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REVIEW PAPER

Chromatin meets the cell cycle

Cécile Raynaud, Allison C. Mallory, David Latrasse, Teddy Jégu, Quentin Bruggeman, Marianne Delarue, Catherine Bergounioux and Moussa Benhamed*

Institut de Biologie des Plantes, UMR8618 Université Paris-Sud XI, 91405 Orsay, France

* To whom correspondence should be addressed. E-mail: moussa.benhamed@u-psud.fr

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Abstract

The cell cycle is one of the most comprehensively studied biological processes, due primarily to its significance in growth and development, and its deregulation in many human disorders. Studies using a diverse set of model organisms, including yeast, worms, flies, frogs, mammals, and plants, have greatly expanded our knowledge of the cell cycle and have contributed to the universally accepted view of how the basic cell cycle machinery is regulated. In addition to the oscillating activity of various cyclin-dependent kinase (CDK)–cyclin complexes, a plethora of proteins affecting various aspects of chromatin dynamics has been shown to be essential for cell proliferation during plant development. Furthermore, it was reported recently that core cell cycle regulators control gene expression by modifying histone patterns. This review focuses on the intimate relationship between the cell cycle and chromatin. It describes the dynamics and functions of chromatin structures throughout cell cycle progression and discusses the role of heterochromatin as a barrier against re-replication and endoreduplication. It also proposes that core plant cell cycle regulators control gene expression in a manner similar to that described in mammals. At present, our challenge in plants is to define the complete set of effectors and actors that coordinate cell cycle progression and chromatin structure and to understand better the functional interplay between these two processes.

Key words: Cell cycle, chromatin, epigenetic, gene expression, histone modification, replication.

Introduction

One major aspect of the cell cycle is the faithful duplication and transmission to daughter cells of the genetic and epigenetic information contained within nuclear DNA. The first cytological studies of cell division clearly highlighted the link between chromatin and the cell cycle. As early as during the 19th century, while studying the process of cell division, W. Flemming observed that the material found in the nucleus, which he called chromatin because he could stain it with basophilic dyes, formed thread-like structures in dividing cells: he thus called cell division mitosis, from the Greek word for thread. Chromatin modifications are epigenetic outputs that are key determinants of cell fate and genome stability: microscopic observation of nuclei clearly shows heterogeneity in chromatin staining. Weakly stained regions were called euchromatin and correspond to transcriptionally active parts of the genome, whereas strongly stained regions, called heterochromatin, correspond to transcriptionally inactive regions. Heterochromatin is instrumental in the maintenance of genome integrity because it facilitates the silencing of transposons and repetitive elements. It also permits the expression of different sets of genes in different cell types. In animals, cell fate is determined early in development: they retain stem cells that are capable of dividing and giving rise to a precise cell type. Plants, in contrast, possess stem cells in meristems that lead to the formation of whole organs containing several different cell types. Nevertheless, the position of cells in meristems, especially in the root, establishes the identity of their progeny. Hence, in both animals and plants, completion of the cell cycle allows transmission of both genetic and epigenetic information to daughter cells.

One distinctive feature of plants compared with animals is the more frequent occurrence of endoreduplication (a succession of S phases without mitosis). Interestingly, this process is often associated with cell differentiation (De Veylder *et al.*, 2011; Fox and Duronio, 2013), suggesting that this particular type of cell cycle may be concomitant with the deposition of epigenetic marks required for the specification of cell identity.

The molecular bases of epigenetics have been described extensively, even though the connections between individual chromatin modifications and their functions are not always clear. Chromatin is the association between DNA and nucleosomes that allows the compaction of centimetres or even metres of DNA in a nucleus that is only a few micrometres wide. Nucleosomes are histone octamers containing two copies of each of the four histone proteins, H2A, H2B, H3, and H4, around which 146 bp of DNA are wrapped, forming the basic unit of chromatin. The structure of chromatin is regulated by a variety of mechanisms including histone modifications, direct alterations of histone–DNA interactions, DNA methylation, non-coding RNA-directed silencing, and the replacement of canonical histones by variants.

According to the histone code hypothesis, histone post-translational modifications are placed and removed by proteins called writers and erasers, respectively, while reader proteins recognize these modifications and interpret them into functional outcomes (Jenuwein and Allis, 2001). The most common histone modifications involved in the regulation of chromatin condensation are methylation and acetylation of lysine residues. In plants, SET-domain proteins are responsible for the methylation of histones, whereas histone demethylases belong to two classes: LSD1-like and JMJ proteins (Liu *et al.*, 2010). Histone acetylation and de-acetylation is achieved by histone acetyltransferases (HAT) and histone deacetylases (HDACs), respectively, which are each grouped into four classes based on primary homologies in yeast and mammals (Pandey *et al.*, 2002). All of these families of histone modifiers are extremely diverse: for example, the *Arabidopsis* and rice genomes encode 41 and 37 SET domain proteins, respectively (Liu *et al.*, 2010). This diversity probably allows specific regulation of gene expression during development or in response to environmental changes.

Reader domains and histone modification domains often are associated either on the same protein or on two subunits of a single protein complex, allowing pre-existing histone marks to influence further chromatin modifications. Well known examples are the Polycomb repressor group protein complexes (PRCs) involved in the repression of gene expression: in animals, PRC2 is responsible for the deposition of H3K27me3 (trimethylation of Lys27 of histone H3), which allows recruitment of PRC1, leading to the deposition of the monoubiquitin mark on Lys119 of histone H2A and to chromatin compaction (Aloia *et al.*, 2013). Similar mechanisms exist in *Arabidopsis*: most of the PRC2 core subunits have several plant homologues (Hennig and Derkacheva, 2009). The existence of a plant PRC1-like complex was for a long time a matter of debate, but LHP1 (LIKE-HETEROCHROMATIN 1) was shown to bind PRC2-mediated H3K27me3 through its chromodomain and to function like the animal PRC1 component Polycomb (Pc) in stabilizing H3K27me3-mediated transcriptional silencing (Berr *et al.*, 2011). Also, Pc has been shown recently to bind directly the PRC2 subunit MSI-1 (Multicopy Suppressor of Ira 1) (Derkacheva *et al.*, 2013). This repressive heterochromatin mark is often associated with DNA methylation (Roudier *et al.*, 2011), which is deposited by DNA methyltransferases such as MET1, and removed either via active mechanisms involving base excision repair proteins or via passive mechanisms (Saze *et al.*, 2012).

Another important group of proteins involved in the control of chromatin structure is chromatin remodellers, which use the energy of ATP hydrolysis to modify DNA–histone interactions and alter the location or conformation of nucleosomes. Four classes of ATP-dependent chromatin remodellers, characterized by core ATPase subunits (SWI/SNF, INO80, ISWI, and NURD/Mi-2/CHD), have been isolated in eukaryotes, and all of these proteins have homologues in plant genomes (Clapier and Cairns, 2009). Chromatin remodellers alter chromatin structure in various way: they can promote nucleosome sliding, ejection, or unwrapping and facilitate the exchange of histone variants (Clapier and Cairns, 2009).

Chromatin structure and cell cycle regulation are connected intimately. As described above, the most obvious link is that chromatin metabolism and structure are at the heart of cell cycle progression, for both the duplication and the segregation of the genome. However, cell cycle regulation and chromatin are intertwined in many other ways: genetic evidence for this comes from the observation that many mutants deficient for chromatin modifiers display defects in the control of cell proliferation and, inversely, many mutants primarily affected in cell cycle regulation also display anomalies in the maintenance of transcriptional gene silencing. Recent examples are the antagonistic roles of the remodelling factor PICKLE (PKL) and the Polycomb group (PcG) protein CURLY LEAF (CLF) in the control of root meristem activity (Aichinger *et al.*, 2011), and the involvement of the *Arabidopsis* DNA replication factor C (RFC) in the maintenance of gene silencing (Liu *et al.*, 2010). Other examples will be discussed in detail below. In this review, we will describe the relationships between chromatin and the cell cycle, first by illustrating how chromatin structure changes during DNA replication and mitosis, and how these changes both follow and govern cell cycle progression. In the second part of this review, we will focus on the regulation of cell cycle gene expression via epigenetic mechanisms, and on the role of core cell cycle regulators in the control of gene expression and cell fate.

Chromatin dynamics during the cell cycle: an effector and an actor of cell cycle progression

In all eukaryotes, DNA replication begins at precise positions of the genome. These sites are termed replication origins and are defined by the binding of the origin recognition complex (ORC). In late G_1, CDT1 and CDC6 are recruited to ORCs, allowing the loading of mini-chromosome maintenance (MCM) proteins, which are considered as DNA helicases,

to form the pre-replication complex (pre-RC). Subsequent phosphorylation by cyclin-dependent kinases (CDKs) and CDC7-Dbf4 and the loading of other factors such as CDC45 and GINS (go ichi ni san) allow formation of the pre-initiation complex, which recruits DNA polymerases to form the replisome (reviewed in Diffley, 2011). This model, established mainly in yeast and animals, probably applies to plants because homologues of all of these factors have been identified in plant genomes (Shultz *et al.*, 2007; Sanchez Mde *et al.*, 2012).

Chromatin modifications such as histone modifications probably play an important role throughout the S phase because both loading of the pre-RC and progression of the replication fork require local loosening of chromatin structure (Fig. 1). Consistent with this idea, chromatin marks play critical roles in the positioning of replication origins, the timing of replication, and the progression of the replication fork (see below). Our knowledge regarding the respective roles of chromatin modifications in all of these processes comes largely from studies performed in yeast or animal cells, although evidence for conservation of these mechanisms in plants continues to accumulate. Indeed, immunofluorescence studies in various plant species have revealed the dynamics of various epigenetic marks in S-phase nuclei. For example, as S phase progresses, *Arabidopsis* nuclei display an increase in H3K18ac (acetylation of Lys18 of histone H3) and H4K16ac, whereas barley nuclei become enriched in H4K5ac, H4K8ac, and H4K12ac (reviewed in Costas *et al.*, 2011b).

Role of chromatin organization in the specification and activation of replication origins

In all eukaryotes, except budding yeast, a clear consensus sequence required for the binding of ORC proteins on origins has not been identified. Although genome-wide studies have established that yeast replication origins appear to be AT rich, in contrast to metazoan and plants origins, which appear to be GC rich (Mechali *et al.*, 2013), sequence information alone is not sufficient to specify replication origins. As a general rule, replication origins are located preferentially in accessible genomic regions: metazoan origins often are found in the vicinity of gene promoters (Cadoret *et al.*, 2008; Sequeira-Mendes *et al.*, 2009; Cayrou *et al.*, 2011), and ~77% of the identified origins in *Arabidopsis* are located in genes (Costas *et al.*, 2011a). Several epigenetic features probably contribute to the positioning of origins on the genome: replication origins tend to be located in nucleosome-depleted regions and enriched in the histone variants H3.3 and H2A.Z (Mechali *et al.*, 2013). Although it is unknown whether replication origins are also enriched for specific histone marks, a great number of histone modifications have been reported to be over-represented at metazoan or yeast origins (Dorn and Cook, 2011). Among those modifications, H3K4me3 and H4K5ac as well as the histone variant H2A.Z have been found to be enriched in *Arabidopsis* replication origins (Costas *et al.*, 2011a). It is tempting to speculate that these histone modifications may be required both to specify and to activate replication. Indeed, recent results obtained in *Drosophila* suggest that recruitment of the HAT complex SAGA and chromatin remodeller Brahma is required to generate an open chromatin state favourable for ORC binding (Vorobyeva *et al.*, 2013). In addition, several chromatin modifiers are required for the assembly or activation of the pre-RC after ORC loading. For example, H3K4 methylation positively regulates assembly of the pre-RC in mammals (Tardat *et al.*, 2010) and in yeast (Rizzardi *et al.*, 2012). Likewise, both the chromatin remodelling complex SNF2H and the HAT HBO1 interacts with

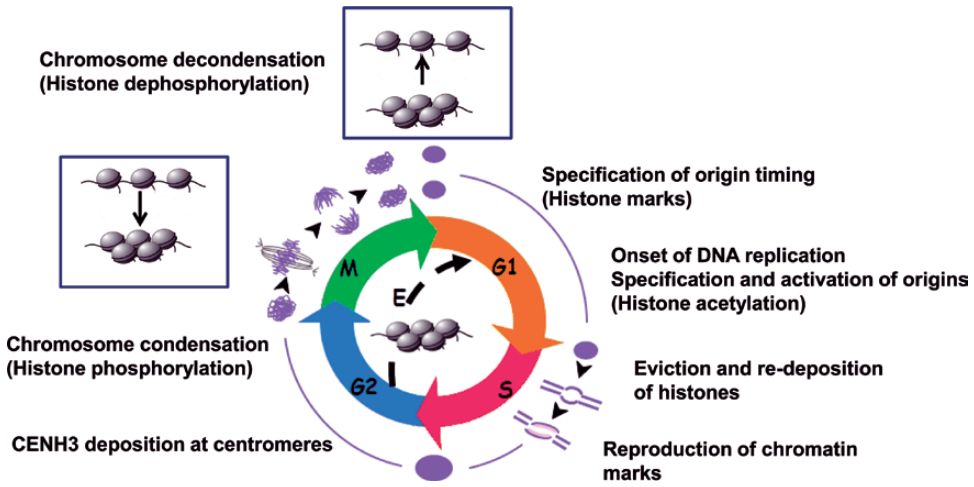

Fig. 1. Interplay between chromatin structure and cell cycle regulation. All steps of cell cycle progression from the initiation of DNA replication to mitosis depend on chromatin modifications. Indeed, the deposition of several histone marks (mainly histone acetylation) possibly as early as mitosis or G_1 governs replication timing of each region of the genome. At the G_1/S transition, histone acetylation is also required for the specification and activation of replication origins. During S phase, the chromatin structure has to be loosened to allow fork progression, and to be reconstructed behind the fork. This implies nucleosome dynamics as well as reproduction of pre-existing chromatin marks. During the G_2 phase, deposition of the CENH3 variant at centromeres prepares mitosis. During mitosis, chromosome condensation is mediated by histone modifications, mainly phosphorylation. Finally heterochromatin may function as a barrier against re-replication or endoreduplication (E), avoiding re-entry into S phase without mitosis (dashed line).

CDT1, and these interactions are required for the recruitment of the MCM complex in animal cells (Miotto and Struhl, 2010; Sugimoto *et al.*, 2011). HBO1 is not conserved in yeast, suggesting that its role in DNA replication evolved recently (Miotto and Struhl, 2008); whether this function is conserved in plants remains to be established. Interestingly, HAM1 and HAM2, two HATs belonging to the MYST family, like HBO1, are redundantly required for gametophyte development: pollen grains and embryo sacks lacking both genes fail to undergo mitosis (Latrasse *et al.*, 2008), a phenotype reminiscent of the one observed in *cdt1* mutants (Domenichini *et al.*, 2012).

Role of chromatin structure in the control of replication timing

In addition to their contribution to the specification and activation of replication origins, epigenetic marks and chromatin organization also appear to play a crucial role in the setting of replication timing. Indeed, as early as the 1970s, observations that short bromodeoxyuridine (BrdU) labelling resulted in clear banding of human chromosomes rather than dispersed staining led to the recognition that large regions of the genome replicate synchronously (Latt, 1975). This idea was generalized rapidly to other eukaryotes, including plants (Van't Hof and Bjerknes, 1981). Since then, extensive progress has been made in our knowledge of replication timing in various eukaryotes, and the recent development of next-generation sequencing techniques has allowed genome-wide analysis of replication timing in several species and cell types (Farkash-Amar and Simon, 2010). As a general rule in metazoa, early-replicating regions appear to be gene rich, and actively expressed, whereas late-replicating regions seem to correspond to heterochromatin (Farkash-Amar and Simon, 2010). Again, analyses performed in plants provide evidence for the conservation of this mechanism (Lee *et al.*, 2010).

In animals and yeast, several mechanisms appear to contribute to the regulation of replication timing, including the availability of initiation factors, histone modifications, and even *cis*-acting sequences (Mechali *et al.*, 2013). Indeed, initiation factors must be recycled from early replicated regions to activate late origins, and overexpression of initiation factors alters the replication programme in yeast (Mantiero *et al.*, 2011; Tanaka *et al.*, 2011). The accessibility of these initiation factors to different origins is regulated by epigenetic mechanisms, particularly by histone acetylation: specific histone marks such as H3K18ac or H3K27ac are associated with early replication in *Drosophila* (Eaton *et al.*, 2011), and, in yeast, deletion of the HDAC Rpd3 advances the replication of late-firing origins (Aparicio *et al.*, 2004). In *Arabidopsis*, the early-/mid-replicating domains are enriched for H3K56ac and depleted of 5-methylcytosine (5mC) and H3K9me2, whereas the late-replicating domains display repressive epigenetic marks characteristic of heterochromatin, namely H3K9me2 and 5mC (Lee *et al.*, 2010). Interestingly, the control of replication timing seems to involve elements of DNA damage stress checkpoints that play an important role in delaying the activation of late origins (Mechali *et al.*, 2013). Consistently, many *Arabidopsis* mutants with defects in chromatin remodelling that may affect the progression of replication forks (see below) show constitutive activation of the DNA damage response (Cools and De Veylder, 2009).

In addition, replication timing translates into three-dimensional organization of the chromatin: circular chromatin conformation capture and fluorescence *in situ* hybridization (FISH) experiments recently showed that early-replicating regions are associated physically in the nucleus of human cells (Moindrot *et al.*, 2012). The function of this spatial organization remains to be established, but it is tempting to speculate that it may facilitate synchronous replication of distant regions by generating nuclear subdomains enriched in initiation factors. Investigation of replication timing in plants is still in its infancy; however, the development of efficient EdU (ethynyl deoxyuridine) labelling *in vivo* (Kotogány *et al.*, 2010), together with FACS (fluorescence activated cell sorting) technology and next-generation sequencing techniques should soon allow breakthroughs in this field.

Further studies will be required to elucidate fully the epigenetic marks and chromatin modifiers or readers that contribute to the specification of the initiation site of replication and replication timing. Results obtained in plants and other eukaryotes suggest that the exact nature of the epigenetic marks involved is not necessarily conserved between organisms, but that the same structural features are associated with active or inactive replication origins, consistent with chromatin accessibility being a key factor both for the specification of origins and for their activation.

Progression of the replication fork: disrupting and reconstructing chromatin structure

Chromatin structure needs to be loosened to allow DNA replication. One universal mechanism that contributes to this process is likely to be the phosphorylation status of histone H1, which is involved in the establishment of higher order chromatin structure and is regulated by CDK2 via CDC45-dependent targeting (Alexandrow and Hamlin, 2005). In addition, progression of the replication fork requires disruption of nucleosomes into two H2A–H2B heterodimers and a (H3–H4) tetramer ahead of the fork, followed by transfer of parental histones to the leading or the lagging strand and *de novo* histone incorporation. This process is likely to involve ATP-dependent chromatin-remodelling complexes of the SWI/SNF family, although it is not clear whether they play a role in the eviction of nucleosomes ahead of the fork, the formation of chromatin behind the fork, or both (Groth et al, 2007b).

Other important actors in replication fork progression in mammalian cells are histone chaperones, which can potentially act as histone acceptors and facilitate the transfer of histones onto the daughter strands. Consistently, both the H2A–H2B chaperone FACT (Gambus *et al.*, 2006; Tan *et al.*, 2006) and the H3–H4 chaperone Asf1 (Groth *et al.*, 2007a) associate with MCM proteins and are required for replication fork progression, probably by coordinating histone supply (parental and new) with the replication fork. This role of Asf1 is likely to be conserved in plants because *Arabidopsis*

mutants lacking the two proteins ASF1a and ASF1b display reduced cell proliferation and DNA damage response activation (Zhu *et al.*, 2011). In addition, ASF1a and ASF1b are targets of E2F transcription factors, and their expression is up-regulated during S phase (Lario *et al.*, 2013).

Another histone chaperone, CAF-1, is probably responsible for *de novo* histone deposition (Groth *et al.*, 2007b). Indeed, CAF-1 specifically associates with the major S-phase histones H3.1 and H4, but not the H3.3 variant, which is incorporated into chromatin independently of DNA replication. CAF-1 is recruited on the replication fork via its interaction with the processivity factor of DNA polymerase proliferating cell nuclear antigen (PCNA; Shibahara and Stillman, 1999). Again, this mechanism is probably conserved in plants. In *Arabidopsis*, CAF-1 is composed of three subunits: FASCIATA1 (FAS1), FASCIATA 2 (FAS2), and MULTICOPY SUPPRESSOR OF IRA1 (MSI1) (Ramirez-Parra and Gutierrez, 2007b), and *Arabidopsis* mutants lacking CAF-1 activity display severe growth defects, some of which are due to a reduction of cell proliferation (Ramirez-Parra and Gutierrez, 2007a). Nevertheless, an interesting observation is that *Arabidopsis fas* and *asf1ab* mutants are viable, which is in sharp contrast to the situation observed in metazoan cells where CAF-1 and ASF1 appear to be essential for viability (Quivy *et al.*, 2001; Sanematsu *et al.*, 2006). This intriguing observation suggests the involvement of other factors in chromatin dynamics in the vicinity of the replication forks, or higher plasticity of replication-associated chromatin metabolism in plants. Indeed, at least two other groups of proteins have been proposed to participate in histone chaperoning during DNA replication. Nucleosome assembly proteins (NAPs) interact with histones H2A and H2B and probably are involved in their incorporation into chromatin during S phase (Zhu *et al.*, 2006), and TONSUKU/BRU1 has functions that are partially overlapping with those of CAF-1, although its molecular function remains to be determined (Takeda *et al.*, 2004).

How are chromatin marks reproduced once histones are incorporated in newly synthesized DNA? Histone modifications present on parental nucleosomes, if they are maintained through disassembly and reassembly of nucleosomes, may serve as a template for the reproduction of chromatin domains during replication. Data available thus far in animals and yeast provide evidence for complex mechanisms involved in the maintenance of heterochromatin during replication. PCNA appears to play a prominent role in this process, by functioning as a hub coupling chromatin restoration to replication. Indeed, in animal cells, PCNA interacts with various chromatin modifiers including SNF2H, Dnmt1 (a DNA methylase), HMTs, and HDACs, and recruits CAF-1, which can, in turn, recruit proteins involved in histone H3 methylation (Groth *et al.*, 2007b). Some of these enzymes are considered as general chromatin maturation factors [e.g. HDACs are probably required for the transient acetylation of newly synthesized histones (Bhaskara *et al.*, 2013)], while others clearly are specifically involved in the deposition of repressive marks at particular loci. In plants, two SET-domain proteins have been isolated for their ability to interact with PCNA (Raynaud *et al.*, 2006), and further analysis confirmed that they function as H3K27 methyltransferases and are likely to be involved in the reproduction of this mark during replication (Jacob *et al.*, 2009). Based on the mechanisms described in mammals, plant PCNA proteins probably recruit MET1 (the plant homologue of Dnmt1) to allow faithful reproduction of DNA methylation during replication, and possibly other HMTs.

In addition to PCNA, other proteins of the plant replication complex have been shown to recruit chromatin modifiers. Indeed, mutations in several core replication proteins including DNA polymerases α and ε result in loss of transcriptional gene silencing (reviewed in Liu and Gong, 2011), suggesting that they all contribute to the maintenance of heterochromatin, although the molecular mechanisms are incompletely described and are largely inferred from studies performed in animals and yeast. Finally, DNA replication may also be regarded as a window of opportunity to modify the epigenome of cells and commit them to a new cell fate. It has been suggested that during the replication timing decision point, very early in G_1, major changes occur in the chromatin structure in response to extracellular cues that set the replication timing, which in turn impacts the epigenetic marks and, thus, the future expression status of genes (Gilbert, 2010). In agreement with this hypothesis, in mouse, parental imprinting is erased by passive de-methylation during S phase, because Dnmt1 is not recruited by PCNA (Kagiwada *et al.*, 2013). Similar events may well be at work in plants: H3K27me3 has been shown to be a key determinant of tissue-specific expression patterns (Lafos *et al.*, 2011), but whether this mark is deposited during S phase in dividing initials and endoreduplicating cells, or independently of the cell cycle has not been explored.

Together, current models for the initiation and progression of S phase highlight the huge diversity of chromatin modifications that either contribute to the regulation of S phase or, in contrast, are targets of S-phase-regulated proteins. In addition, there is now accumulating evidence that specific chromatin marks play a role in the maintenance of genome integrity during DNA replication by avoiding origin re-firing.

Histone modifications as a barrier to both re-replication and endoreduplication

During the faithful transmission of the genome to the daughter cells, it is critical that replication is not initiated more than once at a given origin during S phase. A wealth of mechanisms targeting various pre-RC subunits that prevent origin re-firing have been described (reviewed in Costas *et al.*, 2011c), but other pathways may exist that target histones. For example, in animals, H4K20me1 decreases during S phase, whereas H4K20me2 increases. These changes in the degree of methylation of the same histone have been proposed to inhibit origin re-firing: H4K20me2 could maintain an inactive chromatin state with respect to pre-RC formation until this mark is removed during the next G_1 phase (Dorn and Cook, 2011). Intriguingly, in *Arabidopsis*, maintenance of heterochromatin seems to play an important role not only in the maintenance

of transcriptional gene silencing, but also in the regulation of DNA replication itself. Indeed, mutations in the H3K27 methyltransferases *ATXR5* and *ATXR6* lead to decondensation of chromocentres as well as partial re-replication of heterochromatin regions (Jacob *et al*., 2010). Overexpression of the CDK inhibitor KRP5 in the *atxr5 atxr6* double mutant increases chromatin decondensation and promotes endoreduplication, suggesting that condensation of heterochromatin functions as a barrier to DNA replication initiation and possibly endoreduplication (Jégu *et al.*, 2013).

Chromosome condensation and mitosis

After duplication of the genome, its equal repartitioning between the two daughter cells requires extreme compaction of the chromosomes to untangle chromatin and form robust structures that can withstand the forces pulling the DNA toward opposite poles of the cell during mitosis. In both yeast and metazoa, chromatin condensation during mitosis is associated temporally with an increase in several histone modifications including phosphorylation of Ser10 on histone H3, H2B ubiquitination, and phosphorylation of histone H1 (Xu *et al.*, 2009). These modifications are catalysed by different enzymes: Aurora kinases phosphorylate histone H3, whereas histone H1 is a substrate of CDK2 (Xu *et al.*, 2009). Several immunolabelling experiments have been performed on various plant species to follow histone modifications throughout mitosis, and these studies have shown that H3S10 phosphorylation is conserved in plants, but that other histone modifications (both phosphorylation on different residues and other covalent modifications) occur during plant mitosis (reviewed in Costas *et al.*, 2011b). Surprisingly, the role of H3S10P in plants may relate more to the control of sister chromatid cohesion than to chromosome condensation (Kaszas and Cande, 2000). Nevertheless, Aurora kinases are conserved in plants, preferentially phosphorylate H3S10 (Demidov *et al.*, 2005), and are required for normal cell proliferation (Petrovska *et al.*, 2012). Other conserved effectors of chromosome condensation are condensins, multisubunit complexes that confer the ability to supercoil DNA positively via an ATP-dependent mechanism (Thadani *et al.*, 2012). They are regulated by various mechanisms including phosphorylation by CDKs, Aurora kinase, and Polo kinase (Thadani *et al.*, 2012). Plant cohesins belong to large gene families with specific functions in chromosome condensation but also in DNA repair, homologous recombination, etc. (Schubert, 2009). Hence, histone modifications and specific proteins governing chromatin organization cooperate to allow chromosome condensation during mitosis in all eukaryotes including plants, although some differences in the molecular mechanisms exist.

Finally, a particular chromatin organization is required for centromere function and, thus, for sister chromatid segregation. Plants, and more specifically maize, have long been used as a tool to study centromeres. Typical epigenetic marks of functional centromeres include the binding of a conserved variant of the conventional histone H3, termed CENH3 in plants (Lermontova *et al.*, 2011a), as well as the presence of H2AT133P (Dong and Han, 2012) and H3S10P (Houben *et al.*, 1999). The numerous reports of inactive centromere sequences in various genomes, and the observation that reintroduction of centromeric sequences is not sufficient to generate active centromeres, support the hypothesis that epigenetic modifications of centromeres are essential to their function (Birchler *et al.*, 2011). Cytological analyses clearly show that CENH3 is deposited at centromeric regions during G_2 (Lermontova *et al.*, 2011b), but how this deposition is governed remains largely unknown. One likely mechanism to avoid premature incorporation of CENH3 into chromatin is the repression of the CENH3 gene by EF2 transcription factors (Heckmann *et al.*, 2011). In humans, ORC proteins have also been found to be associated with centromeres. This association may reflect their role in the formation of heterochromatin (see below), or a direct role in centromere function because many ORC mutations activate the spindle checkpoint mutation in yeast (Gibson *et al.*, 2006). The role of ORC proteins in centromere function has not been reported in plants, partly due to the fact that *orc* null mutants are lethal (Collinge *et al.*, 2004; de la Paz Sanchez and Gutierrez, 2009).

Interestingly, some chromatin events occurring during mitosis appear to be important for the next S phase: the activity of the HMT PR-Set7 responsible for H4K20 monomethylation is cell cycle regulated and peaks in late G_2 and early M. Deposition of H4K20me would create a burst of histone acetylation, which would in turn allow the formation of the pre-RC during the next S phase (Brustel *et al.*, 2011).

The data summarized so far describe the dynamics of chromatin structure during the cell cycle. Some of these changes are the consequence of cell cycle progression, whereas others appear to function as regulators of the cell cycle. Interestingly, the same reciprocal interaction exists in the control of cell cycle genes via chromatin changes, as we will discuss below.

Interplay between cell cycle regulators and regulation of gene expression by chromatin modifiers: pathways to control cell proliferation and cell fate

Histone modifications and cell cycle gene regulation: the E2F/RBR module as a switch to regulate entry into the cell cycle and cell fate

In almost all eukaryotes, the pRB–E2F pathway is considered as a major mechanism bridging the activity of the cell cycle machinery with transcription, particularly at the G_1/S transition. In quiescent cells (G_0) and during early G_1 phase, pRb or related proteins called pocket proteins bind and inactivate the E2F transcription factors. Later, during late G_1 phase, phosphorylation of pRB by CDKs results in its release from the promoter by altering the E2F–pRB interaction, leading to transcription activation, which irreversibly commits the cell to undergo DNA replication (Dimova and Dyson, 2005).

This regulatory module is conserved in plants (Zhao *et al.*, 2012): like in animals, plant E2Fs have been shown to target many genes involved in DNA replication (Naouar *et al.*, 2009) and, in *Arabidopsis*, E2Fa and E2Fb are

positive regulators of cell proliferation (De Veylder *et al.*, 2002; Magyar *et al.*, 2005; Sozzani *et al.*, 2006). Conversely, loss of RBR (the plant homologue of pRb) is gametophytic lethal and results in supernumerary divisions in developing embryo sacs (Ebel *et al.*, 2004). Down-regulation of RBR expression or targeted inactivation of the protein in various plants and tissues has shown that RBR restricts cell proliferation in developing embryos (Gutzat *et al.*, 2011) and leaves (Park *et al.*, 2005; Desvoyes *et al.*, 2006), as well as endoreduplication in leaves (Desvoyes *et al.*, 2006; Borghi *et al.*, 2010) and endosperm (Sabelli *et al.*, 2013), suggesting that a canonical RB pathway is likely to be required for the specification of stem cells (Wildwater *et al.*, 2005).

However, this relatively simple model for the regulation of cell cycle onset conceals a far more complex situation (Fig. 2). First, E2F transcription factors can be grouped into two classes differing by the molecular mechanisms allowing them to bind DNA. (Lammens *et al.*, 2009). Secondly, some E2Fs function primarily as repressors of cell proliferation and can promote terminal differentiation (Trimarchi and Lees, 2002). For example, in *Arabidopsis*, E2Fc overexpression inhibits cell proliferation (del Pozo *et al.*, 2002): E2Fc is thought to associate constitutively with RBR to repress cell proliferation in dark-grown seedlings and to be degraded upon re-illumination to allow plant growth (Lopez-Juez *et al.*, 2008). Thirdly, it is now clear that pocket proteins repress the activity of E2Fs not only by masking their transactivation domain but also by modifying chromatin structure on E2F target genes. In animals, pRB recruits chromatin remodelling factors such as HDAC and Pc proteins, which induce chromatin condensation and repress the promoter activity of E2F-DP target genes (Trimarchi and Lees, 2002), as well as different sets of genes via its interaction with other types of transcription factors (Skapek *et al.*, 2006). This allows pRB to modify chromatin structure stably at given loci, thereby playing a key role in the determination of cell fate and differentiation. This chromatin function of RBR is at least partly uncoupled from the canonical CDK-regulated E2F/RBR module because the role of RBR in the regulation of cell proliferation and in the control of gene expression can be uncoupled (Johnston *et al.*, 2010; Sabelli *et al.*, 2013).

The role of Rb proteins in the control of cell fate probably relates to their ability to interact directly with chromatin modifiers: both animal pRB and plant RBR interact with the conserved RPA48/MSI1 protein, which is a subunit of PRC2 complexes (Ach *et al.*, 1997). Consistently, mutants deficient for PRC2 complex subunits display fertilization-independent proliferation of female gametophyte cells (Kiyosue *et al.*, 1999; Ohad *et al.*, 1999), reminiscent of the defects observed in *rbr* mutants (Ebel *et al.*, 2004). At the molecular level, inactivation of RBR results in a reduction of H3K27 trimethylation, and its interaction with MSI1 appears to be required for imprinting (Jullien *et al.*, 2008). Likewise, suppression of the embryonic developmental programme after germination requires RBR-dependent H3K27me3 to silence embryonic genes permanently (Gutzat *et al.*, 2011). An additional layer of complexity comes from the fact that many genes encoding subunits of the PRC2 complex [namely *CURLY LEAF* (*CLF*), *VERNALIZATION 2* (*VRN2*), and *MET1*] are actually RBR targets, while PRC2-specific H3K27 trimethylation activity represses paternal RBR allele development, indicating that RBR and PRC2 complexes are part of a reciprocal regulatory circuit that controls gametophyte development (Johnston *et al.*, 2008, 2010).

Finally, some E2Fs, such as the human protein E2F6 and all atypical E2Fs, lack an RB binding domain, suggesting that at least some E2Fs function independently of RB. Indeed, E2F1 recently has been shown to be differentially phosphorylated upon DNA damage, resulting in the production of pRb-bound and pRb-free E2F1 forms: in this context, pRb–E2F complexes are required for cell cycle arrest, while both populations of E2F1 are required for full expression of pro-apoptotic genes (Carnevale *et al.*, 2012). Likewise, E2F6 has been found to bind PcG proteins (Ogawa *et al.*, 2002), and in this way could directly regulate genes involved in cell cycle progression (Attwooll *et al.*, 2005). Whether plant E2Fs can directly bind chromatin remodelling factors remains to be established, but recent results have shown that both free E2Fa and RBR-bound E2Fa regulate cell proliferation and endoreduplication separately (Magyar *et al.*, 2012).

Thus, both E2Fs and RB proteins are associated intimately with chromatin remodelling factors to control not only cell cycle progression but also cell fate and developmental programmes. Similarly, epigenetic changes probably play an important role in the regulation of the G_2/M transition, although only one example has been described so far: mutants lacking the *HISTONE MONO-UBIQUITINATION 1* (*HUB1*) gene show down-regulation of several *CYCA*, *CYCB*, *CDKB*, and kinesin genes as well as genes encoding other factors required for microtubule dynamics (Fleury *et al.*, 2007). Chromatin modifications also induce cell cycle arrest, as shown for the chromatin remodelling factor PROPORZ1, which regulates the expression of cell cycle inhibitors (Anzola *et al.*, 2010). Many chromatin modifiers involved in cell cycle progression probably remain to be identified, including non-coding RNAs. Indeed, a role for non-coding RNAs in cell cycle regulation has been recognized only recently in animals (Hung *et al.*, 2011). Interestingly, recent findings suggest that additional core cell cycle regulators have a chromatin function to control cell division and differentiation (see below).

Involvement of core cell cycle regulators in the control of gene expression: new functions for old proteins

In mammals, it has been clearly demonstrated that, in addition to associating with cyclin–CDKs, the CDK inhibitor p21 participates in a number of other specific protein–protein interactions and exerts functions that are cell type and context dependent. Indeed, p21 both binds to the E2F-1 transcription factor and inhibits its activity (Delavaine and La Thangue, 1999) and to the N-terminus of c-Myc suppressing c-Myc-dependent transcription by interfering with c-Myc–Max association (Kitaura *et al.*, 2000). Moreover, Devgan and colleagues have shown that in keratinocytes,

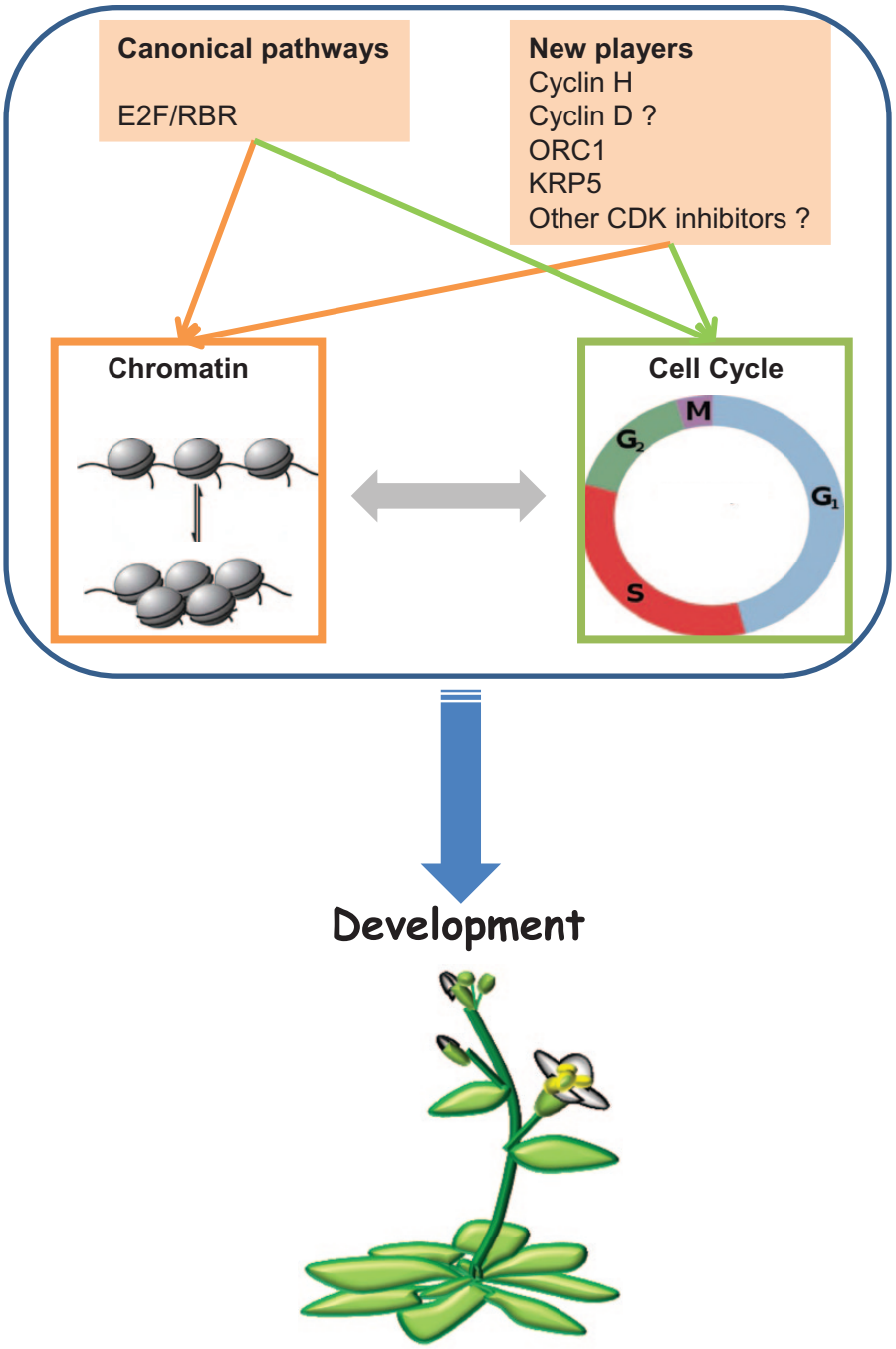

Fig. 2. Regulation of gene expression via chromatin modification governs cell cycle progression and cell fate during plant development. The E2F–Rb pathway is a well known pathway that controls not only the G_1/S transition, but also cell fate and genome imprinting; however, recently, core cell cycle regulators such as ORC1 or KRP5 have been shown to play a direct role in the control of target gene expression, raising the possibility that other cell cycle regulators may play a similar role to that observed in animal cells. These intimate connections allow the coordination of the cell cycle and development as well as adaptation of plant growth to changes in environmental conditions.

p21 functions together with E2F-1 as a selective negative regulator of transcription that inhibits expression of Wnt4 independently of its effects on the cell cycle (Devgan *et al.*, 2005). Until recently, evidence was lacking for a similar role for CDK inhibitors in plants, although several KRP proteins have been reported to display a punctuate accumulation pattern in nuclei (Bird *et al.*, 2007), suggesting that they may bind specific chromatin regions. We have demonstrated that KRP5 binds chromatin and, more precisely, both euchromatin and heterochromatin, possibly via its interaction with the SWI/SNF complex subunit BAF60. Furthermore, comparison of ChIP-seq and transcriptomic data obtained from the analysis of a KRP5 overexpression line revealed that KRP5 binds to genes involved in cell growth and differentiation and stimulates their expression (Jégu *et al.*, 2013), proving that like p21, KRP5 acts not only as a CDK inhibitor but also

as a transcriptional cofactor. Interestingly, systematic tandem affinity purification identified an interaction between a CDK inhibitor from a different family, SMR1, and the ATPase of the SWI/SNF complex BRAHMA, as well as several interactions between CDK inhibitors and transcription factors (Van Leene *et al.*, 2010), suggesting that this chromatin connection may not be restricted to KRP5.

In animals, it is well documented that cyclin D1, in addition to its role as a CDK-dependent cell cycle regulator, also has CDK-independent functions. Cyclin D1 binds and regulates transcription factors and co-activators such as HATs, thereby playing an important role in cellular processes such as hormone response or differentiation (Fu *et al.*, 2004). Like other eukaryotes, plant genomes encode several non-cell cycle-related CDKs and cyclins such as CDKC (Kitsios and Doonan, 2011) and CDKD–cyclin H complexes (Zhou *et al.*, 2013) that are involved in the regulation of gene expression. To date, a role for canonical CDKs or cyclins in the control of gene expression or chromatin metabolism outside of their classical cell cycle-related roles has not been reported in plants. However, plant CDKs and cyclins are exceptionally diverse (Inagaki and Umeda, 2011); thus, future research is likely to demonstrate that they function in a much more complex way than we currently acknowledge.

Finally, several lines of evidence support the idea that subunits of the pre-replication complex also play a role in the regulation of gene expression.

First, ORC proteins are involved in gene silencing independently of their role in DNA replication: ORC1 proteins of yeast and human recruit proteins involved in heterochromatin formation such as HP1 (Sasaki and Gilbert, 2007). Similarly, a genetic interaction between the *Arabidopsis ORC2* and the PcG gene *MEA* (encoding a PRC2 subunit) has been described (Collinge *et al.*, 2004). Surprisingly, plant ORC1 proteins seem to have an opposite effect on gene expression. Indeed, they have the unique feature of harbouring a PHD (plant homeodomain), and *Arabidopsis* ORC1b specifically binds H3K4me to regulate positively the expression of several cell cycle genes such as MCM3, CDT1, and ORC3 (de la Paz Sanchez and Gutierrez, 2009). Secondly, CDT1a and CDT1b interact with the GEM protein, which participates in the maintenance of the repressor histone H3K9 methylation status of root patterning genes (Caro *et al.*, 2007). Lastly, it has been proposed that plant MCM6 homologues are members of the ZF-HD (zinc finger-homeodomain) transcription factor family because they possess a zinc finger motif, and the overexpression of *PsMCM6* confers salt tolerance to tobacco plants, possibly by activating the expression of stress-related genes (Dang *et al.*, 2011). Together, although they are fragmentary, these results open up wide-ranging and exciting prospects regarding the role of DNA replication proteins involved in the control of gene expression.

Concluding remarks

Interplay between chromatin remodelling and cell cycle regulation is critical for plant development, but the links between the cell cycle and chromatin metabolism are just beginning to be established. Even though changes occurring at the chromatin level during the cell cycle are better described in animal cells than in plants, their function in animals is still poorly understood. Future challenges in plants include better description of the system in terms of replication initiation and timing in various cell types and documenting the identity and function of the associated chromatin marks. Other challenges include determining how these mechanisms relate to the maintenance of genome integrity and integrating of all these complex mechanisms into developmental processes such as the formation of organs, reprogramming of germline cells, embryogenesis, or dedifferentiation. In this respect, comparisons with animal systems will probably provide valuable clues regarding the molecular basis for the determination of cell fate, the transition from proliferation to differentiation, and the reactivation of cell cycle progression in non-dividing cells.

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