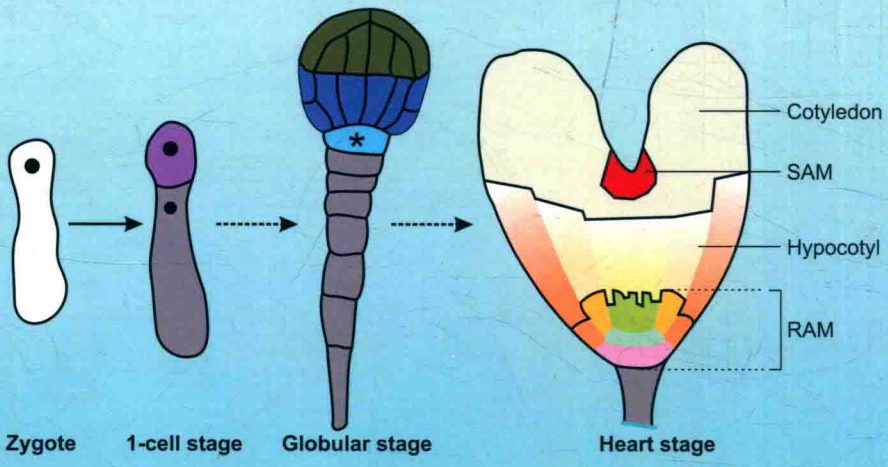
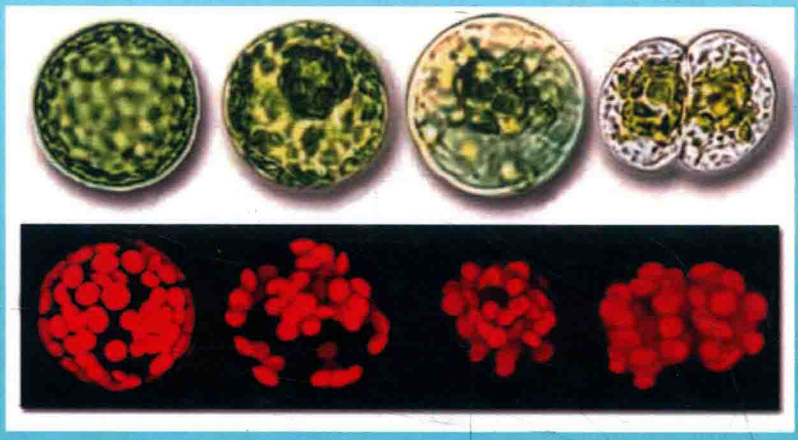
Molecular Cell Biology of the Growth and Differentiation of Plant Cells

Editor: **Ray J. Rose**





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Preface

Plants provide humankind with food, fibre and timber products, medicinal and industrial products as well as ecological and climate sustainability. Understanding how a plant grows and develops is central to providing the ability to cultivate plants to provide a sustainable future. 'Molecular Cell Biology of the Growth and Differentiation of Plant Cells' encompasses cell division, cell enlargement and differentiation; which is the cellular basis of plant growth and development. Understanding these developmental processes is fundamental for improving plant growth and the production of special plant products, as well as contributing to biological understanding. The dynamics of cells and cellular organelles are considered in the context of growth and differentiation, made possible particularly by advances in molecular genetics and the visualization of organelles using molecular probes. There is now a much clearer understanding of these basic plant processes of cell division, cell enlargement and differentiation. Each chapter provides a current and conceptual view in the context of the cell cycle (6 chapters), cell enlargement (5 chapters) or cell differentiation (9 chapters).

The cell cycle section examines the regulation of the transitions of the cell cycle phases, proteins of the nucleus which houses most of the genomic information, the division of key energy-related organelles - chloroplasts, mitochondria and peroxisomes and their transmission during cell division. The final chapter in this section deals with the transitioning from cell division to cell enlargement.

The cell enlargement section considers the organisation of the cell wall, the new technical strategies being used, the biosynthesis and assembly of cellulose microfibrils and signaling dependent cytoskeletal dynamics. There are then chapters on the regulation of auxin-induced, turgor driven cell elongation and hormonal interactions in the control of cell enlargement

The cell differentiation section considers the regulation of the cell dynamics of the shoot and root apical meristems, the procambium and cambial lateral meristems as well as nodule ontogeny in the legume-rhizobia symbiosis. There are chapters on asymmetric cell divisions, stem cells, transdifferentiation, genetic reprogramming in cultured cells and the paradox of cell death in differentiation. The final chapter deals with the protein bodies and lipid bodies of storage cells.

Each chapter is written by specialists in the field and the book provides state of the art knowledge (and open questions) set out in a framework that provides a long term reference point. The book is targeted to plant cell biologists, molecular biologists, plant physiologists and biochemists, developmental biologists and those interested in plant growth and development. The chapters are suitable for those already in the field, those plant scientists entering the field and graduate students. The cover images are taken from Chapters 5 and 12.

Ray J. Rose

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The Plant Cell Cycle

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Plant Cell Cycle Transitions

*José Antonio Pedroza-Garcia, Séverine Domenichini and Cécile Raynaud**

Introduction

Plant development is largely post-embryonic, and relies on the proliferative activity of meristematic cells that can form new organs and tissues throughout the life cycle of the plant. Tight control of cell proliferation is therefore instrumental to shape the plant body. In the root meristem, the quiescent centre cells have a low division rate; they play a key role in the self-maintenance of the stem cell pool and function as a reservoir of stem cells that can divide to replace more actively dividing initials (Heyman et al. 2014). The shoot meristem, although less strictly organized than the root meristem, also contains a pool of slowly dividing cells at its centre. On the sides of the meristem, an increase in mitotic index precedes or at least accompanies primordium outgrowth to initiate leaf development (Laufs et al. 1998). Finally, cell proliferation gradually ceases from the tip of the developing leaf to its base as cells progressively differentiate (Andriankaja et al. 2012). This brief summary of the basic mechanisms underlying plant development perfectly illustrates that tight control of the cell cycle plays a central role in this process (Polyn et al. 2015).

Study of the cell cycle began in the second half of the XIXth century with the discovery of cell division and the understanding that cells originate from pre-existing cells. With the identification of chromosomes as the source of genetic information at the beginning of the XXth century, the cell cycle was placed at the centre of the growth, development and heredity for all living organisms (Nurse 2000). Next, in the 1950s the elucidation of the structure of the DNA molecule, and the use of radioactive labelling led to the finding that in eukaryotes, DNA is duplicated during a restricted phase of the cell cycle in interphase that was called S-phase (for synthesis). The cell cycle was thus divided in four phases, S-phase, M-phase or mitosis and two so-called Gap phases, G1 before S-phase and G2 before mitosis. After these crucial

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conceptual advances, further dissection of the cell cycle and notably of its regulation had to wait until technical progresses allowed its genetic analysis. This was achieved in the 1970s: combination of genetics, biochemistry and molecular biology allowed the identification of Cyclin Dependent Kinase (CDK)-cyclin complexes as the universal motors of cell cycle regulation in all eukaryotes. CDKs are protein kinases that phosphorylate various substrates to promote transitions from one cell cycle phase to the next. Their activity is modulated by their association with the regulatory subunits called cyclins that are characterized by their cyclic accumulation during the cell cycle. In 2001, L. Hartwell, P. Nurse and T. Hunt were awarded the Nobel prize in Physiology or Medicine for their complementary achievements: their work not only unravelled the role of CDK/cyclin complexes but also introduced the concept of checkpoints to explain the observation that impairing one phase of the cell cycle inhibits subsequent progression.

Basic mechanisms regulating cell cycle progression, DNA replication and mitosis are conserved in all eukaryotes including plants. This high degree of conservation allowed fast progress in the understanding of cell cycle regulation in all organisms. For example, the first plant CDK was isolated by functional complementation of a yeast mutant with an Alfalfa cDNA (Hirt et al. 1991), and considerable progress has been made in the last 35 years in our understanding of plant cell cycle transitions. In spite of this conservation of molecular effectors, the plant cell cycle has a number of specificities. One obvious difference concerns plant mitosis that is characterized by the absence of centrosomes and mechanisms governing cytokinesis. Another hallmark of the plant cell cycle is the relatively frequent occurrence of endoreduplication, a particular type of cell cycle consisting of several rounds of DNA replication without mitosis, and leading to an increase in cell ploidy. Although this process can be found in animals, it is generally restricted to relatively specific cell types such as the salivary glands in Drosophila and hepatocytes in mammals (Fox and Duronio 2013). By contrast in plants, it is widely distributed in various organs such as fruits in tomato, endosperm in cereals or even leaves in plants such as Arabidopsis (Fox and Duronio 2013). In addition, there are also differences in terms of molecular mechanisms regulating cell cycle transitions between plants and other eukaryotes. In the present chapter, we will describe plant cell cycle regulation with a specific emphasis on the molecular mechanisms that control cell cycle transitions, and we will briefly discuss how these basic mechanisms are modulated during plant development or according to external stimuli.

Plant CDKs and Cyclins, Motors of Cell Cycle Progression with an Intriguing Diversity

Core CDK/Cyclin complexes

One feature of plants is the surprisingly high diversity of core cell cycle regulators encompassed by their genomes. Indeed, the Arabidopsis genome encodes 5 CDKs distributed in two sub-classes (a single A-type CDK and four B-type CDKs) and 31 Cyclins belonging to three families (10 CycA, 11 CycB and 10 CycD), whereas *Saccharomyces cerevisiae* has a single CDK and 9 Cyclins, and *Homo sapiens* has

4 CDKs and 9 Cyclins (Van Leene et al. 2010). The number of putative CDK/Cyclin pairs is thus very large in plants, making the elucidation of their role problematic. One important step forward in the understanding of how plant CDK/Cyclin complexes control cell cycle transitions has been the comprehensive analysis of their expression in synchronized cell suspensions (Menges et al. 2005) followed by the systematic analysis of interactions between core cell cycle regulators using Tandem Affinity Purification (Van Leene et al. 2010). These results led to a global picture of CDK/cyclin complexes around the cell cycle (Fig.1). According to these studies, CDKA;1 is expressed throughout the cell cycle and stably associates with D-type cyclins and S-phase expressed A-type cyclins as well as with CYCD3;1 in G2/M, suggesting it could be involved in the control of the G1/S as well as the G2/M transition. Consistently, expression of a dominant negative form of CDKA;1 drastically inhibits cell proliferation (Gaamouche et al. 2010). Likewise, CDKB2s are required for normal cell cycle progression and meristem organisation (Andersen et al. 2008). More recently, analysis of *cdka* and *cdkb* knock-out mutants revealed that CDKA;1 is required for S-phase entry, while it redundantly controls the G2/M transition with B-type CDKs (Nowack et al. 2012).

The large size of Cyclin families complicates the genetic analysis of their respective functions, but as for CDKs, a global view of their respective roles has been obtained by compiling information about their expression during the cell cycle and ability to bind to different CDKs. Very schematically, D-type Cyclins are thought to control cell cycle onset whereas A-type cyclins would be involved at later stages during the S and G2-phases in complex with CDKA1;1 or CDKBs and B-type cyclins bound to CDKBs would control the G2 and M phases [Fig.1, (Van Leene et al. 2010)]. However, Cyclin D3;1 has the particularity of peaking both at the G1/S and at the G2/M transition (Menges et al. 2005), and genetic analysis supports its role

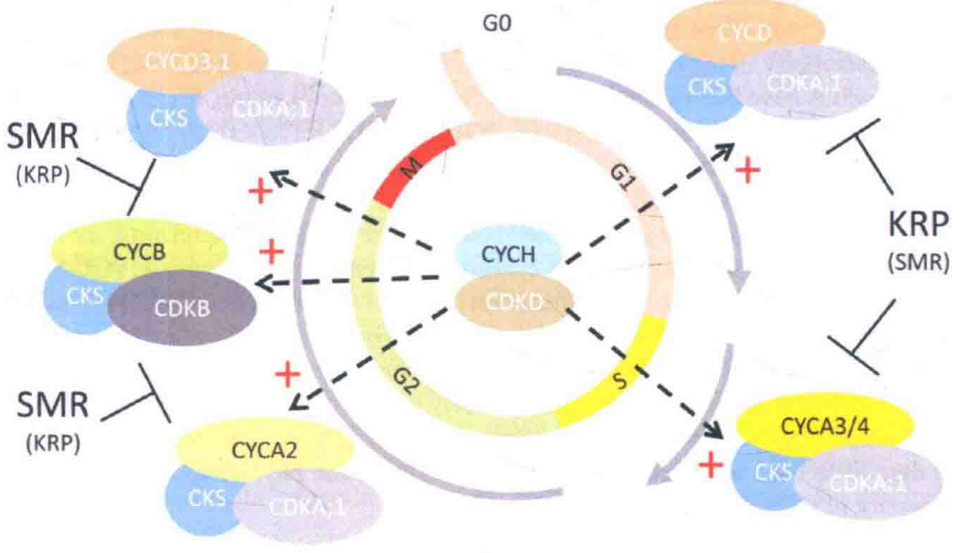

FIGURE 1 Succession of CDK/Cyclin complexes during the cell cycle (adapted from Van Leene et al. 2010). CYCD/CDKA, CYCA/CDKA and CYCB/CDKB sequentially accumulate and are activated to allow progression through the various phases of the cell cycle. CKS sub-units are scaffolding proteins associated with all complexes. Likewise, all CDK/Cyclin complexes are activated by the CYCH/CDKD kinase.

as a positive regulator of both cell cycle transitions (Riou-Khamlichi et al. 1999). Conversely, triple mutants lacking the whole *CYCD3* family show premature exit of cell proliferation towards endoreduplication (Dewitte et al. 2007). Very few genetic studies have been performed on A-type cyclins, and their respective roles are thus largely inferred from expression and interaction data. Nevertheless, the proposed role for Cyclin A3 during S-phase is supported by the observation that down-regulation of CYCA3;2 in Tobacco leads to reduced cell proliferation and endoreduplication (Yu et al. 2003). Two more members of the CycA family have been studied in more detail in Arabidopsis: Cyclin A;1 has thus been shown to be required for the meiotic cell cycle (d'Erfurth et al. 2010), although it could also have functions in vegetative cells (Jha et al. 2014), while Cyclin A2;3 negatively regulates endoreduplication (Imai et al. 2006) by associating with CDKB1;1 and activating cell division (Boudolf et al. 2009). Loss of function studies have allowed this role to be extended to the whole CYCA;2 subfamily: *cycA2;2,3,4* triple mutants show a global reduction of cell proliferation in both shoots and roots (Vanneste et al. 2011). Finally, B-type cyclins are involved in the control of the G2/M transition. This view is supported by their expression pattern that peaks in G2/M, their ability to form complexes with the B-type CDKs, and the observation that ectopic expression of CYCB1;2 is sufficient to induce cell division instead of endoreduplication in developing trichomes (Schnittger et al. 2002). It is worth noting that this model may be over-simplified. For example, CYCD4-1 which has been found by Van Leene et al. (2010) to behave like other D-type cyclins and to bind CDKA;1, has been reported to interact with CDKB2;1 and to be expressed in G2 (Kono et al. 2003). Authors hypothesize that this finding may reflect transient interactions due to the ability of CYCD4/CDKA complexes to regulate CDKB-containing complexes, but clearly, more detailed functional analysis of the various Cyclins will be required to reconcile sometimes conflicting experimental data.

As stated above, the large size of Cyclin gene families hampers the genetic dissection of their respective function. In addition, transcriptomic analysis revealed little tissue specificity in the expression pattern of cyclins (Menges et al. 2005). However, a few cyclins have been assigned specific functions. For example, CYCD6;1 has been shown to act downstream of SCARECROW and SHORTROOT to regulate the formative divisions required for root patterning (Sozzani et al. 2006), nevertheless, loss of CYCD6;1 alone is not sufficient to fully compromise these formative divisions, and even triple cyclin mutants still retained some degree of normal patterning, indicating a large level of redundancy between cyclins in this pathway. Likewise, CYCD4-1 and 2 have been involved in stomata formation (Kono et al. 2007), and CYCD4-1 appears to be specifically involved in the regulation of the pericycle cell cycle and during lateral root formation (Nieuwland et al. 2009). Globally, results available so far suggest that a lot of redundancy exists between closely related cyclins. However, the potential role of specific cyclins in response to stress or changes in external conditions have to date little been explored, and could shed light on the physiological role of such a diversity of CDK/cylin complexes.

Atypical CDKs and Cyclins are involved in basal activation of core complexes and in the regulation of gene expression

According to (Menges et al. 2005), the list of Arabidopsis CDKs and Cyclins can be further extended to 29 CDKs and 49 Cyclins by including other sub-groups: CDKC-G and CDK-like (CKL) proteins and CycH, L, P and T. CDKC (in complex with CYCT) and CDKE classes of CDKs are likely involved in the control of gene expression rather than cell cycle progression, and will thus not be further discussed, with the exception of CYCP2;1 (see below) (Barroco et al. 2003, Wang and Chen 2004, Cui et al. 2007, Kitsios et al. 2008). Likewise, CDKG-Cyclin L complexes are involved in chromosome pairing during meiosis, either by directly regulating the meiotic cell cycle or more indirectly by regulating gene expression (Zheng et al. 2014).

By contrast, CDKD-CycH and CDKF are considered as core cell cycle regulators: they are the CDK Activating Kinases (CAK). These proteins can activate CDKs by phosphorylating a conserved threonine in their T-loop. Their accumulation is constant throughout the cell cycle and they are probably not involved in the regulation of one specific cell cycle phase. Consistently, CDKD-deficient mutants show gametophytic lethality, suggesting that CDKD-CycH complexes are required to phosphorylate and activate all core CDKs (Takatsuka et al. 2015). Interestingly, although *cdkf* mutants show reduced cell proliferation, this effect does not seem to be mediated by reduced CDKA or B activity, suggesting that the CDKF could control cell cycle progression via a different pathway that may rely on the regulation of basal transcription (Takatsuka et al. 2009).

Various post-translational mechanisms control CDK/Cyclin complexes activity

In addition to the activating phosphorylation by the CAK, multiple mechanisms acting at the post-translational level modulate CDK/Cyclin activity. The WEE1 protein kinase can inhibit CDKs by phosphorylating them on Tyr15 and Thr14 (Berry and Gould 1996). This phosphorylation plays an important role in the control of the G2/M transition in eukaryotes and functions to avoid premature division of cells that have not sufficiently expanded as well as to delay mitosis after DNA damage. However, in Arabidopsis, the WEE1 kinase seems to be predominantly involved in DNA stress response, and not in growth regulation under normal conditions (De Schutter et al. 2007, Cools et al. 2011). Finally, CDK/cyclin complexes can be inhibited by binding of small proteins called CDK inhibitors (or CKI). In plants they are distributed between two unrelated families: the KRP (for KIP-related Proteins) that share homology with the human cell cycle inhibitor p27 and the SMR (for SMR related) (Van Leene et al. 2010). Like CDKs and cyclins, these inhibitors are extremely diverse: the Arabidopsis genome encompasses 7 KRPs and 14 SMRs. KRPs (also called ICKs for Inhibitors of Cyclin-dependant Kinases) were the first identified plant cell cycle inhibitors (Wang et al. 1998). They associate preferentially with CYCD or CYCA/CDKA;1 complexes (Van Leene et al. 2010). Consistently, over-expression of a number of KRPs induces the same phenotypic defects including reduction of cell division and endoreduplication, reduction of lateral root formation and dramatically enlarged cell size (Wang et al. 2000, Jasinski et al. 2002). The respective roles of the various KRPs remain to be elucidated, and a high level of redundancy between these cell

cycle inhibitors is likely to exist. Consistently, quintuple *krp1,2,3,4,7* mutants show only a mild increase in organ size due to the activation of cell proliferation via the E2F pathway (Cheng et al. 2013), however, multi-silenced KRP lines with reduced levels of KRP1-7 show severe developmental defects and ectopic callus formation, providing further evidence for the role of KRPs as negative regulators of cell proliferation (Anzola et al. 2010). Until now, only one member of the KRP family seems to play a distinctive role that cannot be fulfilled by other KRPs: KRP5 is required for the regulation of hypocotyl cell elongation in the dark (Jégu et al. 2013), and cell expansion in the root (Wen et al. 2013). Interestingly, it seems to function at least partly by binding chromatin and regulating the expression of genes involved in cell elongation and endoreduplication, providing evidence for yet unsuspected functions of plant cell cycle inhibitors (Jégu et al. 2013). Whether other KRPs may function as positive regulators of endoreduplication despite their ability to reduce the activity of G1/S CDK-Cyclin complexes remains to be fully established, but this hypothesis is supported by the observation that mild-over-expression of KRP2 results in an increase in endoreduplication (Verkest et al. 2005). SIAMESE (SIM), the founding member of the SMR family, also appears to positively regulate endoreduplication: *sim* mutants display multicellular trichomes, indicating that the SIM protein is required not only to promote endoreduplication but also to inhibit cell proliferation (Churchman et al. 2006). SIM-RELATED proteins (SMRs) have been proposed to play a role in cell cycle arrest during stress response (Peres et al. 2007). Consistently, SMR5 and SMR7 are involved in cell cycle arrest caused by reactive oxygen species, for example during high light stress (Yi et al. 2014), and contribute to the growth reduction caused by chloroplasts dysfunction (Hudik et al. 2014).

Control of the G1/S Transition: The E2F/RBR Pathway

As previously described, CYCD/CDKA complexes are the first CDK/Cyclin complexes activated for cell cycle onset. Consistently, expression of a number of CycDs responds to external cues (see below). In all eukaryotes, CYCD/CDKA complexes promote the G1/S transition by phosphorylating the Retinoblastoma (Rb) protein and alleviating its inhibitory action on E2F transcription factors that can in turn activate genes involved in DNA replication (Berckmans and De Veylder 2009) (Fig. 2). This pathway is conserved in plants, and the Arabidopsis genome encompasses a single Rb homologue (RBR, RetinoBlastoma Related) and six E2Fs (Lammens et al. 2009). Interestingly, most defects of the *cdka;1* mutant are rescued in a *cdka;1 rbr* double mutant, indicating that CDKA;1 regulates cell cycle progression mainly by targeting RBR (Nowack et al. 2012). Plant E2F transcription factors can be divided in two sub-groups: canonical E2Fs (E2Fa, b and c) require a Dimerization Partner (DP) to efficiently bind DNA, whereas atypical E2Fs (E2Fd, e and f) function as monomers. Plant E2Fs also differ by their function in cell cycle regulation, E2Fa and b being activators of the cell cycle whereas E2Fc behaves as a negative regulator (Berckmans and De Veylder 2009). Genome-wide identification of E2F target genes by combining promoter analysis for E2F binding sites and transcriptomic analysis performed on E2F over-expressing lines identified genes involved in DNA replication, DNA repair and chromatin dynamics further supporting the notion that E2Fa and b positively

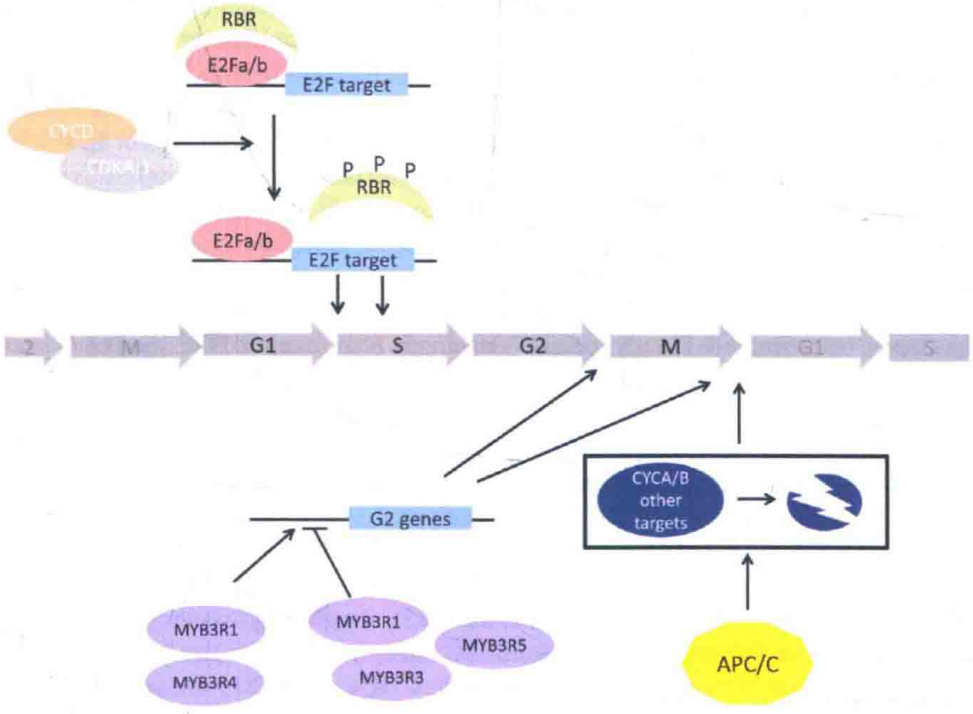

FIGURE 2 Regulation of cell cycle transitions. Activation of CYCD/CDKA complexes leads to phosphorylation of RBR and release of its inhibitory action on E2F factors thereby allowing expression of S-phase genes. G2 and M genes are under the control of MYB3R transcription factors. Activation of the APC/C is required to degrade various targets and allow exit from mitosis.

regulate the G1/S transition (Ramirez-Parra et al. 2003, Vandepoele et al. 2005). By contrast, over-expression of E2Fc inhibits cell proliferation (del Pozo et al. 2002) and its down regulation activates cell division (del Pozo et al. 2006), although it is not clear whether E2Fc acts antagonistically to E2Fa and b during S-phase or if it is more specifically involved in regulating the balance between cell proliferation and endoreduplication. This relatively simple model is made complex by the observation that E2Fa also controls a number of genes involved in cell differentiation: the maintenance of proliferative activity in meristems therefore requires partial inactivation of E2Fa by RBR (Magyar et al. 2012, Polyn et al. 2015). Finally, E2Fe and f are involved in the control of cell expansion: E2Fe prevents endocycles onset and thereby delays cell elongation whereas E2Ff is directly involved in cell expansion (Lammens et al. 2009).

Upon RBR release, activating E2Fs stimulate the expression of genes required for DNA replication, including the ones encoding the pre-replication complex (pre-RC). Assembly of the pre-RC on the replication origin and DNA replication licencing are key steps to the regulation of the G1/S transition. ORC (origin replication complex) proteins bind to replication origins and recruit CDC6 and CDT1 that in turn allow binding of MCM proteins that function as helicases to open the replication fork (DePamphilis 2003). All these factors are conserved in Arabidopsis, and interactions between the various constituents of the pre-RC have been observed in the yeast two-hybrid system (Shultz et al. 2007). In addition, there is genetic evidence that

the function of CDC6, CDT1, MCM2 and MCM7 in DNA replication is conserved in plants (Springer et al. 2000, Castellano et al. 2001, Castellano et al. 2004, Ni et al. 2009, Domenichini et al. 2012). Licencing of replication origins has to be tightly controlled so that it occurs once and only once per cell cycle in order to avoid incomplete DNA replication or re-replication of fractions of the genome (Xouri et al. 2007). Although these regulatory mechanisms are very well described in animals, it is much less clear how they function in plants. However, CDT1 that is the target of many regulatory pathways in animals also appears to be regulated by proteolysis in plants (Castellano et al. 2004). In addition, plant genomes encode homologues of the CDC7/Dbf4 kinase involved in replication licencing (Shultz et al. 2007), but their function has never been studied. Finally, origin licensing is also regulated between early and late-firing origins, early replicating regions corresponding mainly to euchromatin while heterochromatin is replicated at the end of the cell cycle (Hayashi et al. 2013, Bass et al. 2014). How replication timing is controlled in plants remains to be elucidated, but chromatin modifications such as histone marks are likely to play a role in this process (Raynaud et al. 2014). Consistently, mutants deficient for the deposition of the repressive mark H3K27me1 show re-replication of constitutive heterochromatin regions (Jacob et al. 2010), and this defect is aggravated by the over-expression of the cell cycle inhibitor KRP5 (Jégu et al. 2013), suggesting that heterochromatin not only specifies late replicating regions but could also function as a barrier against endoreduplication. Once pre-RC are activated, CDC6 and CDT1 are released from replication origins and inactivated. MCM proteins open the replication fork bi-directionally and are associated with replicative DNA polymerases via CDC45 and the GINS (go ichini san, also called PSF1, 2, 3 and SLD5), which are instrumental to the stabilization of the replication fork (Friedel et al. 2009). Data regarding the function of these factors in plants is scarce but down-regulation of CDC45 in meiocytes results in DNA fragmentation independently of programmed double-strand breaks that form during meiosis, suggesting that CDC45 is required for DNA replication to proceed normally (Stevens et al. 2004). Although data available so far support the notion that plant DNA replication functions in the same way as what is described in yeast and animals, it is worth noting that CDT1 homologues were found to form complexes with DNA polymerase ε, the replicative polymerase that synthesizes the leading strand (Pursell and Kunkel 2008), suggesting that the molecular events occurring during pre-RC formation or fork progression may differ in plants and other eukaryotes (Domenichini et al. 2012).

Regulation of G2 and Mitosis

Many genes expressed during the G2 and M phases harbour a specific regulatory sequence in their promoter called MSA (mitosis-specific activator) (Ito et al. 1998, Menges et al. 2005) that is recognized by MYB3R transcription factors (Haga et al. 2011). The Arabidopsis genome encodes 5 MYB3R: MYB3R2 which appears to be involved in the control of the circadian clock, but MYB3R1, 3, 4 and 5 have all been reported to control cell cycle progression (Fig. 2). MYB3R1 and 4 activate the expression of G2/M specific genes such as *KNOLLE* to allow proper cytokinesis (Haga et al. 2011); whereas MYB3R3 and 5 are repressors of G2/M genes (Kobayashi