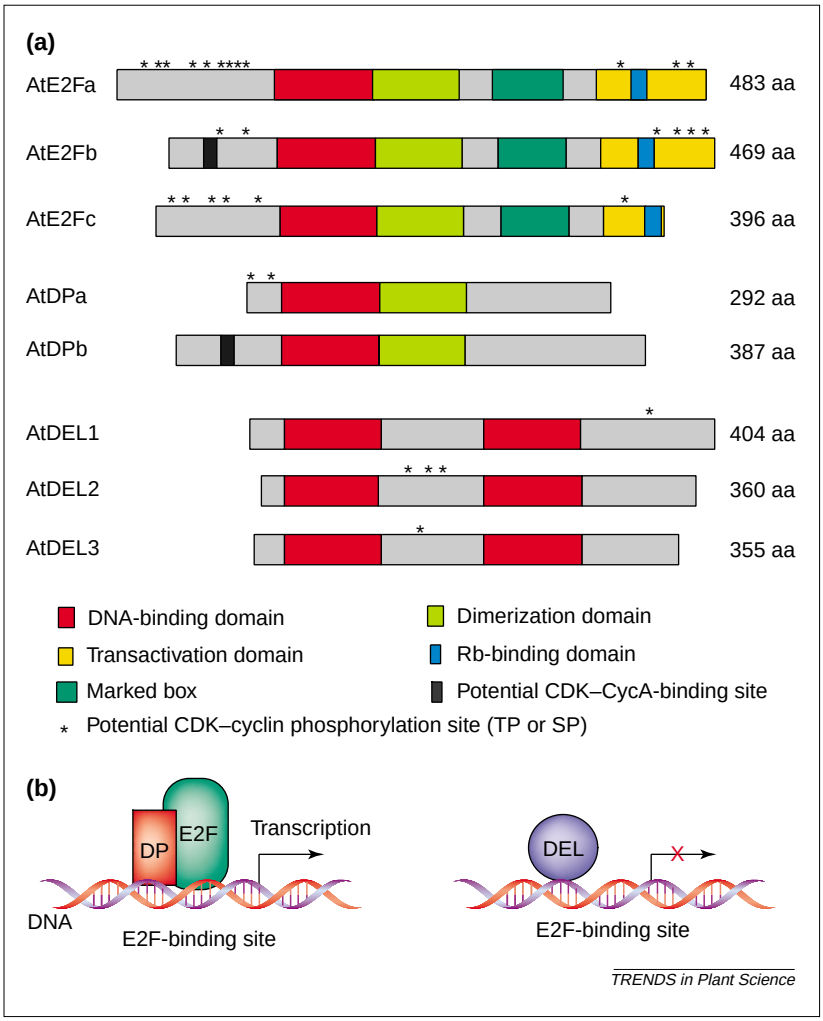
Retinoblastoma (Rb)
First identified as a suppressor of retinoblastoma, a rare pediatric eye cancer, Rb (also called RB or pRB) is a member of the pocket-protein family, which, in humans, also includes p107 and p130. Among the proteins bound and regulated by the Rb family are the E2F-family transcription factors.

SET-domain-containing histone methyltransferases
First identified as a conserved domain of ~130 amino acids in the three *Drosophila* genes involved in epigenetic control [*SU(VAR)3-9*, *Enhancer of zeste* and *Trithorax*], the SET domain is currently recognized as a signature for a class of histone methyltransferases (HMTases). The SET-domain HMTase catalyses the methylation of specific lysine residues of histones using adenosylmethionine as substrate.

SWI-SNF
Switch sucrose non-fermenting (SWI-SNF) is a DNA-dependent multiple-subunit ATPase that can alter nucleosome structures.

whether the different localization patterns correlate with different phases of the cell cycle. Interestingly, AtDPa but not AtDPb can greatly enhance the nuclear translocation of AtE2Fa and AtE2Fb [19]. Therefore, a cooperative interaction between plant E2F and DP proteins appears to control their intracellular localization and consequently their subcellular functions.

Heterodimerizations between plant E2F and DP proteins have been demonstrated by the use of yeast two-hybrid and *in vitro* binding assays [11,12,19]. The plant E2F–DP complexes bind, *in vitro*, a specific DNA sequence – the E2F-binding site (Fig. 1b) – with much higher affinity than E2F alone [12,15,16,19]. In addition, co-production of plant E2F and DP considerably increases the expression of a reporter gene containing in its promoter an E2F-binding site [15,16,19]. Together, these results support the notion that plant E2F and DP proteins function as heterodimer complexes like their animal counterparts. By contrast, AtDEL1–AtDEL3 bind to DNA as monomers and their overproduction inhibits the transcriptional activity of E2F–DP complexes [14,16]. Thus, AtDEL1–AtDEL3 are antagonists of E2F–DP dimers, probably by titration of the E2F-binding site.



Rb repressors and E2F-responsive gene expression

Rb and its relatives play an essential role in the control of E2F transcriptional activity in animals [4,5]. Only one Rb homologue could be identified in

Fig. 1. Structural organization and DNA-binding properties of the *Arabidopsis* E2F-family proteins. (a) The DNA-binding, dimerization, Marked-box and transactivation domains of the E2F-family proteins. The Rb-binding domain and the potential cyclin-dependent-kinase–cyclin-A (CDK–CycA)-binding domain and CDK–cyclin phosphorylation site are also indicated. Based on the conservation of different domains, the eight *Arabidopsis* proteins are classified into E2F, DP and DEL groups (nomenclature according to Ref. [17]). (b) DNA-binding properties of *Arabidopsis* E2F, DP and DEL proteins. E2F-group proteins bind DNA as heterodimers with DP-group proteins, whereas DEL-group proteins bind DNA as monomers. The DNA sequences specifically recognized by E2F–DP dimers and by DEL monomers are similar and match the animal E2F-binding sites, with the consensus sequence TTT(C/G)(C/G)CGC. Because of the lack of a transactivation domain, the DEL proteins are unable to activate transcription. Their co-production inhibits E2F–DP-mediated transcription, probably through titration of the E2F-binding site [14].

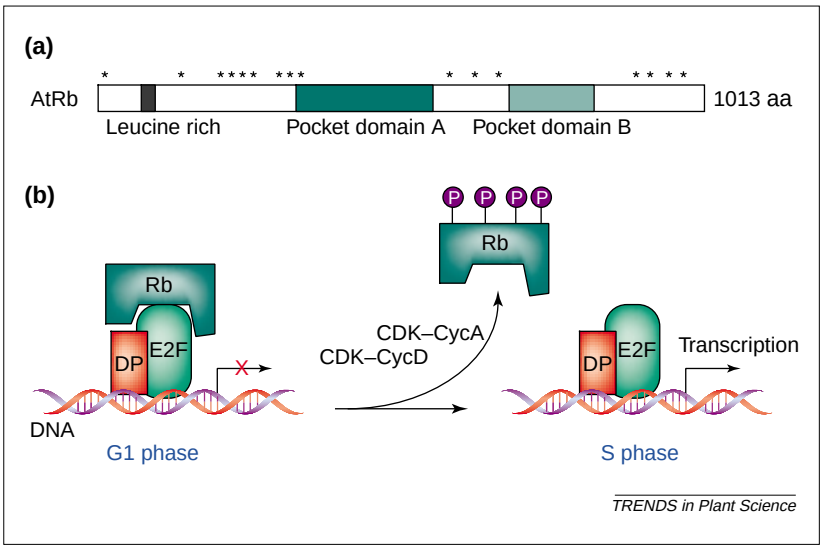


Fig. 2. (a) Structural organization of the *Arabidopsis* retinoblastoma (Rb) protein, showing the conserved pocket domains, the leucine-rich region and the potential cyclin-dependent-kinase–cyclin phosphorylation sites (asterisks). (b) Model for activation of the plant E2F–Rb pathway at the G1-to-S-phase transition. The model is based on results obtained in plants and on parallels with the mammalian E2F–Rb pathway. In growth-arrested cells and during early G1 phase, hypophosphorylated Rb binds E2F–DP dimers and consequently inhibits the E2F transcriptional activity. During late G1 and early S phase, Rb is (hyper)phosphorylated, first by CDK–cyclin-D (CycD) and then by CDK–cyclin-A (CycA) kinases, resulting in the dissociation of Rb from the Rb–E2F–DP complex. The released E2F–DP complex actively promotes transcription of E2F-target genes involved in cell-cycle regulation, DNA synthesis and replication, and chromatin assembly.

Arabidopsis, but there are at least three different ones in maize [17,20]. Not only higher plants but also the unicellular green alga *Chlamydomonas* contain a Rb homologue that is essential for cell-cycle progression [21]. Plant and animal Rb proteins exhibit a similar domain organization, with highest conservation within the pocket domains A and B (Fig. 2a). Physical interaction between the plant E2F and Rb has been shown by the use of yeast two-hybrid assays [8,9]. In animals, Rb (through its pocket domains) binds E2F at a site within the C-terminal transactivation domain and consequently inhibits the E2F transcriptional activity [4,5]. The conserved features of Rb and E2F suggest that a similar type of inhibition might also operate in plants. This is supported by the finding that maize Rb can inhibit the transactivation activity of human E2F [22].

Although the repression of E2F activity by Rb in plants needs more study, the observation that repressed *PCNA* promoter containing an E2F-binding site is activated upon geminivirus infection strongly supports such a role [23]. In animals, DNA tumour viruses induce host-gene transcription through the binding of a viral protein (e.g. adenovirus E1A) to Rb, which dissociates Rb from the Rb–E2F complex, leading to E2F activation [4]. In plants, the geminivirus protein AL1 binds Rb, which might disrupt Rb–E2F complexes and result in the derepression of the *PCNA* promoter [23]. In support of this assumption, high levels of Rb have been detected in differentiated leaf tissues [22].

In mammals, highly orchestrated Rb phosphorylation events, first by CDK–cyclin-D (CycD), then by CDK–cyclin-E (CycE) and later by CDK–cyclin-A (CycA), result in the dissociation of Rb from the E2F–DP complex during late G1 and S phase [4,5]. The highly conserved domain organization suggests that plant Rb proteins are also subject to phosphorylation by CDK–cyclin. Although homologues of CycE could not be identified, both CycD and CycA have been identified in plants. There are ten homologues of CycD in *Arabidopsis*, most of which contain a conserved Rb-binding motif (LxCxE, where x represents any amino acid) [17]. Some of the plant *CycD* genes are induced early upon mitogenic-signal stimulation [1–3], suggesting a primary role for these genes in the activation of the E2F–Rb pathway. In addition, plants contain many A-type cyclins (*Arabidopsis* has ten) [17,24]. Some of the plant *CycA* genes are upregulated during late G1 and early S phase [24]. This is consistent with a possible role for these proteins in the activation of the E2F–Rb pathway during the G1-to-S-phase transition. Indeed, both plant A- and D-type cyclins interact with Rb, and their corresponding CDKs can phosphorylate Rb *in vitro* [25–27]. Overall, it appears that CDK–CycD and subsequently CDK–CycA phosphorylate Rb, resulting in the activation of the plant E2F–Rb pathway (Fig. 2b).

Sequence searches revealed that >30 *Arabidopsis* genes involved in cell-cycle regulation and DNA replication and repair contain one or two E2F-binding sites in their promoters [15]. E2F-binding sites have also been found in promoter regions of cell-cycle-regulated genes involved in the defence, metabolism and development of plants [6]. Mutation analyses have shown that the E2F-binding sites are important for the tissue-specific and cell-cycle-regulated patterns of expression of several plant genes involved in DNA replication [15,23,28–30]. Although one of the two E2F-binding sites in the tobacco *RNR2* promoter functions as an activator for the upregulation at the G1-to-S-phase transition, the other functions rather as a repressor of expression during G1 phase [28]. Similarly, one of the E2F-binding sites in the *Arabidopsis MCM3* promoter has been found to repress expression during G2 phase [30]. Therefore, E2F-binding sites allow both negative and positive regulation, probably depending on higher-order complex structures.

E2F–Rb pathway and epigenetic control connection

In all eukaryotes, including plants and animals, genomic DNA is packaged around octamers of histones to form the basic structural units of chromatin, the nucleosomes. The regulation of nucleosome structures constitutes a crucial control mechanism for gene transcription [31,32]. Recent findings indicate that the Rb–E2F complex actively represses transcription by interacting with different chromatin-remodelling factors (Table 1, Fig. 3).

Table 1. Chromatin-remodelling factors found in complexes with E2F–Rb in mammals and their probable homologues in *Arabidopsis*

Class	Function	Rb-recruited protein		
		Animal protein	Characteristics and function	*Arabidopsis* homologues
SWI-SNF	Modification of nucleosome structures	Brahma (BRM) and brahma-related gene 1 (BRG1)	Homologues of the yeast SWI2, DNA-dependent ATPase subunits of SWI-SNF complexes	At2g28290 (Splayed, SYD, AtCHA3), At3g06010 (AtCHA12), At5g19310 (AtCHA23)
HDAC	Deacetylation of histones	RbAp48	WD-40 repeat-containing protein, subunit of chromatin assembly factor-1 (CAF-1), associating with RPD3-family HDACs	At5g58230 (MSI1), At2g16780 (MSI2), At4g35050 (MSI3), see Ref. [39] for conserved RPD3-family HDACs in plants
HMTase	Methylation of histones	SuvH39H1	Human homologue of the *Drosophila* Su(var)3-9, first SET-domain-containing protein discovered with HMTase activity, catalysing methylation of the lysine 9 of histone H3	At5g04940 (SUVH1), see Ref. [45] for SUVH2–SUVH10
DNMT	Methylation of DNA	DNMT1	Major DNA methyltransferase responsible for the maintenance of methylation patterns in mammals	At5g49160 (DMT1, MET1, DDM2), At5g14140 (DMT2, MET2), At4g13610 (DMT3, MET3)

Abbreviations: DNMT, DNA methyltransferase; HDAC, histone deacetylase; HMTase, histone methyltransferase.

E2F–Rb and chromatin-remodelling factors

The first mechanism to alter or remodel chromatin structure involves ATP-dependent complexes that change the location and/or conformation of the nucleosomes. Amongst various complexes, SWI-SNF is the best studied; this contains 8–12 subunits in animals and yeast [31]. The central subunit of the SWI-SNF complex, SWI2 (also called SNF2) in yeast, is a DNA-dependent ATPase that can alter chromatin structure in the absence of the other subunits. Humans have two homologues of SWI2, BRM and BRG1 (Table 1). Rb can interact with both BRM and BRG1 (Fig. 3a), and this interaction has been shown to be essential for maintaining repression during early S phase of *CDK1* and *CycA* genes, whose products are primarily required for G2- and M-phase progression [4,33]. The *Arabidopsis* genome encodes several SWI2-related proteins, with three of them (including SYD) showing highest homologies to BRM and BRG1 (Table 1) [34]. SYD has recently been shown to be involved in the regulation of floral homeotic-gene expression [35]. The presence of the conserved Rb-binding motif CxLxE in SYD (amino acids 2032–2036) suggests that SWI-SNF complexes could also have a role in plant E2F–Rb-regulated gene expression.

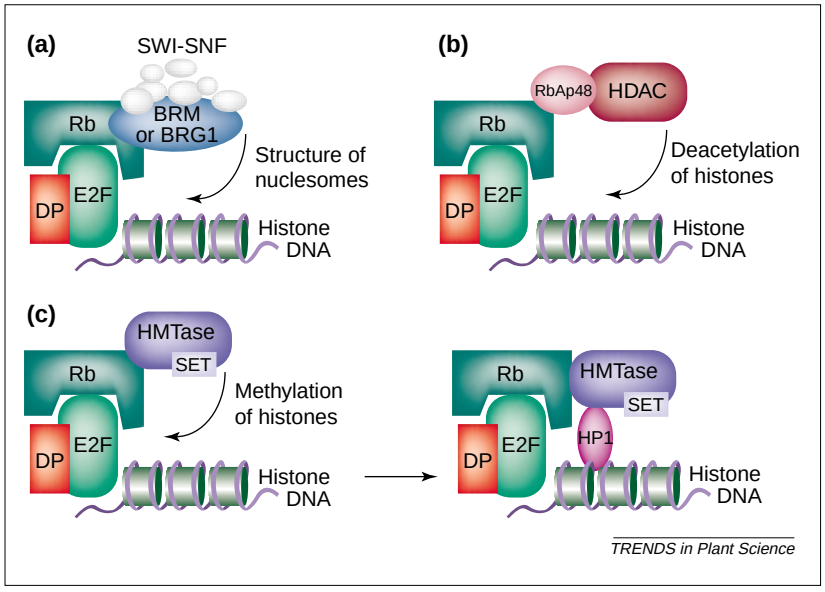


Fig. 3. Models of active repression of transcription by Rb–E2F complexes involving recruitment of chromatin-remodelling factors. (a) Rb can interact with the ATPase subunit (BRM or BRG1 in animals) of the multiple-subunit SWI-SNF complex, which modulates the location and/or conformation of the nucleosomes into a status unfavourable for transcription of E2F target genes. (b) Using the RbAp48 protein, Rb can also recruit the histone deacetylases (HDACs) that catalyse the deacetylation of histone N-terminal tails that protrude from the core nucleosomes, causing a closed chromatin conformation that represses transcription of E2F target genes. (c) Rb can recruit the SET-domain histone methyltransferases (HMTases) (e.g. Suv39H1 in humans) that methylate specific lysine residues of histone N-terminal tails, leading to heterochromatin protein 1 (HP1) binding and silencing of transcription.

The second mechanism for modifying chromatin structure is covalent modification of histone N-terminal tails, which protrude from the nucleosomes [32]. The best-studied covalent modification is acetylation – acetyl groups are added to lysine residues of histones by HISTONE ACETYLTRANSFERASES (HATs) and removed by HISTONE DEACETYLASES (HDACs). In mammals, Rb recruits HDACs through the Rb-associated protein RbAp48 (Table 1, Fig. 3b) [4,36]. Homologues of RbAp48 have been identified in plants [37,38], and an interaction between the tomato protein and the maize or human Rb has been demonstrated [37], suggesting that, in plants, Rb might also recruit the RbAp48-associated HDACs [39]. HDAC–Rb–E2F complexes have been shown in mammals to be required for histone deacetylation of nucleosomes located within promoters and for repression of *CycE* gene during early G1 phase [40,41].

Another, more recently characterized, covalent modification of histones is methylation of lysine residues by SET-DOMAIN-CONTAINING HISTONE METHYLTRANSFERASES (HMTases). SET-domain proteins had long been recognized as key regulators of heterochromatin silencing in yeast, *Drosophila* and mammals. The human SET-domain HMTase Suv39H1 binds to Rb,

specifically methylates lysine 9 of histone H3 and co-represses E2F activity [42–44]. As in heterochromatin silencing, transcriptional repression of E2F target genes by Suv39H1 is mediated through the binding of heterochromatin protein 1 (HP1) to the methylated H3 (Fig. 3c). The *Arabidopsis* genome encodes ~30 SET-domain-containing proteins, and both chromatin association and HMTase activity have been documented for plant SET-domain proteins [45–47]. Homologues of HP1 have also been identified from several plant species [48]. Therefore, HP1–HMTase–Rb–E2F complexes could also repress E2F target genes in plants. In support of this assumption, the *Arabidopsis* SET-domain protein CLF has been shown to bind both the maize and human Rb proteins [49].

DNA methylation is another well-recognized mark for transcriptional repression in animals and plants [50]. DNMT1, the predominant mammalian DNA METHYLTRANSFERASE (DNMT), has been found to bind with HDAC to the Rb–E2F complex and to repress transcription from promoters containing E2F-binding sites (Table 1) [51]. Based on the conservation in *Arabidopsis* [52] of DNMT1 and the methylcytosine-binding protein MeCp2, which is required for maximal repression, DNA methylation could probably also be used in plant E2F–Rb repression.

E2F–Rb repression: an epigenetic process?

Although there are examples of different Rb–chromatin repressor complexes acting on different E2F-target genes, recoveries of SWI-SNF–HDAC, HDAC–HMTase and HDAC–DNMT complexes together associated with Rb–E2F [33,44,51] indicate that different types of modifications (Fig. 3) might communicate with each other. ATP-dependent SWI-SNF might promote the oscillation of nucleosomes for the maintenance of chromatin fluidity, but a more stable repression of Rb–E2F might be achieved by covalent modifications of histones and DNA.

The histone-code hypothesis predicts that a pre-existing modification affects subsequent modifications on histones, and that distinct modifications are read by different proteins or protein complexes to lead to additional downstream events [53]. Considering that *de-novo*-synthesized histones are generally acetylated, methylation by HTMase might require HDAC activity first to remove the acetyl group. However, this ordered intervention might be complicated because acetylation and methylation could occur on different residues of histones. Mutation analyses of SET-domain HMTases in *Neurospora* and *Arabidopsis* have suggested that methylated histones could serve as substrates for DNA methylation [47,54]. A 'chromatin first, DNA methylation second' model is also supported by observation of loss or alteration of DNA methylation in mutants of SWI-SNF and HDAC in *Arabidopsis* [34,50,55].

Another important feature that needs to be considered is the reversibility of covalent modifications. Although HATs and HDACs can carry out reversible acetylation–deacetylation of histones, histone methylation appears to be stable (no histone demethylases have yet been identified). The HP1–HMTase–Rb–E2F complex links the E2F–Rb pathway to heterochromatin-silencing mechanisms. However, there might be differences in the propagation of silencing. In contrast to domain spreading of heterochromatin, Suv39H1–Rb–E2F methylates nucleosomes with a low capacity for spreading [42]. Heterochromatin silencing generally persists through mitotic and meiotic cell divisions [31,32], whereas HP1–HMTase–Rb–E2F would have to be inactivated in each cell cycle. Perhaps more likely, the HP1–HMTase–Rb–E2F complex might be involved in the silencing of proliferation-associated E2F-target genes in differentiating (quiescent) cells. This 'irreversible' inhibition of E2F-regulated genes in differentiated cells might be linked to DNA methylation and reorganization of the genes into a closed, inaccessible structure similar to heterochromatin.

E2F–Rb pathway and plant development

Developmental patterning and morphogenesis in plants are principally determined by post-embryonic regulation of meristems. In contrast to the situation in animals, in which mutants of most chromatin-remodelling factors are embryonically lethal, mutants of plant homologues are viable [55]. Genetic studies of several genes encoding components of SWI-SNF, HDAC, HMTase and DNMT reveal that epigenetic mechanisms are involved in many facets of plant development, including embryonic and meristematic cell proliferation and differentiation [34,50,55]. The connection of chromatin-remodelling factors to the E2F–Rb pathway might provide a mechanism through which epigenetic control affects cell division and differentiation.

The importance of the E2F–Rb pathway in plant development has been addressed directly by overexpressing *AtE2Fa* and *AtDPa* in *Arabidopsis* [18]. Overproduction of AtE2Fa and AtDPa induces extra postembryonic cell divisions. A delayed exit from the cell cycle to differentiation might explain the extra cell divisions observed [18]. Overexpression of *AtE2Fa* and *AtDPa* also causes differentiated leaf cells to re-enter S phase [56]. In addition to the mitotic cell cycle, the endocycle is also affected by high E2F activity, as evidenced from increased ploidy in transgenic plants [18] and *de novo* DNA synthesis of transiently transformed polyploid cells [56]. High levels of transcripts of E2F-responsive genes are detected in *AtE2Fa–AtDPa* transgenic plants [18]. Amongst these E2F-responsive genes, *AtCDC6* plays an important role because its ectopic expression induces endoreplication, resulting in increased ploidy

in transgenic *Arabidopsis* plants [57]. As a result of uncontrolled cell proliferation and delayed differentiation, transgenic *Arabidopsis* plants overexpressing *AtE2Fa–AtDPa* are arrested in growth early during post-embryonic development [18]. A correct balance between cell division and differentiation thus appears to be essential for plant development. A context-dependent role for cell division in plant morphogenesis has also been shown through local modulation of cell-cycle regulators [58].

Concluding remarks

A future challenge will be to understand the specific roles of the individual plant E2F-family members. We need to know whether they regulate the transcription of distinct groups of plant genes and whether and how their function is spatially and temporally integrated with plant development. More molecular and biochemical studies, particularly *in vivo* and under physiological conditions, are required to understand better how the E2F–Rb pathway is activated, repressed and coordinated with plant cell division and differentiation. In spite of their important functions in plant development, as revealed from genetic studies, chromatin-remodelling factors in the E2F–Rb pathway are largely uncharacterized in plants, with regard to both complex composition and mechanism of function.

Acknowledgements
I thank Léon Otten and André Steinmetz for critical reading of the manuscript, and Jérôme Mutterer for drawing the DNA and nucleosome illustrations.

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Evolution of signal transduction in intracellular symbiosis

Catherine Kistner and Martin Parniske

Plant roots form intracellular symbioses with fungi and bacteria resulting in arbuscular mycorrhiza and nitrogen-fixing root nodules, respectively. A novel receptor like-kinase has been discovered that is required for the transduction of both bacterial and fungal symbiotic signals. This kinase defines an ancient signalling pathway that probably evolved in the context of arbuscular mycorrhiza and has been recruited subsequently for endosymbiosis with bacteria. An ancestral symbiotic interaction of roots with intracellular bacteria might have emerged from such a recruitment, in the progenitor of the nodulating clade of plants. Analysis of symbiotic mutants of host plants and bacterial microsymbionts has revealed that present-day endosymbioses require the coordinated induction of more than one signalling pathway for development.

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Arbuscular mycorrhiza (AM), a symbiosis formed between the majority of land plant species (Fig. 1) and zygomycete fungi of the order Glomales [1], leads to an improved uptake of phosphate from the soil [2,3], and its contribution to the acquisition of nitrogen and other macro- and micro-nutrients might be equally significant [4]. During AM formation, fungal hyphae penetrate the epidermis of the root and grow towards the inner cortex, where they form arbuscules: highly branched structures that are thought to be the site of nutrient exchange between the two symbiotic partners. AM-like interactions were detected in early land plants; root nodule symbioses (RNS) with nitrogen-fixing bacteria evolved later (Fig. 1). Legumes engage in RNS with a phylogenetically diverse group of Gram-negative bacteria referred to as rhizobia. Successful infection with rhizobia leading to the development of nitrogen-fixing nodules is generally host–strain-specific [5], in contrast to the high degree of promiscuity exhibited by AM fungi towards plants. Both AM and RNS are endosymbioses, that is, the respective microsymbiont is hosted intracellularly. Rhizobia are recognized by their hosts via specific Nod factors (NF; lipochito-oligosaccharides), and additional components, such as extracellular polysaccharides, lipopolysaccharides and secreted proteins [5]. Equivalent signal molecules of the microsymbiont have not yet been described for AM or for the actinorhizal symbioses, root nodule symbioses formed by plant species of several families with Gram-positive nitrogen-fixing *Frankia* [6]. Investigations of both AM and RNS in legume species have revealed a genetic overlap between the two types of symbioses, supporting the idea that during the evolution of RNS, parts of an evolutionarily older program were recruited from AM [2,7–14].

The predisposition event: the single most crucial, yet enigmatic step in the evolution of root nodule symbioses

Molecular phylogeny has established that all nodulating plants belong to a clade within the Eurosid I, including the orders Fabales, Fagales, Cucurbitales and Rosales (Fa Fa Cu Ro; Fig. 1) [15]. Based on this restricted occurrence of nodulation, it has been postulated that the common ancestor of this clade had acquired a 'predisposition to nodulate' [16] (Fig. 1). However, the nature of this predisposition has remained obscure and might not be related to the development of a nodule itself [13]. Strikingly, intracellular symbiosis of green plants with bacteria is absent outside the Fa Fa Cu Ro clade, with the one exception of the symbiosis between *Gunnera* and cyanobacteria of the genus *Nostoc* (Fig. 1) [17]. This

 1360-1385/02/$ – see front matter PII: S1360-1385(02)02356-7