

Control of the Plant Cell Cycle by Developmental and Environmental Cues

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Plant morphogenesis relies on cell proliferation and differentiation strictly controlled in space and time. As in other eukaryotes, progression through the plant cell cycle is governed by cyclin-dependent kinases (CDKs) that associate with their activator proteins called cyclins (CYCs), and the activity of CYC-CDK is modulated at both transcriptional and post-translational levels. Compared with animals and yeasts, plants generally possess many more genes encoding core cell cycle regulators and it has been puzzling how their functions are specified or overlapped in development or in response to various environmental changes. Thanks to the recent advances in high-throughput, genome-wide transcriptome and proteomic technologies, we are finally beginning to see how core regulators are assembled during the cell cycle and how their activities are modified by developmental and environmental cues. In this review we will summarize the latest progress in plant cell cycle research and provide an overview of some of the emerging molecular interfaces that link upstream signaling cascades and cell cycle regulation.

Keywords: Cell expansion • Cell proliferation • Endoreduplication • Mitotic cell cycle.

Abbreviations: APC/C, anaphase-promoting complex/cyclosome; ARF, AUXIN RESPONSE FACTOR; ARR, ARABIDOPSIS RESPONSE REGULATOR; bHLH, basic helix-loop-helix; CCS52, CELL CYCLE SWITCH 52; CDC20, CELL DIVISION CYCLE 20; CDH1, CDC20 HOMOLOG 1; CDK, cyclin-dependent kinase; CEI, cortex/endodermis initial; ChIP-chip, chromatin immunoprecipitation-based microarray; COP1, CONSTITUTIVE PHOTOMORPHOGENIC 1; CPD, cyclobutane pyrimidine dimer; CUL4, Cullin4; CYC, cyclin; DELLAs, DELLA proteins; DOF, DNA-binding with one finger; DP, dimerization partner; Emi1, Early mitotic inhibitor1; FLP, FOUR LIPS; FZR, FIZZY-RELATED; GIG1, GIGAS CELL1; KRP, Kip-related protein; LBD18, LATERAL ORGAN BOUNDARIES18; LR, lateral root; MSA, M phase-specific activator; MYB3R, three Myb repeats; OSD1, OMISSION OF SECOND DIVISION1; PHR1, PHOTOLYASE 1; PP1, type 1 protein phosphatase; PRZ1, PROPORZ1; PYM,

POLYCHOME; QC, quiescent center; RBR, retinoblastoma-related; RSS1, RICE SALT SENSITIVE 1; SCF, Skp-Cullin1-F-Box; SCR, SCARECROW; SHR, SHORTROOT; SIM, SIAMESE; SMR, SIAMESE-RELATED; UV-B, Ultraviolet-B; UVI4, UV-INSENSITIVE4.

Introduction

Production of new cells through cell proliferation is the primary force that drives organ growth in plants. Cells proliferate by going through the mitotic cell cycle that consists of four distinct phases, Gap 1 phase (G₁ phase), DNA synthesis phase (S phase), Gap 2 phase (G₂ phase) and mitotic phase (M phase). As in other eukaryotic cells, the progression of the plant mitotic cycle is driven by the periodic activation of cyclin-dependent kinases (CDKs) which, in combination with different cyclins (CYCs), triggers the transition from the G₁ to S phase and the G₂ to M phase. What appears to be unique in plants is that they possess many more CYCs and CDKs compared with yeasts and animals (Inzé and De Veylder 2006, Inagaki and Umeda 2011). For example, the Arabidopsis genome encodes 10 A-type CYCs (CYCAs), 11 B-type CYCs (CYCBs) and 10 D-type CYCs (CYCDs), while most yeast and animal genomes encode only one or two of each type of CYC. Both CYCAs and CYCBs can be subdivided into three groups, CYCA1–CYCA3 and CYCB1–CYCB3, while CYCDs are comprised of seven groups, CYCD1–CYCD7. Plants also possess several classes of CDKs and, among them, CDKAs and CDKBs, the latter of which is only found in the plant lineage, are directly involved in cell cycle control. The combinatorial interactions between different CYCs and CDKs have been thought to provide a strategy to recognize distinct substrates and thereby promote different phases of the cell cycle. As recently reviewed by Van Leene et al. (2011), accumulating evidence from several interaction studies indeed suggests that Arabidopsis CDKA;1 primarily bind to CYCDs and CYCA3 to drive the G₁ to S transition and S phase progression, respectively, while CDKA;1 forms a complex with CYCD3 to mediate M phase progression (Schnittger et al. 2002, Dewitte et al. 2007, Boruc

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et al. 2010, Van Leene et al. 2010). In contrast, CDKBs are postulated to interact preferentially with CYCA2 and CYCBs to promote the G₂ to M transition and M phase progression (Boudolf et al. 2009, Xie et al. 2010, Vanneste et al. 2011).

Once plant cells stop proliferating and begin to differentiate, they often enter an alternative cell cycle called the endoreduplication cycle or endocycle in which cells repeat DNA replication without intervening mitoses. It is well established that an increase in ploidy through successive rounds of the endocycle also contributes to growth and development of various plant organs (Sugimoto-Shirasu and Roberts 2003). Substantial progress has been made in recent years to understand how endocycling is controlled, and it is generally accepted now that the key molecular event triggering the onset of the endocycle involves inactivation of the mitotic CYC–CDK complexes (for reviews, see Breuer et al. 2010, De Veylder et al. 2011).

Controlling the activity of individual CYC–CDK complexes is therefore vital in both proliferating and differentiating cells and, most importantly, the progression of both the mitotic and endoreduplication cycles needs to be coordinated during plant development as well as in response to environmental changes. Plants and other eukaryotes share common regulatory mechanisms to control the basic machinery of the cell cycle but, due to their sessile lifestyle, plants have also developed unique strategies to allow growth and survival under environmentally harsh conditions. In this review we will summarize our current understanding of these controls and highlight how developmental and environmental signals connect with the progression of the cell cycle.

Transcriptional Control of Cell Cycle Regulators

Temporal transcription of cell cycle regulators ought to be an important mechanism underlying the phase-specific activation of CYC–CDK complexes, but we are still far from having a comprehensive view on how this control is mediated in plants (Berckmans and De Veylder 2009). Based on the global expression analysis of core cell cycle regulators in synchronized *Arabidopsis* cell cultures, Menges et al. (2005) demonstrated that many cell cycle genes show highly specific profiles of expression during the mitotic cell cycle. As expected from the prominent roles that CYCs play in activating CDKs, expression of almost all CYCs peaks at specific phases of the cell cycle. For example, the expression of several CYCA genes including CYCA3;1, CYCA3;2 and CYCA3;4 is dramatically up-regulated at the G₁ to S transition and S phase, while transcripts of other CYCA genes, such as CYCA2;1 and CYCA2;3, and of all CYCB genes accumulate preferentially at the G₂ to M transition (Fig. 1). Most of the CYCD genes, including CYCD3;3, CYCD4;1, CYCD4;2, CYCD5;1, CYCD6;1 and CYCD7;1, are expressed during G₁ and S phase, but some, such as CYCD3;1, are transcribed during the G₂ and M phase. In agreement with the predicted function of CDKA;1 throughout the cell cycle, its transcript

levels are fairly constant during all phases. In contrast, transcripts of the CDKB1 genes, CDKB1;1 and CDKB1;2, accumulate from S to M phase and those of the CDKB2 genes, CDKB2;1 and CDKB2;2, are detected more specifically at late G₂ and M phase.

Gene expression that supports the progression from G₁ to S phase is generally controlled by the E2F–dimerization partner (DP)–retinoblastoma-related (RBR) pathway (Fig. 1A). The *Arabidopsis* genome encodes three typical E2F transcription factors E2Fa, E2Fb and E2Fc, which form dimers with DPa or DPb proteins to bind to specific E2F sites in promoters of their transcriptional target genes. Both E2Fa and E2Fb act as transcriptional activators to promote the G₁ to S transition and, accordingly, their putative direct target genes include those required for DNA replication, DNA repair and chromatin maintenance (Ramirez-Parra et al. 2003, Vlieghe et al. 2003, Vandepoele et al. 2005, Takahashi et al. 2008, Naouar et al. 2009). During the G₁ phase, CYCD–CDKA complexes phosphorylate RBR and thus release E2Fa–DPa and E2Fb–DPa to allow transcription of their target genes required for the G₁ to S transition (Bonioti and Gutierrez 2001). On the other hand, E2Fc–DPb dimers function as transcriptional repressors and, although their direct targets genes have not been fully described, they appear to repress cell cycle progression through an RBR-independent mechanism (del Pozo et al. 2002, del Pozo et al. 2006, de Jager et al. 2009).

Three atypical E2Fs, E2Fe/DEL1, E2Fd/DEL2 and E2Ff/DEL3, also function as transcriptional repressors in *Arabidopsis*, but so far none of them has been shown to control directly the expression of cell cycle genes. All of these atypical E2Fs possess two DNA-binding domains and they can bind to the target promoters as monomers. The E2Fe/DEL1 protein binds to the promoter of the CCS52A2 gene, an activator of the plant anaphase-promoting complex/cyclosome (APC/C; see also below), and represses entry into the endocycle (Lammens et al. 2008). Based on gain-of-function and loss-of-function studies, the E2Fd/DEL2 protein is implicated in the control of cell proliferation but its direct target genes have not been identified (Sozzani et al. 2010b). Probably most unexpected is that the E2Ff/DEL3 protein directly represses the expression of several cell wall biosynthesis genes, including three expansin genes and a UDP-glucose-glycosyl transferase gene, through direct binding to their promoter, to control cell elongation (Ramirez-Parra et al. 2004).

Genes expressed specifically during G₂ and M phase contain M phase-specific activator (MSA) elements in their promoters (Fig. 1B). The MSA element is recognized by three Myb repeat (MYB3R) transcription factors which were first identified in tobacco as NtMYBA1, NtMYBA2 and NtMYBB (Ito et al. 2001). Among them, NtMYBA1 and NtMYBA2 function as a transcriptional activator of G₂–M-specific genes, including CYCAs and CYCBs, while NtMYBB acts as their transcriptional repressor. The NtMYBB gene is transcribed throughout the cell cycle, while the expression of NtMYBA1 and NtMYBA2 peaks in late G₂ and early M phase. Therefore, it is postulated that NtMYBB represses the expression of G₂–M-specific genes

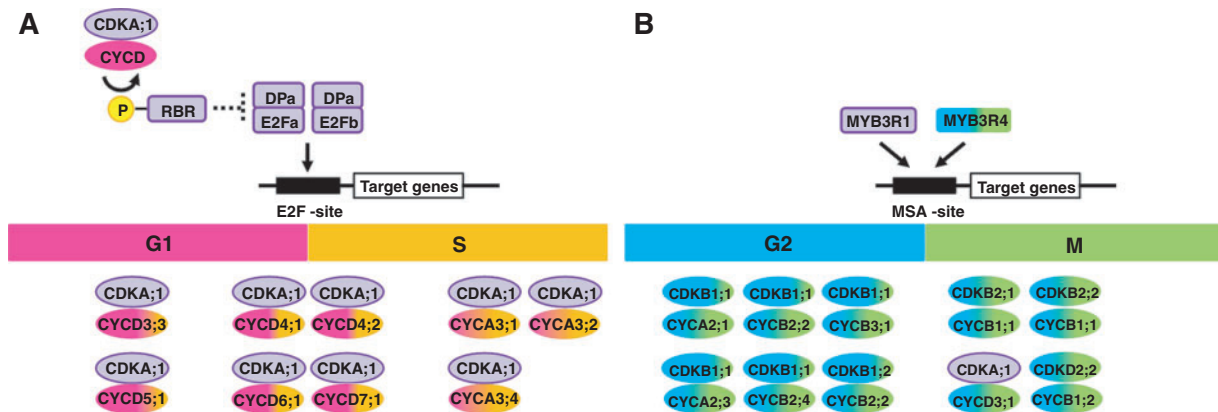


Fig. 1 Many CYCs and CDKs are expressed at specific time points of the mitotic cycle and their combinatorial interactions promote the different phases of the cell cycle. (A) At the G₁ and S phase, many CYCD and CYCA3 genes are transcribed and their gene products assemble preferentially with CDKAs. As cells transit from the G₁ to S phase, the CYCD-CDKA complex phosphorylates RBR to release E2Fa-DPa and E2Fb-DPb. These E2F-DP complexes, as a consequence, bind to the E2F-binding site, marked by a black box in the promoter sequence of target genes, and activate their transcription. (B) At G₂ and M phase, the CYCA2, CYCB1 and CYCB2 genes are strongly expressed and their gene products assemble with CDKB1s and CDKB2s. The CYCD3;1 gene is also transcribed at M phase and its gene products assemble with CDKA. The MYB3R1 and MYB3R4 transcription factors recognize the MSA site, marked by a black box in the promoter sequence of target genes, and activate their transcription. Genes that accumulate at G₁, S, G₂ and M phase are labeled in pink, yellow, blue and green, respectively, and genes expressed throughout the cell cycle are labeled in purple.

during G₁-S phase and, as cells enter G₂ phase, NtMYBA starts to occupy the MSA sites to promote gene transcription. What initiates the transcription of *NtMYBA* genes in the first place is unknown but, once initiated, there are several positive feedback mechanisms that can lead to a quick amplification and establishment of NtMYBA-based gene expression. For instance, the promoter sequence of both *NtMYBA1* and *NtMYBA2* genes also contains the MSA elements and they can activate their own transcription (Kato et al. 2009). It is also reported that together with CDKs, CYCA and CYCB, the direct downstream targets of NtMYBA, phosphorylate NtMYBA itself and thereby hyperactivate its transactivation capacity (Araki et al. 2004).

The Arabidopsis genome encodes five MYB3R proteins named MYB3R1-MYB3R5, of which MYB3R1 and MYB3R4 are the closest homologs of NtMYBA1 and NtMYBA2, but intriguingly there is no obvious homolog of NtMYBB (Haga et al. 2007). While the expression level of MYB3R4 is up-regulated during G₂ to M transition, the transcript level of MYB3R1 does not change throughout the cell cycle, suggesting that MYB3R1 is post-translationally regulated. Expression of many G₂ to M-specific genes carrying the MSA elements is dramatically down-regulated in the *myb3r1 myb3r4* double mutants but it is not completely abolished (Haga et al. 2011). These data illustrate the importance of MYB3R1 and MYB3R4 for the expression of those genes, but also suggest the existence of an alternative mechanism, potentially mediated by other MYB proteins, controlling the transcription of G₂-M phase genes.

In addition to E2Fs and MYBs, several other transcription factors that mediate phase-specific expression of cell cycle genes have been identified. For instance, the DNA-binding with one finger (DOF) transcription factor OBP1 directly

up-regulates the expression of *CYCD3;3* and *AtDOF2;3*, a replication-specific transcription factor (Skirycz et al. 2008). Overexpression of OBP1 shortens the cell cycle, with elevated expression of many other cell cycle genes, implying that OBP1 may act as a key transcriptional regulator in the cell cycle.

Ubiquitin-Mediated Proteolysis of Cell Cycle Regulators

The activity of CYC-CDK complexes is also controlled through several post-translational modification mechanisms and, among others, ubiquitin-mediated degradation of cell cycle proteins is most central for the timely progression of the cell cycle. Several ubiquitin-dependent destruction pathways have been associated with the mitotic cell cycle and, in all cases, ubiquitin E3 ligases mark target proteins by polyubiquitination for their selective proteolysis by the 26S proteasomes. The Skp-Cullin1-F-Box (SCF) E3 ligase primarily regulates the G₁-S transition, while the APC/C, a Cullin-RING finger E3 ligase, is most active from mid-M phase (anaphase) to the end of the G₁ phase during the mitotic cell cycle. Recent studies have also uncovered an involvement of monomeric RING-type E3 ligases and Cullin4 (CUL4)-based E3 ligases in plant cell cycle control (Liu et al. 2008, Roodbarkelari et al. 2010). Functional diversities of these E3 ligases have been recently reviewed in Marrocco et al. (2010) and Hua and Vierstra (2011), thus we focus here on APC/C E3 ligases which now prove to have broader roles in both cell proliferation and cell differentiation.

The APC/C complex is composed of at least 11 subunits in plants and among them its catalytic core proteins, APC2 and

APC11, have structural similarities to the components of the SCF complex. In the Arabidopsis genome, all APC/C components except APC3/CDC27/HOBBIT are encoded by a single gene (Capron et al. 2003), and recent studies have begun to unveil the precise roles of individual APC/C subunits. All mutants of the APC/C subunits studied so far, including *apc2*, *apc3b*, *apc6* and *apc8*, accumulate mitotic CYCs in the embryo sacs (Blilou et al. 2002, Capron et al. 2003, Kwee and Sundaresan 2003, Zheng et al. 2011), suggesting that as in animals, mitotic CYCs are substrates of the plant APC/C. It has been suggested in other organisms that active components of the APC/C vary in different cell types (Huang and Raff 2002). Similarly, single mutants in *apc2* and *apc10* or *apc3a apc3b* double mutants display female gametophytic lethality whereas mutation in APC8 causes defects in male gametogenesis (Capron et al. 2003, Pérez-Pérez et al. 2008, Eloy et al. 2011, Zheng et al. 2011). These data imply that the plant APC/C may also assemble differently in some cell type-specific contexts.

In addition to core components, APC/C also associates with co-activators, known as CELL DIVISION CYCLE 20 (CDC20)/FIZZY and CDC20 HOMOLOG 1 (CDH1)/FIZZY-RELATED (FZR), which confer substrate specificities. These co-activators mediate physical interaction between the APC/C and its substrates, and, according to studies in other organisms, APC/C^{CDC20} and APC/C^{CDH1} exert distinct activities during the cell cycle. Yeast and animal APC/C^{CDC20} is activated during early mitosis and targets mitotic CYCs and securin for degradation to drive sister chromatid separation during the transition from metaphase to anaphase. In contrast, the activity of APC/C^{CDH1} is increased during late anaphase to promote the degradation of other APC/C substrates including mitotic CYCs and CDC20, to trigger an exit from mitosis (Kramer et al. 2000, Pflieger and Kirschner 2000). The Arabidopsis genome contains five CDC20 genes and three CDH1/FZR genes, also called CELL CYCLE SWITCH 52 (CCS52), but how they modify the APC/C activity during the cell cycle is not fully established. A recent work shows that two of the five CDC20 genes, CDC20.1 and CDC20.2, have redundant functions for the mitotic cell cycle and that they are required for meristem maintenance, normal plant growth and male gametophyte formation (Kevei et al. 2011) (Fig. 2). In contrast, it appears that other CDC20 genes, annotated as CDC20.3–CDC20.5, are pseudogenes, since they do not show detectable levels of expression in planta. Among the three CCS52 genes in Arabidopsis, CCS52A1 and CCS52A2 participate in meristem maintenance (Vanstraelen et al. 2009) (Fig. 2). Interestingly, however, they act through different mechanisms and the distinct patterns of their gene expression define their functional divergence. The expression of CCS52A1 starts at the elongation zone of Arabidopsis roots and it stimulates mitotic exit and an entry into the endoreduplication cycle. Conversely, CCS52A2 is expressed at the distal part of the root meristem and is required to maintain the identity of cells in the quiescent center (QC). The QC cells in the wild type divide only occasionally for self-renewal, but the *ccs52a2*

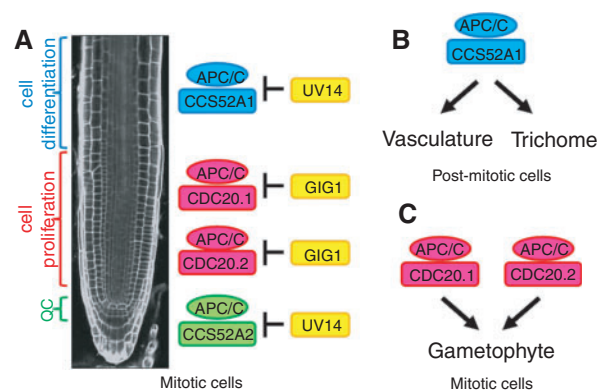


Fig. 2 The APC/C plays vital roles in mitotic, post-mitotic and meiotic cells. (A) The APC/C targets mitotic CYCs for degradation to promote the mitotic cell cycle. The activity of the plant APC/C is controlled by several co-activators such as CDC20.1, CDC20.2, CCS52A1 and CCS52A2 which are expressed in a complementary fashion in the root meristem. UVI4 and GIG1 function as negative regulators of the plant APC/C by directly binding to the co-activators. UVI4 and GIG1 can interact with both CDC20 and CCS52A, but they may have some functional preferences, as indicated. (B) The APC/C activity is required for post-mitotic cell differentiation. The APC/C subunits and its co-activators are expressed in post-mitotic cells and their down-regulation causes severe defects in the development of the leaf vasculature and trichomes. The APC/C is implicated in the control of endocycling in trichomes. (C) The APC/C is present in meiotic cells and participates in the development of male and female gametophytes.

mutation activates the QC cell division, resulting in the loss of their identity. Promoter swap experiments showed that the CCS52A1 expression, driven by the CCS52A2 promoter, fully rescues these phenotypes in *ccs52a2*, indicating that the two CCS52As are functional homologs. These discoveries clearly point to the conserved mechanism of the APC/C activation in plants and raise the next set of questions such as how CDC20/FZY and CDH1/FZR/CCS52 function during the plant cell cycle, what they target at different phases of the mitotic cycle, and how their activities are regulated in the developmental context.

In vertebrates, APC/C activity is also modified by negative regulators called Early mitotic inhibitor1 (Emi1) and Emi2 which directly bind to CDH1/FZR/CCS52 and CDC20/FZY to inhibit APC/C activity (Pesin and Orr-Weaver 2008). Although no proteins orthologous to Emi1 and Emi2 have been identified in plants, two independent studies have recently unveiled that GIGAS CELL1 (GIG1)/OMISSION OF SECOND DIVISION1 (OSD1) and UV-INSENSITIVE4 (UVI4)/POLYCHOME (PYM) act as their functional homologs in plants (Heyman et al. 2011, Iwata et al. 2011) (Fig. 2). Both GIG1 and UVI4 physically interact with plant APC/C activators, CDH1/FZR/CCS52 and CDC20/FZY, and their overexpression leads to an accumulation of CYCB1;2 and CYCA2;3, respectively, by inhibiting the APC/C activity. Intriguingly, however, single mutations of *gig1* and *uvi4* cause similar but distinct modes of polyploidization,

endomitosis in *gig1* and endoreduplication in *uvi4*. The key difference between these polyploidization events is that in endomitosis, cells initiate but do not fully complete mitosis, while cells in endoreduplication omit mitosis entirely. These phenotypic differences therefore suggest that UVI4 functions earlier than GIG1 during the mitotic cell cycle. It should also be noted that the *gig1-1* and *gig1-2* alleles were identified as a new mutation enhancing the *myb3r4* mutant phenotypes we discussed earlier. This genetic evidence reinforces the idea that the levels of CYCs need to be controlled both transcriptionally and post-translationally.

The APC/C was initially discovered for its function in the mitotic cell cycle, but accumulating evidence suggests that it also has pivotal roles in post-mitotic cell differentiation. In animals, for example, the APC/C is required for the development of neuron, muscle and lens, and it targets cell cycle regulators associated with the endocycle or other developmental regulators, e.g. transcription factors, that are not directly linked to cell cycle control (Eguren et al. 2011). Given that most of the Arabidopsis APC/C mutants are gametophytic lethal, an involvement of the plant APC/C in post-mitotic cells has not been extensively studied in the past. Marrocco et al. (2009), however, have recently shown that many APC/C subunits are expressed in mature leaves of Arabidopsis and that treatment with a proteasome inhibitor, MG132, results in the accumulation of mitotic CYCs, suggesting that APC/C activities are also maintained in differentiating cells. The authors further showed that transgenic Arabidopsis plants with reduced expression of APC6 and APC10 display severe defects in vasculature development, strongly suggesting that the APC/C also participates in post-mitotic cell differentiation in plants (Fig. 2B).

The Arabidopsis leaf trichomes also represent a good model system to study an involvement of the APC/C in post-mitotic cells. Wild-type trichomes are made up of a single cell and their cell growth is coupled with several rounds of endocycles, final ploidy levels typically reaching up to 32C. The APC/C activity might be also required for this process because increased or decreased expression of CCS52A1 directly affects ploidy levels and cell growth of trichomes (Larson-Rabin et al. 2009, Kasili et al. 2010) (Fig. 2B). It is also reported, however, that the APC/C is only required for an entry into the trichome endocycle while the CUL4-based ubiquitin E3 ligase plays more predominant roles for its progression (Roodbarkelari et al. 2010). Thus, future studies need to clarify these discrepancies and, more importantly, should identify ubiquitination targets in post-mitotic cells so we can fully understand how the APC/C and other E3 ligases participate in cell differentiation.

Interactions with CDK Inhibitors

Another key mechanism that modulates the CYC–CDK activity involves direct binding of the CYC–CDK complex to a group of proteins called CDK inhibitors, leading to interference with its

substrate phosphorylation. Plants are unique in having two classes of CDK inhibitors—KIP-RELATED PROTEINS (KRPs) and SIAMESE (SIM)/SIAMESE-RELATED (SMR)—which show limited sequence similarities in the C-terminal CYC-binding domain. The Arabidopsis genome encodes seven KRPs, KRP1–KRP7, and at least 13 SIM/SMRs. Recent proteomic analyses in Arabidopsis have uncovered that all seven KRPs co-purify solely with CYCDs and CDKA (Van Leene et al. 2011), strongly suggesting that KRPs inhibit the activity of the CYCD–CDKA complex. These results are consistent with previous reports based on the yeast two-hybrid assay (De Veylder et al. 2001) but do not exclude the possibility that KRPs also inhibit the activity of the CYCD–CDKB complex as suggested by other studies using Arabidopsis and alfalfa (Nakai et al. 2006, Pettkó-Szandtner et al. 2006). All KRPs display overlapping but some distinct expression patterns in the Arabidopsis shoot apex. The KRP4 and KRP5 genes are predominantly expressed in dividing cells, while strong expression of KRP1 and KRP2 is detected in differentiating cells. In contrast, the KRP3, KRP6 and KRP7 genes are expressed in both dividing and differentiating cells (Ormenese et al. 2004). Many of these KRPs are likely to have redundant functions since single loss-of-function mutants of KRPs do not display any visible phenotypes. Down-regulation of multiple KRP genes, in contrast, severely compromises organ growth and, most notably, leads to the formation of callus-like tissues from the shoot apical meristem, clearly demonstrating that KRPs act as a negative regulator of cell proliferation (Anzola et al. 2010). A role for KRPs in driving endocycle onset has been demonstrated by mild overexpression of KRP1 or KRP2 in mitotically active cells since both KRPs inhibit the mitotic CDK activities and promote entry into the endocycle (Verkest et al. 2005, Weinl et al. 2005). When KRP1 or KRP2 is strongly expressed, however, they block both mitotic and S-phase CDK activities, leading to a complete cell cycle arrest (Lui et al. 2000, De Veylder et al. 2001, Schnittger et al. 2003). These observations therefore suggest that different levels of KRPs exert multiple functions in cell cycle control.

The PROPORZ1 (PRZ1) protein is a component of the chromatin-remodeling complex required for histone acetylation in response to auxin (Anzola et al. 2010). Accordingly, the *prz1-1* mutants display pleiotropic phenotypes impaired in various aspects of auxin-mediated morphogenesis. The authors discovered that the expression of several KRP genes including KRP2, KRP3 and KRP7 is reduced in *prz1-1* and that PRZ1 recognizes the promoter sequence of these KRP genes. Interestingly, the reduced expression of KRP7 alone accounts for some of the growth defects in *prz1-1* mutants since overexpression of KRP7 under the 35S promoter partly rescues their dwarf phenotypes. These results suggest that auxin signaling is translated into modified KRP expression through PRZ1-mediated chromatin remodeling.

The SIM/SMR family of CDK inhibitors is found only in plants. The SIM gene was first discovered in Arabidopsis as its loss-of-function mutation causes multicellular trichomes (Walker et al. 2000). SIM is required to repress the mitotic

cell cycle in trichomes, and the direct interaction of SIM with the CYCD–CDKA complex is thought to execute this inhibition (Churchman et al. 2006). Another member of the SIM/SMR family, SMR1/LGO, is implicated in the control of endocycling in sepals (Roeder et al. 2010). The sepal epidermis of wild-type flowers possesses large, highly endoreduplicated cells called giant cells, but these cells are lost in *smr1/lgo* because cells undergo additional cell divisions instead of endoreduplication. Conversely, overexpression of *SMR1/LGO* produces additional giant cells since cells transit into the endocycle earlier than those of the wild type. A recent in vivo protein interaction study revealed that both SIM and SMR/LGO co-purify with CDKB1;1 while other SMRs interact with CDKA;1 (Van Leene et al. 2010). These findings raise the interesting possibility that the direct inhibition of CDKB1;1 by SIM/SMR1 might allow entry into the endocycle.

Cell Cycle Control by Developmental Signals

Highly coordinated progression of the cell cycle is central for the post-embryonic plant development. This is particularly the case when cells need to undergo controlled cell division to form new cell types or new tissues, for example, in root development. Molecular genetic studies over the past decade have uncovered several key regulators, many of which turned out to be transcriptional regulators, involved in these processes, but how these upstream regulators link to cell cycle control has not been well characterized. This is now changing, however, and the first couple of examples recently published show that some of the developmental regulators directly activate or repress core cell cycle genes.

SHORTROOT (SHR) and SCARECROW (SCR) are members of the GRAS family of transcription factors required for the asymmetric division of cortex/endodermis initial (CEI) cells in the root apical meristem (Di Laurenzio et al. 1996, Helariutta et al. 2000). This formative division generates two new cell types, cortex and endodermis, thus controlled division of CEI is a prerequisite for proper root development. An elegant work by Sozzani et al. (2010a), combining cell type-specific gene expression analyses and chromatin immunoprecipitation-based microarray (ChIP-chip) analyses, has revealed that both SHR and SCR directly regulate the expression of *CYCD6;1*, one of the CYCDs present at the G₁ and S phase, by binding to its promoter (Fig. 3A). The *CYCD6;1* gene is expressed specifically in CEI and CEI daughter cells, and the formative asymmetric division of CEI is significantly decreased in the *cycd6;1* mutants. Moreover, ectopically expressed *CYCD6;1* in the *shr* mutant background partially complements its formative division defects, further supporting that *CYCD6;1* acts downstream of the SHR–SCR pathway. Expression of several other cell cycle genes, including *CDKB2;1* and *CDKB2;2*, is also regulated by SHR and SCR, and overexpression of these CDKs in endodermal cells promotes their formative cell division. Interestingly, however, they do not appear to be direct targets of SHR and SCR,

suggesting that activation of these genes involves another layer of transcriptional control.

Cell proliferation needs to be reactivated in the xylem-pole pericycle cells for lateral root (LR) initiation, and this process can be induced by auxin in many plant species including Arabidopsis. The LR development starts via auxin-dependent degradation of IAA14/SLR, leading to the derepression of two related AUXIN RESPONSE FACTORS (ARFs), ARF7 and ARF19 (Benková and Bielach 2010). Both ARF7 and ARF19 are required for the subsequent expression of LATERAL ORGAN BOUNDARIES18 (LBD18) and LBD33 transcription factors. A recent study by Berckmans et al. (2011b) demonstrated that a heterodimer composed of LBD18 and LBD33 directly binds to the promoter of *E2Fa*, one of the *E2F* genes induced at LR initiation, to activate its expression (Fig. 3B). The *E2Fa* expression is increased by auxin treatment at the LR initiation site and this auxin-dependent *E2Fa* expression is lost in the *iaa14/slr-1* mutant background. As expected, the number of LR primordia is decreased in the *e2fa* mutants, confirming a clear requirement for *E2Fa* in LR initiation. Therefore, the LBD18/LBD33–*E2Fa* pathway represents one molecular interface linking auxin signaling with cell cycle progression during LR development. Another *E2F* family protein, *E2Fb*, might also play a role in LR initiation, although no significant increase in the *E2Fb* expression level was observed by auxin treatment (Berckmans et al. 2011a).

An independent cell cycle pathway that also contributes to the auxin-induced LR formation involves down-regulation of KRP2 by auxin (Sanz et al. 2011) (Fig. 3B). The authors show that under low auxin conditions the CYCD2;1–CDKA activity is repressed by the presence of KRP2. Upon auxin treatment, both gene expression and protein accumulation of KRP2 are reduced, leading to an increase in CYCD2;1–CDKA activity and thus enhanced LR induction. It is therefore hypothesized that the activated CYCD2;1–CDKA complex may hyperphosphorylate RBR, resulting in the activation of *E2Fb* without its transcriptional induction (Berckmans et al. 2011a). Interestingly, auxin acts as a trigger for the assembly of CYCD–CDKA complexes in tobacco (Harashima et al. 2007). This effect of auxin may also contribute to the auxin-induced LR formation.

Formation of stomata, a pair of guard cells, is another post-embryonic developmental process that relies on local activation of cell proliferation. In Arabidopsis, stomata form after at least one asymmetric division and one symmetric division. FOUR LIPS (FLP) and MYB88 genes, encoding closely related atypical two-MYB-repeat transcription factors, are required to restrict the final symmetric division to one and, accordingly, loss of FLP alone or in combination with MYB88 causes additional symmetric divisions of guard cells (Lai et al. 2005). Two recently published studies now demonstrate that this final division is terminated through the transcriptional repression of *CYCA2;3* and *CDKB1;1* by FLP and MYB88 (Xie et al. 2010, Vanneste et al. 2011) (Fig. 3C). The ChIP-chip analysis showed that FLP and MYB88 directly bind to the promoters

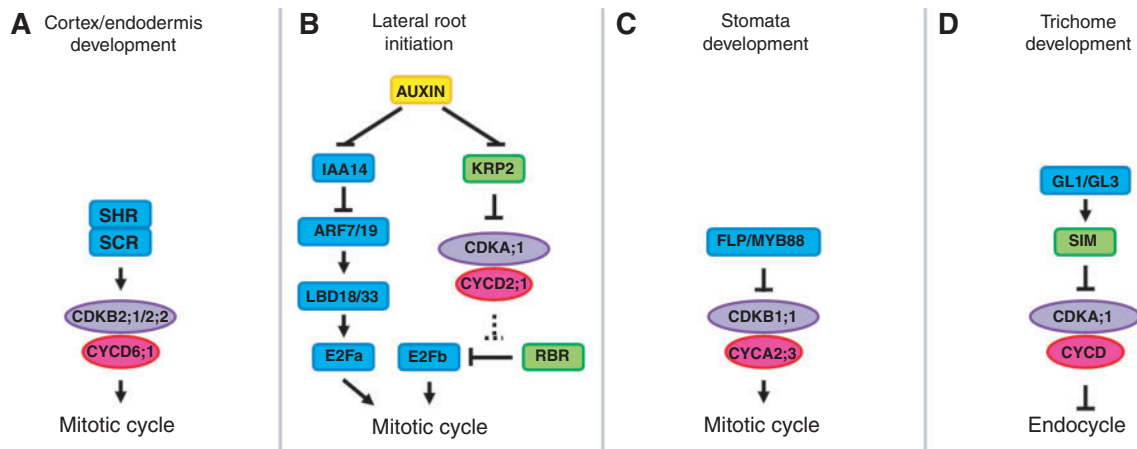


Fig. 3 Cell cycle regulation mediated through developmental signals. (A) The SHORTROOT (SHR)/SCARECROW (SCR) transcription factor complex binds directly to the *CYCD6;1* promoter to activate its expression in cortex/endodermis initial (CEI) and CEI daughter cells. The *CDKB2;1* and *CDKB2;2* genes are not the direct targets of SHR/SCR but their gene products act with *CYCD6;1* in the same pathway to promote the mitotic cycle. (B) Auxin controls lateral root (LR) initiation through the E2F pathway. Auxin promotes the degradation of IAA17 through ubiquitin-mediated proteolysis and activates the ARF7 and ARF19 transcription factors to initiate LR formation. ARF7 and ARF19 induce the expression of LBD18 and LBD33 transcription factors which subsequently bind to the promoter of the *E2Fa* gene to drive its expression. As an alternative pathway, auxin also down-regulates KRP2, leading to an activation of the *CYCD2;1*–CDKA complex and enhanced LR initiation. How *CYCD2;1*–CDKA promotes LR initiation is not established, but one hypothesis is that *CYCD2;1*–CDKA hyperphosphorylates RBR and releases E2Fb for its activation. (C) The FOUR LIPS (FLP) and MYB88 transcription factors repress the expression of *CDKB1;1* and *CYCA2;3* to restrict the final symmetric division of guard cells to one. (D) *GLABLA1* (GL1) and GL3 regulate trichome development in part through the induction of *SIAMESE* (SIM) expression. SIM is thought to inhibit the *CYCD*–CDKA activity to repress the mitotic cycle and promote the transition into the endocycle. Solid lines represent experimentally confirmed functional relationships, and dashed lines represent hypothesized relationships.

of a large number of cell cycle genes, including components of the pre-replication complex, *CYC* and *CDK* genes (Xie et al. 2010). Among them, loss-of-function mutations of *CDKB1;1* or *CYCA2;3* (together with its close homologs *CYCA2;2* and *CYCA2;4*) suppress overproliferation phenotypes of the *flp* mutants, genetically confirming that both *CYCA2;3* and *CDKB1;1* act downstream of FLP and MYB88. Similar to FLP and MYB88, *CYCA2;3* and *CDKB1;1* are expressed just before and after the final symmetric division, highlighting that the transient activation of the FLP/MYB88–*CYCA2;3*/CDKB1;1 pathway is the key molecular basis underlying the correct differentiation of stomata.

Controlling the progression of the endocycle is also vital for the coordinated growth of various plant organs. Endocycles are also implicated in the maintenance of cell fate, e.g. in trichomes (Bramsiepe et al. 2010), thus the expression and/or activities of endocycle regulators must also be modified by developmental cues (Breuer et al. 2010). Morohashi and Grotewold (2009) have indeed shown that the trichome initiation factors *GLABLA1* (GL1) and GL3, encoding MYB and basic helix–loop–helix (bHLH) transcription factors, respectively, directly target *SIM*, one of the CDK inhibitors implicated in the control of endocycles (Fig. 3D). Although the functional relevance of this transcriptional regulation needs to be further verified, these data showcase how an early developmental signal connects to endocycle regulation in trichome development. Another potential mediator that acts later in trichome differentiation is the trihelix transcription factor GT2-LIKE1 (GTL1) which negatively

regulates ploidy-dependent cell growth (Breuer et al. 2009). The *GTL1* gene is expressed as trichome cells reach their maximum size and its expression is dependent on the transcriptional network defining the initial trichome patterning. A loss-of-function mutation in *GTL1* leads to extended endocycles and cell expansion, and these defects are accompanied by modified expression of cell cycle genes. Elucidating the direct upstream regulators of *GTL1* expression and its downstream target genes should provide a further comprehensive view of how endocycling is controlled in the developmental context.

Cell Cycle Control by Environmental Signals

To survive during various changes in the environment, plants need to adapt their growth behavior through altering the rate of cell proliferation and differentiation. As expected, the expression of many cell cycle genes is up- or down-regulated by external cues (Peres et al. 2007) but so far very little is understood regarding the molecular basis underlying these transcriptional controls. It is equally possible that the activities of some cell cycle proteins are post-translationally modified under adverse conditions, but our current knowledge of these controls is also very limited. Recent studies, however, have identified several key players in stress-induced cell cycle modifications and have begun to provide the first molecular insights into how environmental signals talk to the mitotic or endoreduplication cycle.

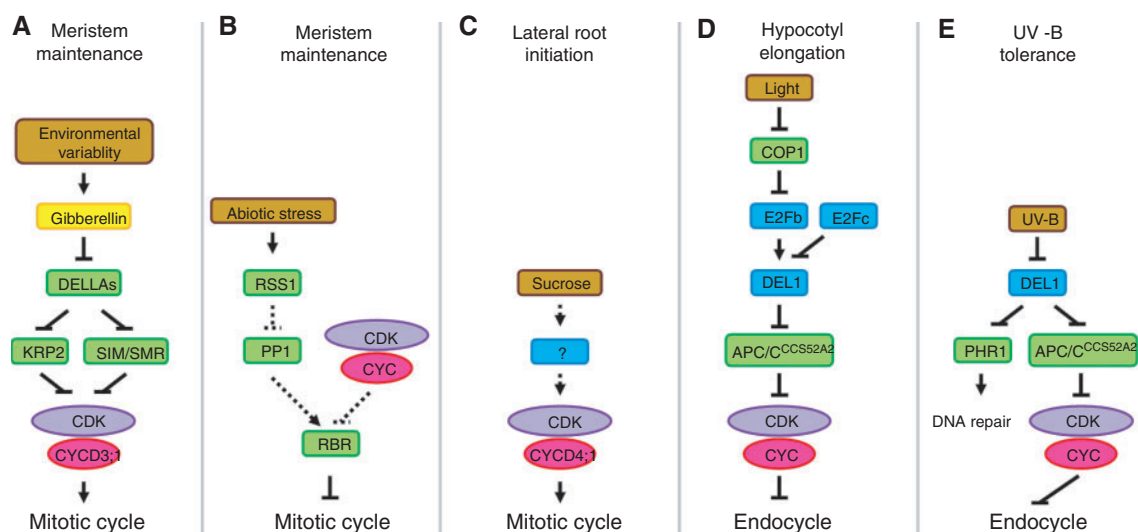


Fig. 4 Cell cycle regulation mediated through environmental signals. (A) The level of DELLA proteins is influenced by various environmental factors including light and temperature. DELLA proteins inhibit the mitotic cycle by enhancing the accumulation of CDK inhibitors. *CYCD3;1* is thought to act downstream of DELLAs. (B) Under various abiotic stress conditions, RICE SALT SENSITIVE 1 (*RSS1*) is required to maintain the mitotic cycle in meristematic cells. *RSS1* interacts with a type 1 protein phosphatase (PP1) and may regulate its activity at the G₁ to S transition. Human PP1 dephosphorylates RBR proteins to prevent the G₁ to S transition, but whether the rice PP1 performs the same function has not been explored. (C) Sucrose up-regulates the expression of *CYCD4;1* in root pericycle cells and promotes LR initiation. The transcriptional control underlying the cell type-specific *CYCD4;1* up-regulation is not currently known. (D) An atypical E2F transcriptional factor, DEL1, participates in the light-mediated control of endocycle onset in the hypocotyls. The expression of DEL1 is positively and negatively regulated by typical E2F transcriptional factors, E2Fb and E2Fc, respectively. In the dark, E2Fb is degraded through the CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1)-mediated proteolysis and E2Fc represses the expression of *DEL1*, resulting in an accumulation of *CCS52A2* transcripts and endocycle onset. (E) DEL1 coordinates endocycle onset and DNA repair by negatively regulating the expression of *CCS52A2* and *PHOTOLYASE 1* (*PHR1*). The expression of *DEL1* is strongly inhibited by UV-B irradiation, leading to increased DNA repair and endocycle progression.

The plant hormone gibberellins promote cell expansion by disrupting growth-inhibitory proteins named DELLAs (Richards et al. 2001). A recent study shows that gibberellins, in addition, promote cell proliferation in Arabidopsis (Achard et al. 2009) (Fig. 4A). In the root meristem of gibberellin-deficient mutants, the rate of cell division is reduced and this phenotype can be recovered by exogenous gibberellin treatment. The DELLA proteins also participate in this regulation since non-degradable forms of DELLA inhibit cell proliferation. The authors further showed that low levels of gibberellins in gibberellin-deficient mutants up-regulate the expression of several CDK inhibitor genes, *KRP2*, *SIM*, *SMR1* and *SMR2*, in a DELLA-dependent manner and that cell proliferation defects of these mutants can be rescued by overexpression of the *CYCD3;1* gene. These results together suggest that gibberellin signaling controls cell proliferation by modulating the activity of CYC–CDK complexes and that this is mediated at least in part through the DELLA-dependent expression of CDK inhibitors. These findings are of particular interest since DELLAs are known to restrict organ growth under adverse conditions (Achard et al. 2006, Achard et al. 2008). Therefore, DELLAs could act as a transducer of the environmental signals with a direct downstream link to cell cycle progression.

The work by Ogawa et al. (2011) has demonstrated that RICE SALT SENSITIVE 1 (*RSS1*) controls cell cycle progression

under various abiotic stress conditions (Fig. 4B). *rss1* mutants do not show any obvious growth defects under normal growth conditions but they display hypersensitivity to high salinity, ionic stress and hyperosmotic stress. Under these unfavorable conditions both shoot and root meristems are severely compromised in *rss1*, with a reduced population of proliferating cells; therefore, *RSS1* is required to maintain cell proliferative competence in the meristem. The *RSS1* gene encodes a novel protein expressed during the S phase of the mitotic cycle and its gene products are degraded via APC/C^{CDC20} during the M–G₁ phase. The yeast two-hybrid assay revealed that *RSS1* interacts with a type 1 protein phosphatase (PP1) implicated in many physiological processes including the cell cycle. In humans, PP1 is known to inactivate retinoblastoma (Rb) proteins through dephosphorylation, which is inhibitory to the G₁ to S transition (Hirschi et al. 2010). This inactivation of Rb is thought to antagonize the hyperactivation of Rb through CYC–CDK-mediated phosphorylation. Although the exact molecular function of *RSS1* is not clear yet, it might also ensure the G₁ to S transition under unfavorable conditions by regulating PP1 activities.

Sugars act as a signaling molecule in various biological processes, and sucrose-induced LR formation represents one good example of sugars engaging in the reactivation of cell proliferation (Nieuwland et al. 2009). The authors showed that the

expression of *CYCD4;1* in root pericycle cells is dependent on the sucrose availability and that reduced *CYCD4;1* levels in *cyc4;1* mutants or wild-type roots grown without sucrose cause the LR density to drop (Fig. 4C). How sucrose activates *CYCD4;1* in a cell type-specific manner remains unclear, but these data suggest that this transcriptional response is a key for the sucrose-dependent regulation of LR density. Interestingly, auxin does not influence *CYCD4;1* expression in pericycle cells and it restores the reduced LR density phenotype of *cyc4;1* mutants, suggesting that *CYCD4;1* does not act in the auxin-mediated LR initiation pathway. Another D-type CYC, *CYCD3;1*, is also responsive to sucrose availability (Planchais et al. 2004), but how directly sucrose impinges on *CYCD3;1* activity is not clear.

The progression of the endocycle is also influenced by various environmental signals, but the underlying molecular mechanisms are not well understood. As discussed earlier, *DEL1* is one of the key regulators that repress entry into the endocycle (Lammens et al. 2008), thus changing the level of *DEL1* expression may provide one mechanism to promote or limit endocycling. Elongation of hypocotyls is linked with an increase in ploidy through the endocycle and it is known that etiolated growth of hypocotyls in the dark is accompanied by an additional round of endocycling (Gendreau et al. 1997, Gendreau et al. 1998). A recent study suggests that the balance between the transcriptional activator E2Fb and repressor E2Fc controls light-dependent endocycling through the antagonistic modification of *DEL1* expression (Berckmans et al. 2011a) (Fig. 4D). The authors show that E2Fb and E2Fc compete for the same DNA-binding site on the *DEL1* promoter to activate or repress *DEL1* expression, respectively. Under light, E2Fb predominantly binds to the *DEL1* promoter and enhances *DEL1* expression, thus resulting in the repression of the endocycle. In the dark, in contrast, E2Fb is degraded by CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1)-mediated proteolysis, permitting E2Fc to bind to the *DEL1* promoter and thus repress *DEL1* expression.

Ultraviolet-B (UV-B) radiation damages DNA molecules by forming cyclobutane pyrimidine dimers (CPDs) which prevent DNA transcription and translation. Plants remove CPDs by photolyases, and these enzymes are encoded by a *PHOTOLYASE 1* (*PHR1*) gene in Arabidopsis. Based on microarray and ChIP analyses, Radziejewski et al. (2011) show that in addition to *CCS52A2*, a known target of *DEL1*, *DEL1* directly represses the transcription of the *PHR1* gene and thereby coordinates DNA repair and endocycle onset (Fig. 4E). Upon UV-B treatment, the expression of *DEL1* is strongly down-regulated, allowing up-regulation of *PHR1* and therefore increasing cellular capacities for DNA repair. Since low *DEL1* expression also promotes endocycle progression due to higher expression of *CCS52A2*, the authors argued that such *DEL1*-mediated DNA repair and ploidy-dependent cell growth may compensate for the compromised cell proliferation under UV-B.

Conclusions and Future Outlook

It is now evident that cell cycle progression is highly correlated with plant morphogenesis under both favorable and unfavorable conditions. Recent large-scale studies have begun to uncover that plant CYCs and CDKs assemble in many different combinations throughout the cell cycle and many of them function at specific phases of the mitotic and endoreduplication cycle. The complex intersecting pathways fine-tuning the CDK activities are well conserved among eukaryotes, but plants have also implemented some unique regulatory mechanisms, e.g. by recruiting plant-specific CDK inhibitors into the CYC–CDK complex. Post-translational modifications, such as phosphorylation and ubiquitination, of the cell cycle regulators is crucial for both cell proliferation and cell differentiation, but their target proteins and the underlying molecular mechanisms still remain largely unknown. Systematic identification of protein targets modified by various CYC–CDK complexes and ubiquitin E3 ligases in proliferative or differentiating cells should provide further molecular insights into how cell cycle progression is controlled and how it links to other physiological processes such as cell growth and metabolism.

We are also beginning to witness how developmental or environmental signals connect to the cell cycle and in some cases how short the regulatory cascades are. Direct control of the cell cycle regulators by developmental or stress-related transcription factors, for example, highlights one of the most effective strategies that plants have adopted to respond quickly to upstream signaling. Given that these regulations are often temporal and probably involve only subsets of cells within a tissue, cell type-specific analyses of gene expression and transcription factor-binding sites, using the laser microdissection technique or cell sorting (Deal and Henikoff 2010), will be very powerful to dissect the transcriptional control of the cell cycle further. Many individual regulators of the cell cycle (instead of E2Fs or MYBs that can modify the expression of cell cycle genes more globally) appear to be directly activated or repressed by upstream regulators, implying that the molecular interface where developmental or environmental signals meet the cell cycle is probably very diverse in plants. One would predict that at least some of the transcriptional regulators in plant hormone signalling, such as ARFs and ARABIDOPSIS RESPONSE REGULATORS (ARRs), also directly activate or repress core cell cycle regulators. Gaining more holistic insights into these regulatory cascades will be one of the most exciting targets in future studies for our full understanding of plant growth and stress response.

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