the plant journal

SEB SOCIETY FOR EXPERIMENTAL BIOLOGY

The Plant Journal (2009) **59**, 645–660 doi: 10.1111/j.1365-313X.2009.03893.x

TECHNICAL ADVANCE

Systematic analysis of cell-cycle gene expression during Arabidopsis development

Janice de Almeida Engler[1,2,†], Lieven De Veylder[1,2], Ruth De Groodt[1,2], Stephane Rombauts[1,2], Véronique Boudolf[1,2], Bjorn De Meyer[1,2], Adriana Hemerly[3,4], Paulo Ferreira[3,4], Tom Beeckman[1,2], Mansour Karimi[1,2], Pierre Hilson[1,2], Dirk Inzé[1,2,*] and Gilbert Engler[5,†]

[1]*Department of Plant Systems Biology, Flanders Institute for Biotechnology (VIB), Technologiepark 927, 9052 Gent, Belgium,*
[2]*Department of Plant Biotechnology and Genetics, Ghent University, Technologiepark 927, 9052 Gent, Belgium,*
[3]*Laboratório de Biologia Molecular de Plantas, Instituto de Bioquímica Médica, Universidade Federal de Rio de Janeiro, CEP 21941-590, Rio de Janeiro RJ, Brazil,*
[4]*Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Diretoria de Pesquisa Científica, CEP 22460-030, Rio de Janeiro, RJ, Brazil, and*
[5]*Laboratoire Associé de l'Institut National de la Recherche Agronomique, Ghent University, Technologiepark 927, 9052, Belgium*

Received 15 January 2009; revised 16 March 2009; accepted 31 March 2009; published online 22 May 2009.
*For correspondence (fax +32 9 3313809; e-mail dirk.inze@psb.ugent.be).
†Present address: Institut National de la Recherche Agronomique, Interactions Biotiques et Santé Végétale, BP 167, F-06903 Sophia Antipolis Cedex, France.

SUMMARY

The steady-state distribution of cell-cycle transcripts in *Arabidopsis thaliana* seedlings was studied in a broad *in situ* survey to provide a better understanding of the expression of cell-cycle genes during plant development. The 61 core cell-cycle genes analyzed were expressed at variable levels throughout the different plant tissues: 23 genes generally in dividing and young differentiating tissues, 34 genes mostly in both dividing and differentiated tissues and four gene transcripts primarily in differentiated tissues. Only 21 genes had a typical patchy expression pattern, indicating tight cell-cycle regulation. The increased expression of 27 cell-cycle genes in the root elongation zone hinted at their involvement in the switch from cell division to differentiation. The induction of 20 cell-cycle genes in differentiated cortical cells of etiolated hypocotyls pointed to their possible role in the process of endoreduplication. Of seven cyclin-dependent kinase inhibitor genes, five were upregulated in etiolated hypocotyls, suggesting a role in cell-cycle arrest. Nineteen genes were preferentially expressed in pericycle cells activated by auxin that give rise to lateral root primordia. Approximately 1800 images have been collected and can be queried via an online database. Our *in situ* analysis revealed that 70% of the cell-cycle genes, although expressed at different levels, show a large overlap in their localization. The lack of regulatory motifs in the upstream regions of the analyzed genes suggests the absence of a universal transcriptional control mechanism for all cell-cycle genes.

Keywords: cell cycle, *Arabidopsis thaliana,* endoreduplication, lateral root initiation, *in situ* hybridization.

INTRODUCTION

Regulation of the cell cycle requires the expression and activation of a significant number of genes, some of which are already quite well characterized and others still to be identified (Inzé and De Veylder, 2006). A central role in cell-cycle control is played by the group of cyclin-dependent kinases (CDKs) and their regulatory cyclin subunits. In *Arabidopsis thaliana*, 12 different CDKs (designated *CDKA* to *CDKF*) and up to 49 cyclins (types A–D, H, P and T) have been identified (Vandepoele *et al.*, 2002; Wang *et al.*, 2004). At the transcriptional level, most cyclins show quite a strong oscillatory behavior, especially those of types A and B. The CYCA3 class is associated specifically with expression in the S phase, while other A-type and B-type cyclins largely show common regulation in G_2/M (Menges *et al.*, 2005). In contrast, CDKs display little cell cycle phase-dependent transcriptional regulation (Inzé, 2005), with the exception of the B-type CDK

genes that peak during G_2 and M (Fobert *et al.*, 1996; Mironov *et al.*, 1999; Menges and Murray, 2002; Andersen *et al.*, 2008).

At the G_1–S transition, CDKA;1 forms a complex with D-type cyclins (CYCD). D-type cyclins are typically induced by mitogens and are thought to keep cells in a dividing state by repressing the retinoblastoma-related 1 (RBR1)–E2F pathway. As long as RBR1 is hypophosphorylated, it interferes with the onset of DNA replication through its interaction with the E2F–DP transcription factors (Park *et al.*, 2005), crucial regulators of genes required for DNA synthesis. Classical E2F target genes include the *ORIGIN RECOGNITION COMPLEX* (*ORC*), *MINICHROMOSOME MAINTENANCE* (*MCM*) and the replication-licensing factor genes *CDC6a* and *CDT1* that all contribute to the initiation of DNA replication (Masuda *et al.*, 2004; Vandepoele *et al.*, 2005). Upon phosphorylation by the CDKA;1–CYCD complexes, the repressing activity of RBR1 is alleviated, activating the E2F–DP transcription factors. E2F factors are involved in regulating many S-phase genes, thus controlling cell-cycle-regulated gene expression (Combettes *et al.*, 1999; Castellano *et al.*, 2001; de Jager *et al.*, 2001). However, not all E2F–DP heterodimers are expected to have a positive effect on the cell cycle. In Arabidopsis, three *E2F*, two *DP* and three DP–E2F-like (*DEL*) genes have been identified (Vandepoele *et al.*, 2002). Whereas E2Fa and E2Fb are potential transcriptional activators (De Veylder *et al.*, 2002; Rossignol *et al.*, 2002; Kosugi and Ohashi, 2003), E2Fc acts as a repressor of E2F target genes (del Pozo *et al.*, 2002, 2006). Also the DEL proteins might counteract E2F-activated pathways, because they are thought to operate as transcriptional repressors competing with the E2F–DP complexes for the same DNA-binding sites (Ramirez-Parra *et al.*, 2004; Vlieghe *et al.*, 2005).

Whereas our knowledge about the control of the G_1–S transition is steadily increasing, little is known on how the G_2–M transition is controlled (De Veylder *et al.*, 2003; Inzé and De Veylder, 2006). Because of their observed peak in expression and activity during G_2–M, the B-type CDKs, together with the A-type CDKs, are generally assumed to participate in this transition (Magyar *et al.*, 1997; Porceddu *et al.*, 2001; Sorrell *et al.*, 2001; Boudolf *et al.*, 2004a,b). The possible cyclin partners belong to the A- and B-type cyclins expressed during the S–G_2 and G_2–M phases, respectively.

Because of its core importance to life, cell-cycle control has to be strictly regulated. Timely activation of CDK activity is not only controlled by cyclin binding but also by the phosphorylation status of the CDK. Phosphorylation at Thr161 (or any equivalent residue) is a prerequisite for CDK activity, a reaction catalyzed by the CDK-activating CDKs, represented in plants by the D-type and F-type CDKs (Vandepoele *et al.*, 2002). D-type CDKs are distinct from F-type CDKs because they are only active when associated with H-type cyclins (Shimotohno *et al.*, 2003). The activity of CDKs might also be negatively regulated through phosphorylation of the CDK subunit by the WEE1 kinase (Sun *et al.*, 1999; Sorrell *et al.*, 2002; De Schutter *et al.*, 2007). In yeast and mammals, this reaction is counteracted by the CDC25 phosphatase, but whether a similar mechanism is functional in plants is unclear (Boudolf *et al.*, 2006). WEE1 and CDC25 themselves are also controlled at different levels, the latter through a change in its structural configuration by a prolyl isomerase encoded by the *PARVULIN 1* gene of Arabidopsis (*AtPIN1*) (Stukenberg and Kirschner, 2001) for which a putative plant ortholog has been reported (Landrieu *et al.*, 2000).

Many other mechanisms regulate CDK activity that can be inhibited through their interaction with CDK inhibitory molecules, designated Kip-related proteins (KRPs), also known as interactors of CDC2 kinases (ICKs) (De Veylder *et al.*, 2001). The docking factors CKS1 and CKS2 are also essential regulators of CDK activity, because they help in the binding of CDK substrates (Pines, 1996; Patra *et al.*, 1999). In particular, CDK activity is controlled at the post-transcriptional level by organized protein destruction that occurs via the anaphase-promoting complex (APC/C), which is highly conserved in all eukaryotes (Harper *et al.*, 2002). This proteolytic pathway involves the conjugation of a polyubiquitin chain to a protein and subsequent degradation by a multicatalytic protease complex, called the proteasome. Conjugation of ubiquitin to the protein involves three enzymes: E1 (ubiquitin activating), E2 (conjugating) and E3 (ubiquitin ligase). CDC27a and CDC27b are predicted subunits of the APC, a particular E3 ligase that regulates M-phase progression. Proteins of CDC20/Fzy- and CCS52/Cdh1/Fzy-related families control the APC activity and its substrate specificity spatially and temporally. The large 26S proteasome comprises a 20S catalytic unit and a regulatory subunit, the 19S complex (Coux *et al.*, 1996; Tanaka and Tsurumi, 1997) consisting of different proteins. Twenty-three Arabidopsis genes coding for 14 subunits of the 20S proteasome have been identified (Parmentier *et al.*, 1997; Fu *et al.*, 1998), for instance the *17A-2A* gene (Vercauteren *et al.*, 2001).

The cell cycle is partly regulated at the transcriptional level. Therefore, deciphering the spatial mRNA localization *in planta* of all core cell-cycle genes could help to better understand how cell division functions in relation to plant growth. Until now, mRNA *in situ* hybridization is still the most refined method for spatial and temporal transcript localization. With gene-specific probes, we investigated the expression pattern of a large set of core cell-cycle-regulated genes of different classes in a range of plant tissues of Arabidopsis seedlings and attempted to identify the cell-cycle genes involved in different developmental processes. In an attempt to provide more detailed tissue-specific information, our studies were extended by using *Raphanus sativus* (radish), being closely related to Arabidopsis. Cell-cycle gene expression in root and shoot meristems provided a direct link to cell proliferation. Roots induced to form lateral roots identified genes implicated in lateral root

formation and cell-cycle re-entry. To correlate cell-cycle gene expression with endoreduplication, we systematically compared expression patterns in hypocotyls from seedlings grown in the light and the dark.

RESULTS

The expression patterns of 61 core cell-cycle genes (grouped as a set of CDKs, cyclins and additional genes involved in cell-cycle control and regulation) were studied in different plant tissues with mRNA *in situ* hybridization. All genes analyzed were expressed at detectable levels. A schematic illustration of the expression patterns observed within the different gene classes is depicted in Figure 1 using color codes for the different tissues and organs (Figure 2). All *in situ* experiments were carried out with antisense probes of Arabidopsis genes on seedlings of Arabidopsis and the closely related radish. Radish seedlings were used when particular details of hybridization signals were difficult to discern in certain tissues. Data generated by radish seedlings extend our results in Arabidopsis, and the location of the transcripts was often similar in both species. Expression levels for each gene were estimated by observing signal intensities of tissue sections developed at the same time point and by estimating silver grain deposition over the plant tissues (Figure 1). Some representative images illustrating *in situ* localization data are presented in Figures 3–5. A complete catalogue of approximately 1800 *in situ* hybridization images can be queried on-line via the Plant Systems Explorer Image Database at http://www.psb.ugent.be/ishi/, either by gene names or AGI codes. Control hybridization images can be visualized by entering the Contr* keyword. Image numbers (sample ID reference, for example S152023) indicated throughout the text give direct access to the *in situ* hybridization results via the query interface.

Distinct seedling tissues were used as a marker for particular plant processes. For example, genes expressed in the root elongation zone (EZ) might suggest their involvement in endoreduplication. To link certain cell-cycle genes with endoreduplication, we compared expression profiles between cortical cells of light- and dark-grown plantlets. Pericycle cells primed by 1-naphthylacetic acid (NAA) for lateral root initiation were compared with roots arrested by *N*-1-naphthylphthalamic acid (NPA) to identify genes specifically expressed during the initial steps of lateral root formation. In Table 1, genes were grouped on the basis of their expression pattern:meristematic tissues and meristematic and/or differentiated tissues, showing patchy patterns, induced in dark-grown seedlings, active in pericycle cells at the protoxylem poles, or in synchronized pericycle cells.

Gene expression in the shoot apex

Expression of all cell-cycle genes analyzed was detected at the shoot apex, although at different levels. Because of the small size of the Arabidopsis shoot apical meristem (SAM), gene expression in the different cell layers was difficult to distinguish and only an interpretation for L1 of the three typical SAM layers is presented (Figure 1). Localization patterns for mRNA in the shoot apex were often homogeneous, although *CYCA2;1*(S152023) had an expression pattern which was clearly patchy in individual cells. *CDKB2;1*(S152812) and *CDKB2;2*(S152829; S152830; S152847) also showed patchy patterns in the shoot apex of germinating seedlings of Arabidopsis and radish, validating recently published results (Andersen *et al.*, 2008). High signals were often seen in leaf primordia (Figures 1 and 3a–g for *CDKF;1*, *CYCA2;1*, *CYCB2;3*, *CYCH;1*, *ICK1/KRP1*, *KRP6*, *AtPIN1* in Arabidopsis; Figure 3k–m for *ICK2/KRP2*, *E2Fc*, *AtPIN1* in radish), and low expression was seen for *KRP6* (Figure 3f). In young leaves, nearly all cell-cycle genes were expressed throughout the lamina (YL in Figure 1; e.g. Figure 3a,b,d,e,g,l,m). All cell-cycle genes were also expressed in a variety of patterns in expanding leaves (ML in Figure 1; see database). Although expression was more common in developing vascular bundles, a low and homogeneous or patchy expression of particular genes was seen in the leaf mesophyll tissue (Figure 1 and 3a,b,m). Interestingly, a large set of the core cell-cycle genes analyzed were highly expressed in the leaf epidermis (Figure 1), when compared with signals detected in mesophyll tissues (Figure 3k,l). A unique example is the *ICK2*/*KRP2* mRNA which is specifically and strongly expressed in the epidermis of the hypocotyl, young leaves and in the L1 layer of the SAM (Figure 3k,k'). *CDC6a*(S152003) also showed epidermal expression in leaves, including stomatal cells. More specific expression in the latter was often seen for *CDKB1;1*(S153005) as previously observed by Boudolf *et al.* (2004a). In hypocotyls, most cell-cycle genes [*CYCB2;3*(S152519), *CYCH;1*(S152628), *CCS52A1*(S152286), *CCS52B*(S152291), *CYCA2;3*(S152472), *CYCB2;3*(S152519), *CDKA;1*(S152784) and *ORC4*(S152869)] were more highly expressed in the developing vascular tissue (Figure 3c,d,g), in the epidermis (Figure 3b,c,f) and in the numerous stomata present in that region. The same pattern of transcript distribution often extended throughout the cotyledons (Figure 3b,c,e–g). The expression patterns observed in the apical hook of light-grown seedlings varied from low to high and from homogeneous to patchy (Figures 1 and 3; see database) and most cell-cycle transcripts were differently distributed among tissues. Expression patterns observed for radish in light-grown seedlings firmly matched those observed in Arabidopsis, as illustrated for *AtPIN1* (Figure 3g,m for Arabidopsis and radish, respectively). The typical patchy pattern of *CYCA2;1* in the Arabidopsis shoot apex was never observed in control sense hybridizations (Figure 3n).

Gene expression in dark-grown compared with light-grown seedlings

Even though most genes had a detectable level of expression in the hypocotyl during light-grown seedling development,

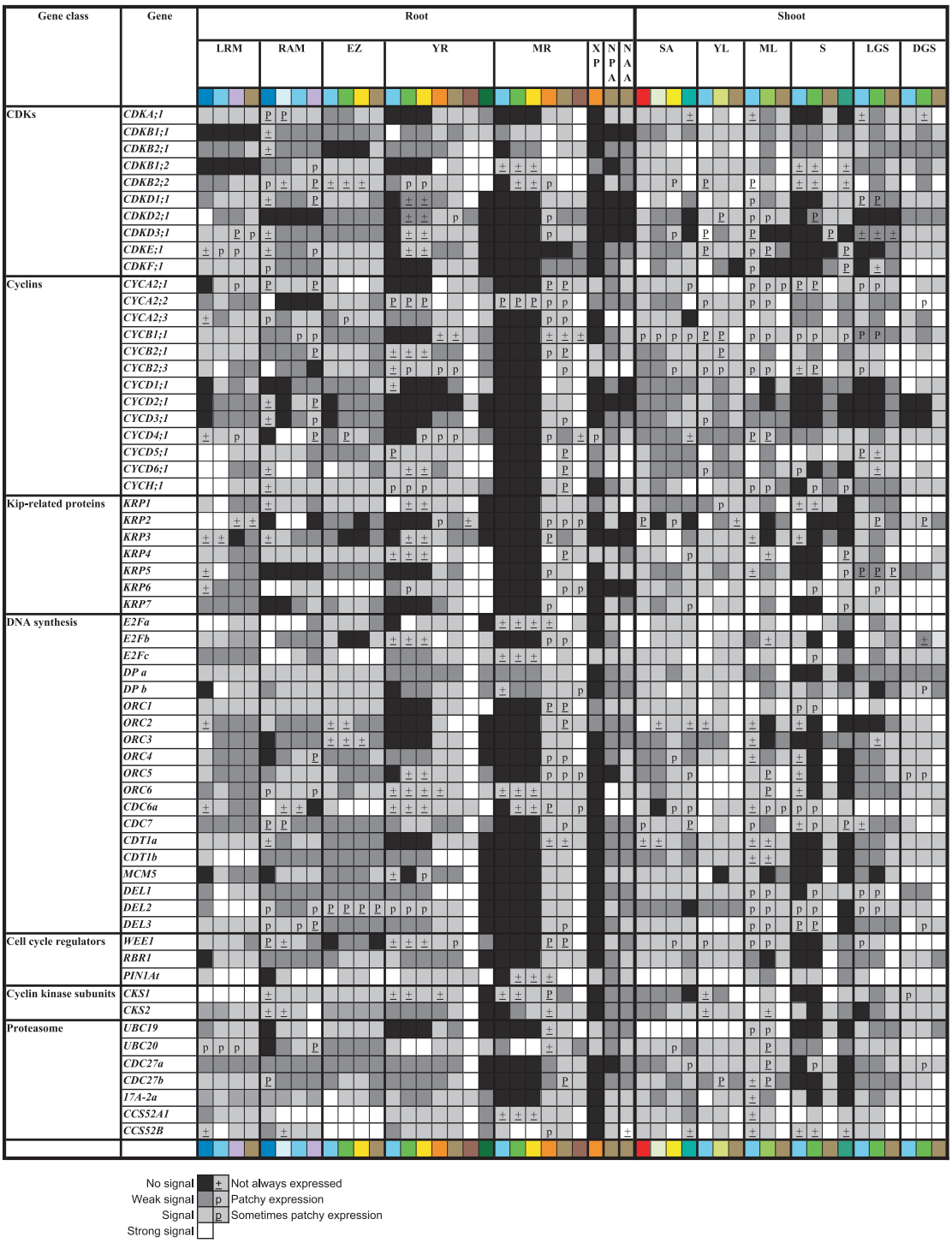

Figure 1. Messenger RNA localization pattern of cell-cycle genes and their relative level of expression.
Genes are grouped in family classes representing their possible activity during cell-cycle progression. The colors shown correspond with those marking organs and tissues in Figure 2. Root and shoot apices were subdivided into LRM (lateral root meristem); RAM (root apical meristem); EZ (elongation zone); YR (young root); MR (maturing root); XP (xylem pole(s)); NPA, roots treated with the auxin transport inhibitor *N*-1-naphthylphthalamic acid preventing pericycle activation; NAA, roots treated with auxin 1-naphthalene acetic acid inducing pericycle cell-cycle reactivation; LGS (light-grown seedlings); DGS (dark-grown seedlings); L1, first layer of the SAM; SAM (shoot apical meristem); LP (leaf primordia); GM (ground meristem); YL (young leaf); ML (mature leaf); S (stem). Relative levels of expression are represented by the following signs: ±, not always expressed; p, patchy expression pattern (either in individual or groups of cells), P, sometimes patchy expression pattern. No sign, expressed.

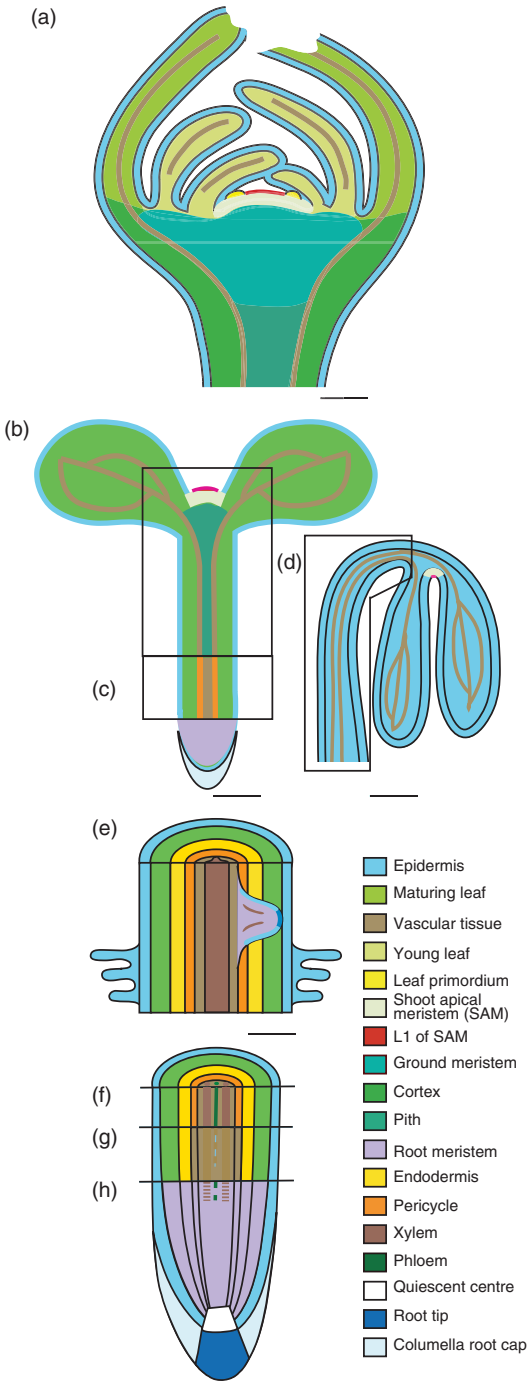

Figure 2. Schematic representation of Arabidopsis organs and tissues. All tissues mentioned below are displayed in a particular color and correspond to the illustration of the expression patterns shown in Figure 1.
(a) Light-grown radish seedling 12 days after sowing (DAS).
(b) Apical hook of light-grown seedling of Arabidopsis 7 DAS.
(c) Root of light-grown seedling of Arabidopsis 7 DAS.
(d) Apical hook of a dark-grown Arabidopsis seedling 7 DAS.
(e) Maturing root containing a lateral root meristem.
(f) Young root.
(g) Root elongation zone.
(h) Root meristem.
Bars = 100 μm (a–d); 30 μm (e–h).

in 43 (Figure 1) out of the 61 analyzed genes the expression level in the apical hook of dark-grown (etiolated) seedlings (Figure 3h–j for *ICK1*/*KRP1*, *KRP6* and *ORC*) was higher than that of light-grown seedlings (Figure 1; see database). We analyzed more particularly cortical cells, in which an increase in expression was more likely to be associated with an extra endocycle (Gendreau *et al.*, 1997). Of the 43 genes, 20 showed a stronger signal in dark- than in light-grown cortical cells (large cross in Table 1). Genes induced in dark-grown plantlets are grouped in Table 1 and examples are shown in Figure 3 [Figure 3h–j; *CYCA2;2*(S152937), *CYCB2;3*(S152495), *CYCD5;1*(S152579), *ICK1*/*KRP1*(S152066), *KRP3*(S152082), *KRP4*(S152088), *KRP7*(S152975), *E2Fa*(S152674), *E2Fc* (S152711) and *ORC6*(S152895)]. Further down the hypocotyl of etiolated seedlings, hybridization signals often decreased in the epidermis and cortical tissues, while persisting in the cells of the vascular cylinder [*KRP5*(S152109), *MCM5*(S152139), *PIN1At*(S152194), *17A2a*(S152249), *CCS52A1*(S152274), *CCS52B*(S152289), *CYCD1;1*(S152526), *CYCD3;1*(S152553), *E2Ff/DEL3*(S152665), *E2Fc*(S152711), *CDKB1;1*(S152793), *CDKB1;2*(S152801) and *CDKB2.2*(S152839)].

Gene expression in the root

All genes analyzed were expressed in lateral root meristems (LRMs) and/or in the root apical meristem (RAM) (Figure 1; see database). A range of transcript distribution (from homogeneous to patchy) of the different core cell-cycle genes could be observed in emerging LRMs (Figure 4a-d) in the EZ (Figure 4e–g) and in the RAM (Figure 4h–l). In addition, the pattern of transcript distribution and levels between emerging and mature RAM often varied for the same gene (Table 1; see database). For example, the *CYCB1;1* and *E2Fd/DEL2* transcripts were homogeneously and highly expressed in young lateral roots but had a patchy and low expression pattern in the mature RAM, respectively [*CYCB1;1*(S152482) for LRM compared with (S152479, S152480, S152481, S152485, S152486, S152487) for RAM and *E2Fd*/*DEL2*(S152660) for LRM compared with (S152657, S152658, S152659) for RAM]. This apparent discrepancy could be explained by the different length of the cell cycle in young lateral meristems versus the mature meristem, where faster cell cycling in younger roots masks a cell-cycle phase-dependent expression pattern. For the *CDKD2;1*, *CYCA2;2*, *CYCB2;3* and *CDC6a* genes [Figure 1; e.g. *CDKD2;1*(S152370), *CYCA2;2*(S152941), *CYCB2;3*(S152501) and *CDC6a*(S152007)], hybridization signals were low or not detectable in the mature RAM. The putative homolog of *AtPIN1* in Arabidopsis (Landrieu *et al.*, 2004) was highly expressed in RAM (S152929, S152934), in accordance with its anticipated role as a regulator of cell division. Genes such as the *KRP* cell-cycle inhibitors were moderately and differently expressed in the LRM and RAM [Table 1; e.g. *ICK1*/*KRP1*(S152065), *ICK2*/*KRP2*(S152078), *KRP3*(S152079), *KRP4*(S152102), *KRP5*(S152117), *KRP6*(S152962) and

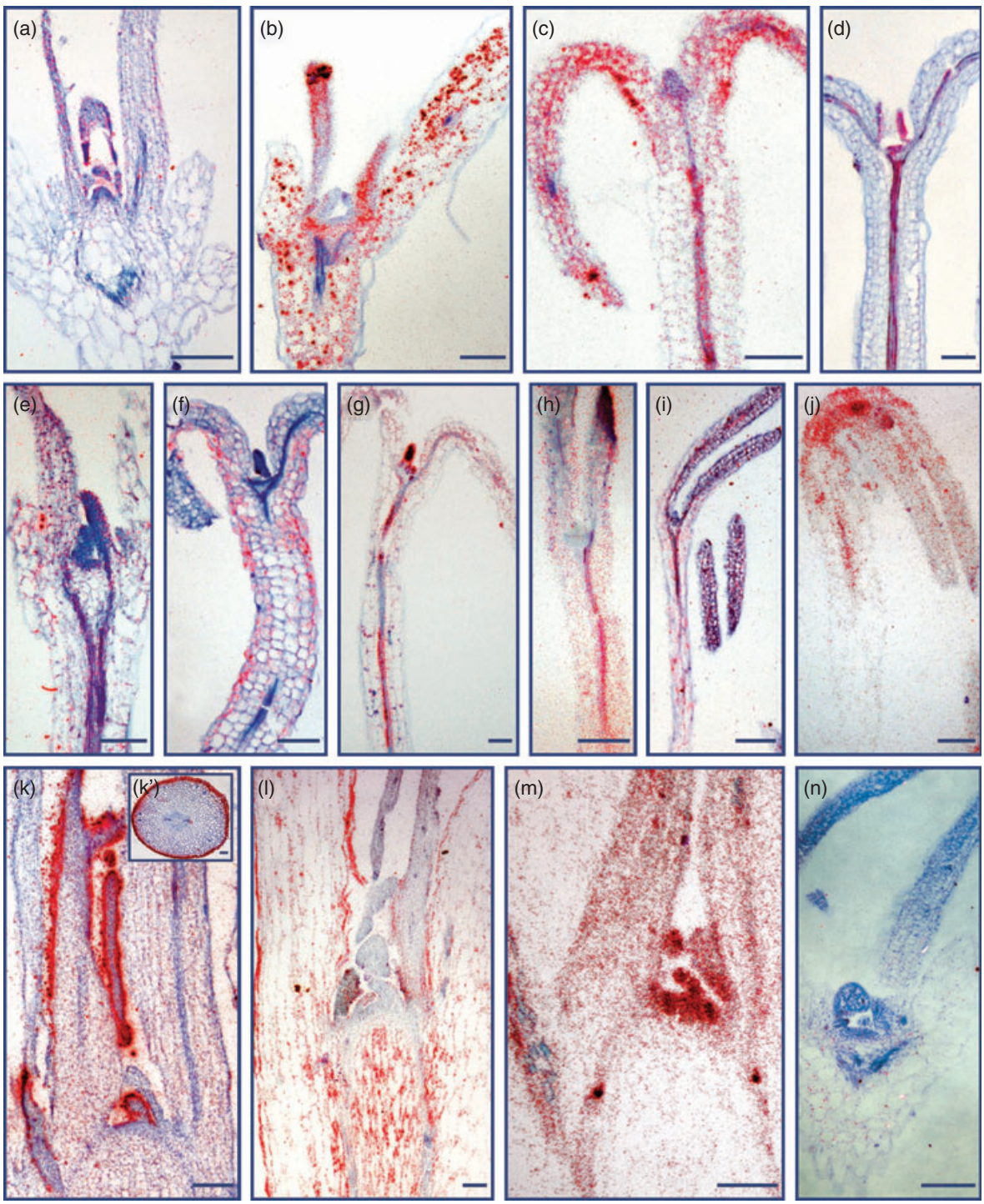

Figure 3. *In situ* localization of cell-cycle transcripts in light-grown seedlings of *Arabidopsis* (a–f) and radish (j–l) and dark-grown Arabidopsis (g–i) seedlings. Longitudinal tissue sections were hybridized with ^{35}S-labeled antisense RNA probes and stained with toluidine blue for morphology. Signal is seen as red dots on a light background. Arabidopsis sections probed for *CDKF;1* (a), *CYCA2;1* (b), *CYCB2;3* (c), *CYCH;1* (d), *ICK1*/*KRP1* (e), *KRP6* (f) and *AtPIN1* (g). Arabidopsis sections of dark-grown seedlings probed for *ICK1*/*KRP1* (h), *KRP6* (i) and *ORC1A* (j). Radish sections *ICK2*/*KRP2* (k, k'), *E2Fc* (l), *AtPIN1* (m) and sense hybridization with *CYCA2;1* in the Arabidopsis shoot apex (n). Bars = 50 μm (a, i), 100 μm (b–h, j–n).

KRP7(S152986)], implying different roles for the distinct KRPs in the root meristems. Interestingly, *ICK2*/*KRP2* was expressed in epidermal and lateral root-cap initial cells within the RAM (Figure 4i). No specific background was seen in sense hybridizations (Figure 4m). For several cell cycle genes, the expression was homogeneous in the RAM

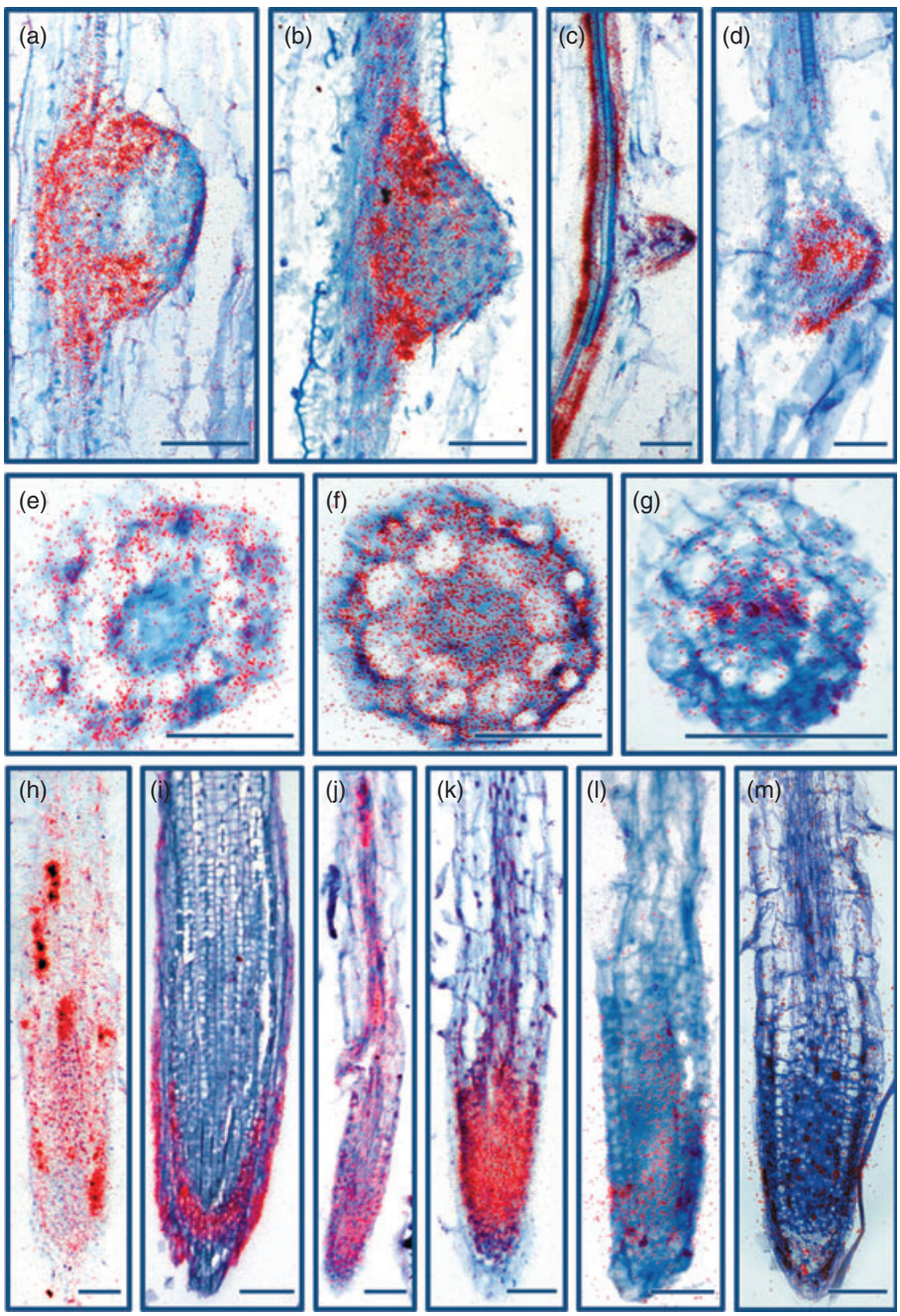

Figure 4. Transcript localization of cell-cycle genes in lateral root meristem, root apical meristem (RAM) and elongation zone (EZ).
Lateral root meristem of radish (a, b) and Arabidopsis (c, d) and root EZ of Arabidopsis (e–g). *CYCA2;1* (a), *CYCD4;1* (b), *E2Fa* (c), *UBC20* (d), Arath;*17A-2A* (e), *CKS1* (f) and *CDC6a* (g). Longitudinal sections of radish (h, i, m) and Arabidopsis RAMs (j–l). *CYCD4;1* (h), *ICK2/KRP2* (i), *ORC4* (j) *CKS1* (k), *UBC20* (l) and sense hybridization with *KRP2* (m). Bars = 50 μm (b, d–h, j–l); 100 μm (a, c, i, m).

(Figure 4k for *CKS1*), whereas for others it was clearly patchy, hinting at a stricter cell-cycle-regulated expression pattern for particular genes (Figures 1 and 4h,l for *CYCD4;1* and *UBC19*). The mRNAs of another set of genes were mainly distributed in particular sectors of the meristem [*CYCA2;3*(S152456, S152457) and *E2Ff*/*DEL3*(S152667, S152664, S152668)]. *CYCD4;1* exhibited mRNA distribution at the base of the LRM [Figure 4b; *CYCD4;1*(S152030, S152572–73, S152576–78 of radish and S153780, S153781 of Arabidopsis], showing changes in patchiness during RAM development (Figure 4h). Expression could be detected in pericycle cells in radish close to one protoxylem pole (Figure 5b) and the expression in vascular tissues varied from patchy to clustered in some cells to a single pericycle

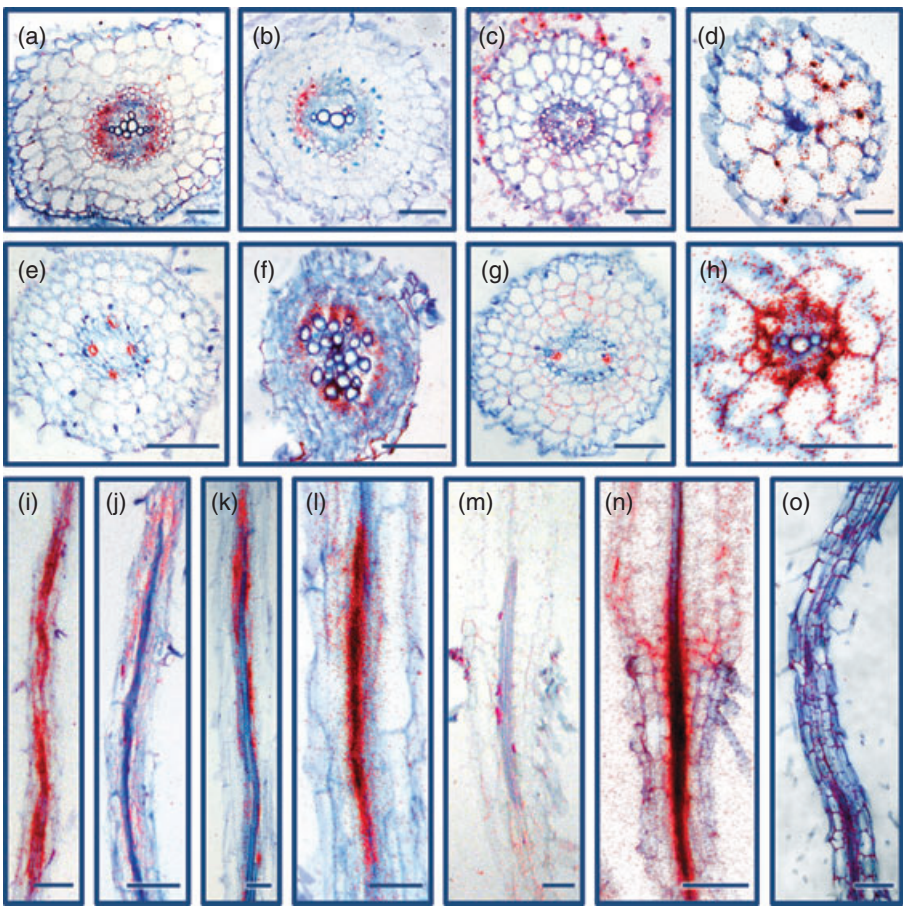

Figure 5. *In situ* localization of cell-cycle transcripts in cross-sections of Arabidopsis (f, h) and radish roots (a–e, g) and in longitudinal sections of Arabidopsis roots, hybridized with antisense (i–l) and sense (o) probes and in pericycle cells blocked (m) or activated (h) to form lateral roots.
CYCA2;1 (a), *CYCD4;1* (b), *CYCD5;1* (c), *KRP6* (d), *CYCD1;1* (e), *E2Fb* (f), *CDC7* (g) and *CKS1* (h). Longitudinal sections of Arabidopsis roots: *CYCA2;2* (i), *CYCB2;3* (j), *E2Fa* (k), *E2Fd/DEL2* (l), *N*-1-naphthylphthalamic acid (NPA)-treated *E2Fb* (m) and NAA-treated *E2Fb* (n); and sense hybridization with *E2Fa* in the Arabidopsis root (o). Bars = 50 μm (f, h–n); 100 μm (a–e, g, o).

cell (De Veylder *et al.*, 1999). As previously observed, *UBC20* had a significant expression in differentiated root tissues (Criqui *et al.*, 2002) (S152772, S152773, S152774).

Surprisingly, most core cell-cycle genes also showed some expression in the root EZ just above the RAM, where endoreduplication presumably occurs (Figure 1). A correlation of expression in the root EZ and strong expression in cortical cells in the apical hook of etiolated seedlings was observed for 15 out of 20 genes highly expressed in etiolated seedlings (Figure 1), further suggesting a link for these genes in the endoreduplication process. Variations in expression levels within the root were observed in the differentiating tissue layers (epidermis, cortex, endodermis and vascular tissue) (Figure 3e–g) and the observed patterns differed from the flanking dividing meristematic tissues (Figure 1). Cell cycle genes such as *CDKD1;1(S152358), E2Fa*(S152684), *E2Fc*(S152724), *CDC6a*(S152007), *PIN1At*(S152929, S152932), *CCS52A1*(S152278, S152280) and *CCS52B*(S152296, S152303) were strongly induced in the root EZ, suggesting their involvement in the switch from cell division (RAM) to differentiation.

More pronounced variations in expression patterns were observed in young and maturing non-meristematic root tissues (Figures 1 and 5a–e,g–l). These differences were also seen within sections of the same gene and might be a consequence of the maturation state of an individual root as well as of the position of the root section (Figure 1; see database). Antisense hybridizations always showed gene-specific expression patterns in the root tissue, whereas sense control hybridizations never displayed a specific background pattern (Figure 5o). During vascular tissue development, all cell-cycle genes were expressed in living xylem cells, not only in the shoot but also in the seedling root (Figures 1 and 4i–l for *CYCA2;2*, *CYCB2;3*, *E2Fa* and *E2Fd/DEL2*). Signals were more rarely detected in mature roots (Figure 5f for *E2Fb*) and in phloem tissues (opposite to xylem poles), and only in particular tissue sections of young

Table 1 Gene assemblage based on expression patterns

		Expression[a]						
Gene class	Gene	I	II	III	IV	V	VI	VII
Cyclin-dependent kinases	*CDKA;1*	x	o	o	o	o	o	o
	CDKB1;1	o	x	o	o	o	o	o
	CDKB2;1	o	x	o	x	o	o	o
	CDKB1;2	o	x	o	o	o	o	x
	CDKB2;2	o	x	o	x	o	x	o
	CDKD1;1	x	o	o	o	x	o	o
	CDKD2;1	x	o	o	o	x	o	o
	CDKD3;1	x	o	o	o	x	o	o
	CDKE;1	x	o	o	o	x	o	o
	CDKF;1	x	o	o	o	x	o	x
Cyclins	*CYCA2;1*	o	x	o	x	x	x	x
	CYCA2;2	o	x	x	x	X	x	o
	CYCA2;3	x	o	o	o	o	o	o
	CYCB1;1	x	o	o	x	o	o	x
	CYCB2;1	o	x	o	x	o	x	x
	CYCB2;3	o	o	x	x	X	x	o
	CYCD1;1	x	o	o	o	x	o	o
	CYCD2;1	x	o	o	o	o	o	o
	CYCD3;1	x	o	o	x	o	o	o
	CYCD4;1	o	x	o	x	x	x	x
	CYCD5;1	o	x	o	x	X	x	X
	CYCD6;1	o	x	o	x	x	x	o
	CYCH;1	o	x	o	o	x	o	x
Kip-related proteins	*KRP1*	o	x	o	o	X	x	X
	KRP2	o	o	x	x	o	o	o
	KRP3	o	x	o	o	X	o	o
	KRP4	o	x	o	x	X	x	o
	KRP5	o	o	x	o	o	o	o
	KRP6	o	x	o	o	X	o	o
	KRP7	o	x	o	o	X	o	o
DNA synthesis	*E2Fa*	o	x	o	o	X	o	X
	E2Fb	o	x	o	o	o	o	X
	E2Fc	o	x	o	x	x	x	o
	DPa	x	o	o	o	o	o	x
	DPb	o	x	o	o	x	o	o
	ORC1a	o	x	o	x	X	x	o
	ORC2	x	o	o	x	x	x	o
	ORC3	x	o	o	o	x	o	o
	ORC4	o	x	o	x	x	o	x
	ORC5	o	x	o	o	o	o	x
	ORC6	o	x	o	o	X	o	o
	CDC6a	o	x	o	o	X	o	o
	CDC7	o	x	o	o	X	o	o
	CDT1a	x	o	o	o	x	o	o
	CDT1b	x	o	o	o	x	o	o
	MCM5	x	o	o	o	X	o	o
	DEL1	o	x	o	o	o	o	o
	DEL2	o	x	o	x	x	o	o
	DEL3	x	o	o	o	o	o	x
Cell-cycle regulators	*WEE1*	x	o	o	x	X	o	o
	RBR1	x	o	o	o	x	o	x
	PIN1At	o	x	o	x	X	x	x
Cyclin kinase subunits	*CKS1*	o	x	o	o	x	o	o
	CKS2	o	x	o	o	x	o	o
Proteasome	*UBC19*	x	o	o	o	x	x	o
	UBC20	o	x	o	x	X	x	x
	CDC27a	x	o	o	o	o	o	x
	CDC27b	x	o	o	o	x	o	o

Table 1 (Continued)

Gene class	Gene	Expression[a] I	II	III	IV	V	VI	VII
	17A-2a	o	o	o	o	X	o	o
	CCS52A1	o	x	o	o	X	o	o
	CCS52B	o	x	o	o	X	o	o

X, strong expression; x, moderate expression; o, insufficient expression to be included.
[a]Cell-cycle genes were grouped based on their recurrent expression mainly in meristematic tissues (I) and in meristematic(II) and/or in differentiated tissues (III) as well as genes regularly showing patchy expression patterns (IV), with induced expression in dark-grown compared to light-grown seedlings (V), active in protoxylem poles (VI) and in synchronized pericycle cells (VII).

roots, indicating a transient activation of the cell cycle during phloem ontogeny [(Figures 1 and 5e for *CYCD1;1*; for others see *CYCB2;3*(S152513), *CYCD6;1*(S152618), *E2Fc*(S152731), *UBC20*(S152772), *CDKB1;2*(S152811), *CDKB2;2*(S152845) and *ORC6*(S152923)]. An overall and high expression was observed in young vascular tissue before secondary growth and only a few genes strongly hybridized to xylem cells of mature roots located around dead xylem vessels [e.g. *CYCA2;3 (*S152464, S152465, S152466); *E2Fa*(S152686, S152687, S152693, S152694); *E2Fb*(S152055); *ORC1a* (S152153); *ORC4*(*S*152858, S152859), *ORC5* (S152887, S152892), *ORC6* (S152912, S152916, S152920, S152921, S152905) and *KRP5* (S152114, S152115)].

Gene expression in the xylem pericycle and in activated pericycle cells

Cell-cycle activation in pericycle cells at the protoxylem poles is a first step in lateral root initiation (De Smet *et al.*, 2006). We observed that 16 of the 61 genes (Table 1) were preferentially expressed in the pericycle at the protoxylem poles where lateral roots normally emerge, and thus are possibly involved in the competence of lateral root formation (Ferreira *et al.*, 1991). Examples are given for *CYCA2;1* and for *CYCD4;1* (Figure 4a and 5b, respectively) [for others, see *E2Fc*(S152721, S152727, S152732), *KRP4*(S152098); *ORC1a*(S152151) and *CYCB2;3*(S152514)]. This pattern was more marked in cross-sections of radish roots than in the tiny Arabidopsis roots.

In order to investigate which genes might be implicated in the first steps of lateral root formation, Arabidopsis seedlings were treated with the auxin transport inhibitor NPA to block the first pericycle cell division and transferred to NAA-containing medium to activate and synchronize the cell cycle in all pericycle cells. Only 19 of the 61 genes were induced in activated pericycle cells (Table 1; Figure 5n for *E2Fb*). Genes such as *CDKA;1, CDKB2;1*, *CDKE;1*, *CYCA2;2*, *CYCA2;3*, *CYCB2;3*, *CYCD6;1*, *KRP5*, *E2Fc*, *ORC1a*, *ORC2*, ORC3, *CDC6a*, *CDC7*, *CDT1a*, *CDT1b*, *MCM5*, *E2Fe/DEL1*, *CKS1*, *CKS2*, *UBC19*, *CDC27b*, *17A-2A* and *CCS52A1* had similar expression levels in roots treated with NPA and NAA. No preferential expression was observed in pericycle cells activated for lateral root formation. The *CYCA2;3*, *CYCB2;3*, *CYCD6;1*(S152603, S152599), *ICK1/KRP1*, *KRP5* and *CDC6a*(S152012, S152011) genes showed high hybridization signals, whereas the *CDKB2;2*, *CYCD1;1*, *ICK2*/*KRP2*, *KRP4*, *KRP7*, *ORC6*, *E2Fd*/*DEL2* and *WEE1* genes were slightly decreased in NAA-activated pericycle cells, suggesting that the induction of these genes is not essential during the initiation of lateral root development. The observed decrease in expression of *ICK2*/*KRP2, KRP4*(S152100, S152101), *KRP7* and *WEE1*(S152999, S153017) indicates that the down-regulation of these cell-cycle inhibitors might be needed for lateral root activation.

In silico analysis of expression patterns of core cell cycle genes

Based on the descriptive mRNA expression levels of the different cell-cycle genes, discrete values were attributed to high or low signals. After appropriate transformation, tissues in which genes were expressed as one criterion and the level of expression as the other criterion were bi-clustered. (average linkage and Euclidean distance measure) (Figure S1 in Supporting Information). The obtained cluster profiles were submitted to searches in an attempt to find common promoter motifs. None of the sets of promoters, each analyzed with a range of settings (see Experimental Procedures), returned any significant common motif. For some genes the timing of transcription might not necessarily be linked to its activity, whereas for others different functions might be exerted during processes such as cell-cycle progression, cell division, tissue expansion and differentiation, indicating that the maneuver of the cell cycle is probably inherent within the cell cycle itself rather than being bound to spatially dependent components.

DISCUSSION

During plant growth and development, cells divide, expand and differentiate into distinct cell types, such as epidermis, cortex, endodermis, pericycle and vascular tissues that build up the mature plant. Stems and roots grow through a complex interplay between cell division in apical meristems and expansion in subapical EZs. Fine regulation of cell-cycle

gene expression plays a key role in cell-cycle progression as well as in the control of plant development. To better understand the possible role of the cell-cycle genes in these processes, the expression profile of a large set of individual Arabidopsis genes belonging to the core cell-cycle machinery was extensively analyzed with mRNA *in situ* hybridization and gene expression patterns collected during Arabidopsis seedling development. Antisense probes of these genes were used to potentially localize analogous transcripts in radish seedlings. Many validated data on cell-cycle genes are available in heterologous and closely related plant species such as radish (Burssens *et al.*, 2000; Criqui *et al.*, 2002; De Veylder *et al.*, 1999, 2001; Doerner *et al.*, 1996; Himanen *et al.*, 2002; Jacqmard *et al.*, 1999; Shaul *et al.*, 1999). Therefore, we believe that our expression data for radish are not just complementary but can provide supplementary information on potential gene functions.

Based on all the mRNA patterns obtained, we were able to identify 23 genes generally expressed in dividing and young differentiating tissues, 34 genes that were mostly expressed in both dividing and differentiated tissues and gene transcripts that were primarily detected in differentiated tissues (Table 1). Twenty-one genes had a patchy expression pattern, suggesting a tighter cell-cycle regulation, of which nine were cyclins as expected for mitosis-specific genes (Menges *et al.*, 2005). In general, the expression patterns of most cell-cycle gene family members overlapped considerably, supporting the idea that the highly interactive nature of the core cell-cycle proteins is essential to drive cell division and endoreduplication. In agreement with our *in situ* data, the expression of most cell-cycle genes has been found not to be highly tissue specific based on microarray analysis (Menges *et al.*, 2005). In addition, the 61 core cell-cycle genes studied here were expressed at variable levels within the various plant tissues. For a set of genes, we compared the *in situ* expression profiles with the gene expression map of Arabidopsis published by Birnbaum *et al.* (2003). Assessment of both data revealed that *ICK1*/*KRP1* is expressed in most root cells, *ICK2*/*KRP2* often in differentiated tissues (such as epidermis) and in the vascular cylinder, *KRP4* is quite strongly expressed in roots, whereas *KRP5* and *KRP7* are both highly expressed at the root cap and vascular tissue. Also the atypical E2F transcription factors *E2Fd*/*DEL2* and *E2Ff*/*DEL3* show weak and homogeneous expression levels over the roots, while the *E2Fa* mRNA localization nicely confirms high expression in the root cap. *CKS1* expression is weaker than that of *CKS2*, but to a lesser extent when compared with the data of Birnbaum *et al.* (2003). This discrepancy could result from the longer exposure times needed for *in situ* detection of the *CKS2* transcript. Expression patterns obtained from the *in situ* hybridization experiments are in full agreement with data obtained from the analysis of promoter activity of previously studied genes, e.g. *CDKA;1* (*cdc2a*; Hemerly *et al.*, 1993), *CYCB1;1* (*Atcyc1*; Ferreira *et al.*, 1994), *E2Ff*/*DEL3* (Ramirez-Parra *et al.*, 2004) and *CYCD3;1* (Dewitte *et al.*, 2003). In addition, our experiments have complemented previously published results of expression analysis carried out in Arabidopsis and its closely related species radish, such as *cdc2* (Doerner *et al.*, 1996), *CYCD4;1* (De Veylder *et al.*, 1999), *UBC19* (Jacqmard *et al.*, 1999), *UBC20* (Criqui *et al.*, 2002), *CYCA2;1* (Burssens *et al.*, 2000) and *ICK2*/*KRP2* (Himanen *et al.*, 2002). Recently, protein localization of *CDC27B* in Arabidopsis seedlings have confirmed our observations of mRNA expression in the RAM, vascular tissue and shoot apex (Pérez-Pérez *et al.*, 2008).

Cell-cycle genes are continually expressed in the vascular tissue

During seedling development, vascular tissues, xylem and phloem are differentiated from procambial initials into different types of vascular cells, such as tracheary elements, xylem parenchyma cells, sieve elements and phloem parenchyma (reviewed by Ye, 2002). Root expansion also involves ordered cell division for the generation of vessels. During secondary growth, vascular cambium initials will give rise to secondary xylem and phloem. The mRNA *in situ* analyses revealed that core cell-cycle genes are often highly expressed in primary and secondary living xylem elements. Later on, xylem cells undergo expansion, endoreduplication, differentiation and cell death (reviewed by Fukuda, 1996). High cell-cycle gene expression was seen in the primary xylem that develops from procambial or cambial initials close to the RAM. Typically, hybridization signals became weaker and were more patchily distributed in xylem cells further away from the RAM. Only a few cell-cycle genes (e.g. *CYCA2;3*, *E2Fb*, *E2Fc*, *ORC5*, *ORC6*, *WEE1* and *KRP5*) were clearly expressed in xylem cells already showing secondary growth.

The phloem forms continuous columns of cells in the vascular system. In contrast to developing xylem, only a few cell-cycle genes were preferentially expressed in phloem cells, and this signal was more well defined in cross-sections of radish roots than in those of the tiny Arabidopsis roots, although a homogeneous hybridization signal for several genes could often be observed throughout the vascular tissue. These data suggest that the expression level of the majority of the cell-cycle genes analyzed could be low in phloem tissue. Cell-cycle gene expression in vascular cells was also prominently observed in leaves. During leaf expansion, the first mitotic activity occurs throughout the lamina using a single basal plate meristem followed by an increase in mesophyll volume with expansion of intercellular spaces and in the vein network (Pyke *et al.*, 1991). In leaf primordia and young leaves, most cell-cycle genes were homogeneously expressed, whereas in differentiated leaves a preferential expression was seen in the vascular bundles

and in epidermal cells. In agreement with our data, Donnelly *et al.* (1999) have shown that cell division stops first in the epidermis and last in the vascular tissue.

Cell-cycle genes can be used as markers of cell division and endoreduplication

Almost all (except the three *CDKD*) genes analyzed are expressed in young seedlings, mainly in the SAM and RAM and in dividing vascular tissue cells. During seedling development, the growth of hypocotyl cells does not involve significant cortical and epidermal (except for stomatal cells) cell division (Raz and Koornneef, 2001; Saibo *et al.*, 2003). Although most of the cell-cycle genes are expressed in epidermal and cortical cells of the apical hook of the hypocotyl, we observed that the *E2Fb*, *ORC1a*, *CDC6a*, *AtPIN1* and *KRP6* genes had the highest expression in the apical hook. Based on this observation, it is tempting to speculate that the involvement of this set of highly expressed genes in cortical cells of the hypocotyl is not directly involved in the process of cell division but rather with endoreduplication and cell elongation. During cellular differentiation, repeated S phases occur in particular cell types of several plant species, including Arabidopsis, causing increased ploidy levels (Nagl, 1976; Galbraith *et al.*, 1991). In Arabidopsis, endoreduplication has been demonstrated to occur mainly in the cortical cells of the hypocotyl and to be differentially regulated in the light and the dark (Gendreau *et al.*, 1997, 1998). In the presence of light, cells reach a maximum of 8C, whereas in the dark, the thinner and longer hypocotyls undergo an additional round of replication resulting in 16C cells. Differently, cells in the vascular cylinder only reach a 2C–4C DNA content and maintain a level of mitotic activity during secondary growth. The specific induction of cell-cycle genes in dark-grown in comparison with light-grown seedlings provided data indicative of genes involved in the endoreduplication cycle. Among the seven CDK inhibitors, five *KRP* genes were upregulated in etiolated hypocotyls, hinting at a role in suppression of the cell-division cycle in this organ. From these five *KRP* genes, four were also expressed in the root EZ, representing the putative site of endoreduplication in the root. Except for *KRP4*, the link of all investigated cell-cycle inhibitors with endoreduplicating cells has also been observed by Ormenese *et al.* (2004) in shoot apices of Arabidopsis. Strong expression of *E2Fc* in etiolated hypocotyls confirms its proposed role in inhibiting cell division and restricting proliferation in the dark (del Pozo *et al.*, 2002). At moderate levels, the six *ORC* genes were induced in endoreduplicating cortical hypocotyl cells as well, but their transcripts were also found in the expanding vascular tissue. *ORC1a*(*S*152149) showed the highest induction in etiolated seedlings, similar to *MCM5*(S152139). Both genes are potential targets of E2F activators that were also highly expressed in dark-grown hypocotyls. The five analyzed genes involved in the proteasome pathway were rather highly expressed in etiolated hypocotyls. The induction of both genes *CCS52A1* and *CCS52B* supports previous results indicating their possible role in differentiating cells undergoing endoreduplication (Cebolla *et al.*, 1999). The low level of expression of *CDKB1;1*(S152793) in endoreduplicating tissues confirms previously published results (Boudolf *et al.*, 2004a), suggesting that a lower activity of this CDK is required to suppress endoreduplication.

Cell-cycle genes are differentially expressed in activated and non-activated pericycle cells

The development of a complete root system depends on newly formed apical meristems that originate from differentiated pericycle cells (Laskowski *et al.*, 1995; Malamy and Benfey, 1997) that re-enter the cell cycle. Lateral root primordia initiate from founder cells in the pericycle layer. Previous studies suggest that xylem pericycle cells proceed to the G_2 phase while remaining pericycle cells stay in the G_1 phase (Beeckman *et al.*, 2001). The competence of a pericycle cell to induce a lateral root depends on its location as well as on the state of maturation of the root (Himanen *et al.*, 2002). We have identified 19 genes preferentially expressed in pericycle cells positioned at the xylem poles that are committed to asymmetric formative divisions to give rise to lateral root primordia. These genes, comprising mainly cyclins, are transcriptionally switched on at this site before pericycle activation. Only *CYCA2;1* was expressed in pericycle cells at both xylem poles. Simultaneously, expression of two inhibitors (*ICK1*/*KRP1* and *KRP4*) might modulate lateral root initiation.

The first formative division in the pericycle depends on basipetal auxin transport, and the auxin derived from the shoot regulates the emergence of the LRM. Inhibition of polar auxin transport blocks the first divisions for lateral root initiation (Casimiro *et al.*, 2001) and auxin seems to determine the positioning and frequency of lateral root initiation (Himanen *et al.*, 2002). A procedure based on NPA and NAA treatment allows the synchronization of pericycle cells and enhances lateral root formation (Himanen *et al.*, 2002; Vanneste *et al.*, 2005). *In situ* mRNA analysis carried out on similarly treated roots showed the induction of 18 cell-cycle genes in synchronized pericycle cells, suggesting their possible involvement in the first steps of lateral root formation. Transcription of the cell-cycle inhibitor *ICK1*/*KRP1* has been shown to be repressed after 6 h of NAA treatment (Himanen *et al.*, 2002). We have observed *ICK1*/*KRP1*(S153001) expression at the xylem poles before lateral root initiation. A slight induction of this *KRP* gene has been observed 12 h after NAA treatment, suggesting that this inhibitor is a modulator of cell division during lateral root formation. However, the differing results might be attributed to sampling time points. Interestingly, only one gene, *ICK2*/*KRP2*, is not expressed in the protoxylem poles where lateral roots, under normal conditions, are never formed, confirm-

ing that the *ICK2*/*KRP2* gene might encode a protein involved in the specific block of cell-cycle induction in the root pericycle (Himanen *et al.*, 2002). Our results also validate the previously observed induction of *E2Fa* and B-type cyclins in NAA-treated pericycle cells (Himanen *et al.*, 2002).

Concluding remarks

Our data provide a comprehensive overview of expression patterns for 61 core cell-cycle genes in Arabidopsis seedlings. The analysis illustrates the complexity of transcript co-localization in tissues of developing seedlings. Although gene control would be expected to reflect activation of genes at the time the gene products are required to function, our data imply that transcript accumulation of most core cell cycle genes seems to be a gradual process. Genes predominantly expressed in meristematic tissues possibly operate only during the mitotic cycle, whereas those expressed in regions of high cell-cycle activity and differentiating tissues might play a role in both the mitotic and the endoreduplication cycle. This hypothesis might explain why patchy expression patterns are more often detected in genes in which transcription is more tightly regulated by the cell cycle. Finally, we hope that this work will provide a useful resource not only for plant biologists working on the cell cycle but also for those interested in other biological problems.

EXPERIMENTAL PROCEDURES

Fixation, embedding and sectioning of plant material

Arabidopsis thaliana (L.) Heyhn. cv. Columbia and *Raphanus sativus* L. (radish) seedlings were germinated in K1 medium (Valvekens *et al.*, 1988) and different plant parts were harvested for fixation. Roots of Arabidopsis and radish were dissected 21 and 42 days after sowing (DAS) and 3 and 12 DAS, respectively. In a set of time points (3 and 7 DAS for radish and 12 and 21 DAS for Arabidopsis), both species had similar developmental stages. Primary roots of seedlings 7 DAS described below were also used for observation of their expression patterns. Seedlings were germinated in the light and the dark (etiolated) as described (Gendreau *et al.*, 1997) and harvested 7 DAS. Hypocotyl cells of light-grown plantlets went through two endocycles and dark-grown seedlings underwent a third endocycle (Gendreau *et al.*, 1997). Therefore, expression patterns in light- and dark-grown seedlings were compared. The shoot apices of light-grown seedlings (7 and 12 DAS) were also compared, but because they are quite small in Arabidopsis, those of radish seedlings were analyzed as well at 12 DAS. Stem (epicotyl) was sectioned in Arabidopsis and radish seedlings 12 DAS.

To obtain roots in which the formative division in the pericycle was prevented, resulting in roots without lateral roots, Arabidopsis seedlings were grown in K1 medium for 36 h, transferred to medium containing NPA (10 μM) and kept for 3 days (72 h). To activate the entire pericycle cell layer for lateral root initiation, seedlings were transferred and kept for 12 h in medium containing NAA (10 μM) and subsequently harvested for fixation. This protocol designed to synchronize pericycle cells enhanced the activation of lateral root initiation (Himanen *et al.*, 2002).

Plant tissues were oriented by placing samples longitudinally within lens paper closed with staples. After fixation in 2.5% gluteraldehyde and dehydration, packages were removed prior to paraffin embedding. Plant tissue was degassed under a vacuum in fixative and incubated in fresh fixative overnight under rotation at 4°C. Arabidopsis and radish seedlings were dehydrated for 20 min and 45 min, respectively, in increasing ethanol series (15–100%). Paraffin-embedded tissues were cross- and longitudinally sectioned at 10 μm thickness and fixed to 3-aminopropyltriethoxysilane-coated slides. Paraffin ribbons of dark- and light-grown seedlings and roots treated with NPA and NAA were placed on the same slide for better comparison and more accurate determination of the effects of growth conditions or treatments on gene induction.

Complementary DNAs of cell-cycle genes used to generate gene-specific probes for *in situ* hybridization

Nearly all probes of the core cell-cycle genes were designed based on the annotation of the gene structure (Vandepoele *et al.*, 2002). To visualize the sequences used to generate probes, one must click on the link 'constructs' under the menu and subsequently click on the link of the construct ID. Remaining sequence information of the probes can be obtained upon request. *CYCD1;1*, *CYCD2;1* and *CYCD3;1* were kind gifts of Jim Murray (University of Cambridge, UK); *UBC19* and *UBC20* of Pascal Genschik (CNRS, Strasbourg, France); and *CCS52A1* and *CCS52B* of Eva Kondorosi (CNRS, Gif sur Yvette, France).

Probe synthesis

Antisense and sense RNA probes were generated from gene-specific cDNA by PCR amplification, containing Sp6 and T7 RNA polymerase promoters. Gene-specific sequences were used for probe synthesis to avoid cross-hybridization between the different family members (see online data at http://www.psb.ugent.be/ishi/). Genes that could potentially show cross-hybridization were *CKS1* and *CKS2*, *ORC1a* and *ORC1b* and *CDT1 and CDT2*, although the stringent post-hybridization washes should normally eliminate potential nonspecific hybrids. Probes used to localize *ORC4* expression cannot distinguish between *ORC4a* and *ORC4b* transcripts because they undergo differential splicing (Masuda *et al.*, 2004). Sequences of the 3′-untranslated region were used for the four *CDKB*s, *CDC6a* (Ramos *et al.*, 2001) and *CDC27b*. Probes were synthesized as previously described (de Almeida Engler *et al.*, 2001) and 15×10^6 c.p.m. per slide were applied (7.5 ng per slide). Higher probe concentration increased the ratio of signal and background in both antisense and sense slides.

Hybridization, washing and probe detection by autoradiography

Nearly all steps of the *in situ* hybridization experiments were essentially as described (de Almeida Engler *et al.*, 2001). During the first *in situ* hybridization, several combinations of fixatives [formaldehyde/acetic acid/ethanol (FAA), 4% formaldehyde (F) and 1% gluteraldehyde (G), 1% or 2.5% G or 4% F] were tested. The best results were obtained with 2.5% G; this was therefore used in all further experiments.

To avoid cross-hybridization of probes within members of a gene family, samples were washed under highly stringent conditions: 50% formamide and 0.1 × SSC for 60 min at up to 48–50°C. Under these conditions, RNA/RNA hybrids with more than 10% randomly distributed mismatches were eliminated by the washing procedure.

After exposure at 4°C in dry boxes in absolute darkness, the slides were developed, fixed, then gently washed for approximately 2 h in double-distilled water to remove any remaining salts and stained with 0.05% toluidine blue. Slides probed with control sense RNA

were developed at the same moment and after a similar exposure time as the corresponding antisense slides. The hybridization intensity for each gene was estimated quantitatively on sections only when applying the same exposure time. Up to 10 months of exposure were needed to detect the hybridization signal when short probes (up to 150 bp) were applied on tissue sections of Arabidopsis and 15 days to 3 months for slides containing radish sections. Images were recorded with a Zeiss AxioCam digital camera (http://www.zeiss.com/) using standard dark- and bright-field optics.

Bioinformatics analysis of cell-cycle gene expression

For the analysis of the *in situ* hybridization data, the expression levels were given as appreciations of mRNA levels and thus depended on observations and subsequent description. The expression values attributed by an expert were not readily usable for clustering algorithms. Attribution of discrete values to these expression levels was not straightforward, because differences had to be translated into reasonable gaps between described levels. Several attempts were made, from attributing to all levels a distinctive value to using a binary system in which all clearly identifiable levels were set to 1 and the others to 0. In this analysis, the binary attribution was adopted where all levels above 'very low' were set to 1, while all others, including patchy expression levels, were set to 0. This translated table of expression level was loaded into the Genesis package (version 1.5) (Sturn *et al.*, 2002) and hierarchically clustered with Euclidean distance measures. Tissue types and expression levels were bi-clustered.

From each of the clusters that could be delineated, upstream sequences were taken up to 1000 nucleotides when the intergenic region allowed it. On each set of upstream sequences corresponding to each cluster found, motifs were searched with a range of parameters. The software used for the motif searches was MotifSampler (Thijs *et al.*, 2002) that uses a heuristic and, therefore, multiple runs for each set, with the same parameters. MotifSampler was run 100 times per sequence and per parameter set, from which the top five best-performing motifs were filtered. The ranges of parameters used were the background models (Markov order 2–4) and motif length varying from 7 to 17. Three motifs had to be returned per run. These results were additionally filtered with the 'find_explanators' software included in the YMF (version 3.0) package (Sinha and Tompa, 2002) to cluster smaller or overlapping motifs into single motifs, because sometimes motifs on opposite strands could be returned by independent runs of the MotifSampler software. From all data sets returned from the word search analysis, none, even for the short word lengths, had a high degree of conservation. As such, the result obtained gave no clearly statistically sound motif.

ACKNOWLEDGEMENTS

We thank Jim Murray, Pascal Genschik and Eva Kondorosi for providing material to generate gene-specific probes from *CYCD*, *UBC* and *CCS52,* respectively, Marleen Brunain for technical assistance, Julie Hopkins for language review and Martine De Cock for help in preparing the manuscript. This work was supported by grants from the University Poles of Attraction Programme (IUAP VI/33), initiated by the Belgian State, Science Policy Office, and the European Community project SY-STEM (MRTN-CT-2004-005336). LDV and VB are postdoctoral fellows of the Research Foundation-Flanders.

SUPPORTING INFORMATION

The following supplementary material is available for this article online:

Figure S1. Bi-Cluster analysis of the cell-cycle genes. The bi-clustering approach was done on the translated results of the visual interpretation of the expression patterns of all genes included in this work. As it proved itself difficult to precisely quantify the expression levels for a finer clustering, discrete values were given according to the described expression levels for each gene in each tissue of the *in situ* hybridization analyses. This figure provides an overview of all the experiments carried out and the expression patterns observed, and shows a significant spatial (and temporal) overlap, illustrating that these genes are expressed simultaneously. As such, this clustering groups tissues that mainly undergo cell division.

Additional data online

An exhaustive catalogue of approximately 1800 *in situ* hybridization images can be queried online via the Plant Systems Explorer Image Database at http://www.psb.ugent.be/ishi/, either by gene names or AGI codes. Images generated from control hybridization experiments can be accessed by entering the Contr* keyword or clicking on the control image keyword. Image numbers (sample ID reference) (for instance S152023) indicated throughout the text give direct access to *in situ* hybridization results via the query interface. Each sample query returns one or several thumbnails showing the corresponding images and the full image opens upon clicking a thumbnail.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

REFERENCES

de Almeida Engler, J., De Groodt, R., Van Montagu, M. and Engler, G. (2001) *In situ* hybridization to mRNA of *Arabidopsis* tissue sections. *Methods*, **23**, 325–334.

Andersen, S.U., Buechel, S., Zhao, Z., Ljung, K., Novák, O., Busch, W., Schuster, C. and Lohmann, J.U. (2008) Requirement of B2-type *cyclin-dependent kinases* for meristem integrity in *Arabidopsis thaliana*. *Plant Cell*, **20**, 88–100.

Beeckman, T., Burssens, S. and Inzé, D. (2001) The peri-*cell*-cycle in *Arabidopsis*. *J. Exp. Bot.* **52**, 403–411.

Birnbaum, K., Shasha, D.E., Wang, J.Y., Jung, J.W., Lambert, G.M., Galbraith, D.W. and Benfey, P.N. (2003) A gene expression map of the *Arabidopsis* root. *Science*, **302**, 1956–1960.

Boudolf, V., Barrôco, R., de Almeida Engler, J., Verkest, A., Beeckman, T., Naudts, M., Inzé, D. and De Veylder, L. (2004a) B1-type cyclin-dependent kinases are essential for the formation of stomatal complexes in *Arabidopsis thaliana*. *Plant Cell*, **16**, 945–955.

Boudolf, V., Vlieghe, K., Beemster, G.T.S., Magyar, Z., Torres Acosta, J.A., Maes, S., Van Der Schueren, E., Inzé, D. and De Veylder, L. (2004b) The plant-specific cyclin-dependent kinase CDKB1;1 and transcription factor E2Fa-DPa control the balance of mitotically dividing and endoreduplicating cells in Arabidopsis. *Plant Cell*, **16**, 2683–2692.

Boudolf, V., Inzé, D. and De Veylder, L. (2006) What if higher plants lack a CDC25 phosphatase? *Trends Plant Sci.* **11**, 474–479.

Burssens, S., de Almeida Engler, J., Beeckman, T., Richard, C., Shaul, O., Ferreira, P., Van Montagu, M. and Inzé, D. (2000) Developmental expression of the *Arabidopsis thaliana CycA2;1* gene. *Planta*, **211**, 623.

Casimiro, I., Marchant, A., Bhalerao, R.P. *et al.* (2001) Auxin transport promotes Arabidopsis lateral root initiation. *Plant Cell*, **13**, 843–852.

Castellano, M.M., del Pozo, J.C., Ramirez-Parra, E., Brown, S. and Gutierrez, C. (2001) Expression and stability of Arabidopsis CDC6 are associated with endoreplication. *Plant Cell*, **13**, 2671–2686.

Cebolla, A., Vinardell, J.M., Kiss, E., Oláh, B., Roudier, F., Kondorosi, A. and Kondorosi, E. (1999) The mitotic inhibitor *ccs52* is required for endoreduplication and ploidy-dependent cell enlargement in plants. *EMBO J.* **18**, 4476–4484.

Combettes, B., Reichheld, J.P., Chabouté, M.E., Philips, G., Shen, W.H. and Chaubet-Gigot, N. (1999) Study of phase-specific gene expression in synchronized tobacco cells. *Methods Cell Sci.* **21**, 109–121.

Coux, O., Tanaka, K. and Goldberg, A.L. (1996) Structure and functions of the 20S and 26S proteasomes. *Annu. Rev. Biochem.* **65**, 801–847.

Criqui, M.C., de Almeida Engler, J., Camasses, A., Capron, A., Parmentier, Y., Inzé, D. and Genschik, P. (2002) Molecular characterization of plant ubiquitin-conjugating enzymes belonging to the UbcP4/E2-C/UBCx/UbcH10 gene family. *Plant Physiol.* **130**, 1230–1240.

De Schutter, K., Joubès, J., Cools, T. *et al.* (2007) *Arabidopsis* WEE1 kinase controls cell cycle arrest in response to activation of the DNA integrity checkpoint. *Plant Cell*, **19**, 211–225.

De Smet, I., Vanneste, S., Inzé, D. and Beeckman, T. (2006) Lateral root initiation or the birth of a new meristem. *Plant Mol. Biol.* **60**, 871–887.

De Veylder, L., De Almeida Engler, J., Burssens, S., Manevski, A., Lescure, B., Van Montagu, M., Engler, G. and Inzé, D. (1999) A new D-type cyclin of *Arabidopsis thaliana* expressed during lateral root primordia formation. *Planta*, **208**, 453–462.

De Veylder, L., Beeckman, T., Beemster, G.T.S., Krols, L., Terras, F., Landrieu, I., Van Der Schueren, E., Maes, S., Naudts, M. and Inzé, D. (2001) Functional analysis of cyclin-dependent kinase inhibitors of Arabidopsis. *Plant Cell*, **13**, 1653–1667.

De Veylder, L., Beeckman, T., Beemster, G.T.S. *et al.* (2002) Control of proliferation, endoreduplication and differentiation by the *Arabidopsis* E2Fa/DPa transcription factor. *EMBO J.* **21**, 1360–1368.

De Veylder, L., Joubès, J. and Inzé, D. (2003) Plant cell cycle transitions. *Curr. Opin. Plant Biol.* **6**, 536–543.

Dewitte, W., Riou-Khamlichi, C., Scofield, S., Healy, J.M.S., Jacqmard, A., Kilby, N.J. and Murray, J.A.H. (2003) Altered cell cycle distribution, hyperplasia, and inhibited differentiation in Arabidopsis caused by the D-type cyclin CYCD3. *Plant Cell*, **15**, 79–92.

Doerner, P., Jørgensen, J.-E., You, R., Steppuhn, J. and Lamb, C. (1996) Control of root growth and development by cyclin expression. *Nature*, **380**, 520–523.

Donnelly, P.M., Bonetta, D., Tsukaya, H., Dengler, R.E. and Dengler, N.G. (1999) Cell cycling and cell enlargement in developing leaves of *Arabidopsis*. *Dev. Biol.* **215**, 407–419.

Ferreira, P.C.G., Hemerly, A.S., Villarroel, R., Van Montagu, M. and Inzé, D. (1991) The *Arabidopsis* functional homolog of the $p34^{cdc2}$ protein kinase. *Plant Cell*, **3**, 531–540.

Ferreira, P.C.G., Hemerly, A.S., de Almeida Engler, J., Van Montagu, M., Engler, G. and Inzé, D. (1994) Developmental expression of the Arabidopsis cyclin gene *cyc1At*. *Plant Cell* **6**, 1763–1774.

Fobert, P.R., Gaudin, V., Lunness, P., Coen, E.S. and Doonan, J.H. (1996) Distinct classes of *cdc2*-related genes are differentially expressed during the cell division cycle in plants. *Plant Cell*, **8**, 1465–1476.

Fu, H., Doelling, J.H., Arendt, C.S., Hochstrasser, M. and Vierstra, R.D. (1998) Molecular organization of the 20S proteasome gene family from *Arabidopsis thaliana*. *Genetics*, **149**, 677–692.

Fukuda, H. (1996) Xylogenesis: initiation, progression, and cell death. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 299–325.

Galbraith, D.W., Harkins, K.R. and Knapp, S. (1991) Systemic endopolyploidy in *Arabidopsis thaliana*. *Plant Physiol.* **96**, 985–989.

Gendreau, E., Traas, J., Desnos, T., Grandjean, O., Caboche, M. and Höfte, H. (1997) Cellular basis of hypocotyl growth in *Arabidopsis thaliana*. *Plant Physiol.* **114**, 295–305.

Gendreau, E., Höfte, H., Grandjean, O., Brown, S. and Traas, J. (1998) Phytochrome controls the number of endoreduplication cycles in the *Arabidopsis thaliana* hypocotyl. *Plant J.* **13**, 221–230.

Harper, J.W., Burton, J.L. and Solomon, M.J. (2002) The anaphase-promoting complex: it's not just for mitosis any more. *Genes Dev.* **16**, 2179–2206.

Hemerly, A.S., Ferreira, P., de Almeida Engler, J., Van Montagu, M., Engler, G. and Inzé, D. (1993) *cdc2a* expression in Arabidopsis is linked with competence for cell division. *Plant Cell*, **5**, 1711–1723.

Himanen, K., Boucheron, E., Vanneste, S., de Almeida Engler, J., Inzé, D. and Beeckman, T. (2002) Auxin-mediated cell cycle activation during early lateral root initiation. *Plant Cell*, **14**, 2339–2351.

Inzé, D. (2005) Green light for the cell cycle. *EMBO J.* **24**, 657–662.

Inzé, D. and De Veylder, L. (2006) Cell cycle regulation in plant development. *Annu. Rev. Genet.* **40**, 77–105.

Jacqmard, A., De Veylder, L., Segers, G., de Almeida Engler, J., Bernier, G., Van Montagu, M. and Inzé, D. (1999) Expression of *CKS1At* in *Arabidopsis thaliana* indicates a role for the protein in both the mitotic and the endoreduplication cycle. *Planta*, **207**, 496–504.

de Jager, S.M., Menges, M., Bauer, U.-M. and Murray, J.A.H. (2001) *Arabidopsis* E2F1 binds a sequence present in the promoter of S-phase-regulated gene *AtCDC6* and is a member of a multigene family with differential activities. *Plant Mol. Biol.* **47**, 555–568.

Kosugi, S. and Ohashi, Y. (2003) Constitutive E2F expression in tobacco plants exhibits altered cell cycle control and morphological change in a cell type-specific manner. *Plant Physiol.* **132**, 2012–2022.

Landrieu, I., De Veylder, L., Fruchart, J.S., Odaert, B., Casteels, P., Portetelle, D., Van Montagu, M., Inzé, D. and Lippens, G. (2000) The *Arabidopsis thaliana PIN1At* gene encodes a single-domain phosphorylation-dependent peptidyl prolyl *cis*/*trans* isomerase. *J. Biol. Chem.* **275**, 10577–10581.

Landrieu, I., da Costa, M., De Veylder, L. *et al.* (2004) A small CDC25 dual-specificity tyrosine-phosphatase isoform in *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA*, **101**, 13380–13385 (Err. *Proc. Natl. Acad. Sci. USA*, 101, 16391).

Laskowski, M.J., Williams, M.E., Nusbaum, H.C. and Sussex, I.M. (1995) Formation of lateral root meristems is a two-stage process. *Development*, **121**, 3303–3310.

Magyar, Z., Mészáros, T., Miskolczi, P. *et al.* (1997) Cell cycle phase specificity of putative cyclin-dependent kinase variants in synchronized alfalfa cells. *Plant Cell*, **9**, 223–235.

Malamy, J.E. and Benfey, P.N. (1997) Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. *Development*, **124**, 33–44.

Masuda, H.P., Ramos, G.B.A., de Almeida-Engler, J., Cabral, L.M., Coqueiro, V.M., Macrini, C.M.T., Ferreira, P.C.G. and Hemerly, A.S. (2004) Genome based identification and analysis of the pre-replicative complex of *Arabidopsis thaliana*. *FEBS Lett.* **574**, 192–202.

Menges, M. and Murray, J.A.H. (2002) Synchronous *Arabidopsis* suspension cultures for analysis of cell-cycle gene activity. *Plant J.* **30**, 203–212.

Menges, M., de Jager, S.M., Gruissem, W. and Murray, J.A.H. (2005) Global analysis of the core cell cycle regulators of Arabidopsis identifies novel genes, reveals multiple and highly specific profiles of expression and provides a coherent model for plant cell cycle control. *Plant J.* **41**, 546–566.

Mironov, V., De Veylder, L., Van Montagu, M. and Inzé, D. (1999) Cyclin-dependent kinases and cell division in higher plants - the nexus. *Plant Cell*, **11**, 509–521.

Nagl, W. (1976) DNA endoreduplication and polyteny understood as evolutionary strategies. *Science*, **261**, 614–615.

Ormenese, S., de Almeida Engler, J., De Groodt, R., De Veylder, L., Inzé, D. and Jacqmard, A. (2004) Analysis of the spatial expression pattern of seven Kip related proteins (KRPs) in the shoot apex of *Arabidopsis thaliana*. *Ann. Bot.* **93**, 575–580.

Park, J.-A., Ahn, J.-W., Kim, Y.-K., Kim, S.J., Kim, J.-K., Kim, W.T. and Pai, H.-S. (2005) Retinoblastoma protein regulates cell proliferation, differentiation, and endoreduplication in plants. *Plant J.* **42**, 153–163.

Parmentier, Y., Bouchez, D., Fleck, J. and Genschik, P. (1997) The 20S proteasome gene family in *Arabidopsis thaliana*. *FEBS Lett.* **416**, 281–285.

Patra, D., Wang, S.X., Kumagai, A. and Dunphy, W.G. (1999) The *Xenopus* Suc1/Cks protein promotes the phosphorylation of G_2/M regulators. *J. Biol. Chem.* **274**, 36839–36842.

Pérez-Pérez, J.M., Serralbo, O., Vanstraelen, M., González, C., Criqui, M.-C., Genschik, P., Kondorosi, E. and Scheres, B. (2008) Specialization of CDC27 function in the *Arabidopsis thaliana* anaphase-promoting complex (APC/C). *Plant J.* **53**, 78–89.

Pines, J. (1996) Cell cycle: reaching for a role for the Cks proteins. *Curr. Biol.* **6**, 1399–1402.

Porceddu, A., Stals, H., Reichheld, J.-P., Segers, G., De Veylder, L., De Pinho Barrôco, R., Casteels, P., Van Montagu, M., Inzé, D. and Mironov, V. (2001) A plant-specific cyclin-dependent kinase is involved in the control of G_2/M progression in plants. *J. Biol. Chem.* **276**, 36354–36360.

del Pozo, J.C., Boniotti, M.B. and Gutierrez, C. (2002) Arabidopsis E2Fc functions in cell division and is degraded by the ubiquitin-SCF^{AtSKP2} pathway in response to light. *Plant Cell*, **14**, 3057–3071.

del Pozo, J.C., Diaz-Trivino, S., Cisneros, N. and Gutierrez, C. (2006) The balance between cell division and endoreplication depends on E2FC-DPB, transcription factors regulated by the ubiquitin-SCF^{SKP2A} pathway in *Arabidopsis*. *Plant Cell*, **18**, 2224–2235.

Pyke, K.A., Marrison, J.L. and Leech, R.M. (1991) Temporal and spatial development of the cells of the expanding first leaf of *Arabidopsis thaliana* (L.) Heynh. *J. Exp. Bot.* **42**, 1407–1416.

Ramirez-Parra, E., López-Matas, M.A., Fründt, C. and Gutierrez, C. (2004) Role of an atypical E2F transcription factor in the control of Arabidopsis cell growth and differentiation. *Plant Cell*, **16**, 2350–2363.

Ramos, G.B.A., de Almeida Engler, J., Ferreira, P.C.G. and Hemerly, A.S. (2001) DNA replication in plants: characterization of a *cdc6* homologue from *Arabidopsis thaliana*. *J. Exp. Bot.* **52**, 2239–2240.

Raz, V. and Koornneef, M. (2001) Cell division activity during apical hook development. *Plant Physiol.* **125**, 219–226.

Rossignol, P., Stevens, R., Perennes, C., Jasinski, S., Cella, R., Tremousaygue, D. and Bergounioux, C. (2002) AtE2F-a and AtDP-a, members of the E2F family of transcription factors, induce *Arabidopsis* leaf cells to re-enter S phase. *Mol. Genet. Genomics*, **266**, 995–1003.

Saibo, N.J.M., Vriezen, W.H., Beemster, G.T.S. and Van Der Straeten, D. (2003) Growth and stomata development of *Arabidopsis* hypocotyls are controlled by gibberellins and modulated by ethylene and auxin. *Plant J.* **33**, 989–1000.

Shaul, O., Mironov, V., Van Montagu, M. and Inzé, D. (1999) Tobacco cultures transformed with cyclin-promoter-*gus* constructs reveal a discrepancy between *gus* mRNA levels and GUS protein activity upon leaving the stationary state. *Plant Sci.* **141**, 67–71.

Shimotohno, A., Matsubayashi, S., Yamaguchi, M., Uchimiya, H. and Umeda, M. (2003) Differential phosphorylation activities of CDK-activating kinases in *Arabidopsis thaliana*. *FEBS Lett.* **534**, 69–74.

Sinha, S. and Tompa, M. (2002) Discovery of novel transcription factor binding sites by statistical overrepresentation. *Nucleic Acids Res.* **30**, 5549–5560.

Sorrell, D.A., Menges, M., Healy, J.M.S. *et al.* (2001) Cell cycle regulation of cyclin-dependent kinases in tobacco cultivar Bright Yellow-2 cells. *Plant Physiol.* **126**, 1214–1223.

Sorrell, D.A., Marchbank, A., McMahon, K., Dickinson, J.R., Rogers, H.J. and Francis, D. (2002) A *WEE1* homologue from *Arabidopsis thaliana*. *Planta*, **215**, 518–522.

Stukenberg, P.T. and Kirschner, M.W. (2001) Pin1 acts catalytically to promote a conformational change in Cdc25. *Mol. Cell*, **7**, 1071–1083.

Sturn, A., Quackenbush, J. and Trajanoski, Z. (2002) Genesis: cluster analysis of microarray data. *Bioinformatics*, **18**, 207–208.

Sun, Y., Dilkes, B.P., Zhang, C., Dante, R.A., Carneiro, N.P., Lowe, K.S., Jung, R., Gordon-Kamm, W.J. and Larkins, B.A. (1999) Characterization of maize (*Zea mays* L.) Wee1 and its activity in developing endosperm. *Proc. Natl Acad. Sci. USA*, **96**, 4180–4185.

Tanaka, K. and Tsurumi, C. (1997) The 26S proteasome: subunits and functions. *Mol. Biol. Rep.* **24**, 3–11.

Thijs, G., Marchal, K., Lescot, M., Rombauts, S., De Moor, B., Rouzé, P. and Moreau, Y. (2002) A Gibbs sampling method to detect overrepresented motifs in the upstream regions of coexpressed genes. *J. Comput. Biol.* **9**, 447–464.

Valvekens, D., Van Montagu, M. and Van Lijsebettens, M. (1988) *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana* root explants by using kanamycin selection. *Proc. Natl Acad. Sci. USA*, **85**, 5536–5540.

Vandepoele, K., Raes, J., De Veylder, L., Rouzé, P., Rombauts, S. and Inzé, D. (2002) Genome-wide analysis of core cell cycle genes in Arabidopsis. *Plant Cell*, **14**, 903–916.

Vandepoele, K., Vlieghe, K., Florquin, K., Hennig, L., Beemster, G.T.S., Gruissem, W., Van de Peer, Y., Inzé, D. and De Veylder, L. (2005) Genome-wide identification of potential plant E2F target genes. *Plant Physiol.* **139**, 316–328.

Vanneste, S., Maes, L., De Smet, I., Himanen, K., Naudts, M., Inzé, D. and Beeckman, T. (2005) Auxin regulation of cell cycle and its role during lateral root initiation. *Physiol. Plant.* **123**, 139–146.

Vercauteren, I., Van Der Schueren, E., Van Montagu, M. and Gheysen, G. (2001) *Arabidopsis thaliana* genes expressed in the early compatible interaction with root-knot nematodes. *Mol. Plant Microbe Interact.* **14**, 288–299.

Vlieghe, K., Boudolf, V., Beemster, G.T.S., Maes, S., Magyar, Z., Atanassova, A., de Almeida Engler, J., De Groodt, R., Inzé, D. and De Veylder, L. (2005) The DP-E2F-like *DEL1* gene controls the endocycle in *Arabidopsis thaliana*. *Curr. Biol.* **15**, 59–63.

Wang, G., Kong, H., Sun, Y., Zhang, X., Zhang, W., Altman, N., dePamphilis, C.W. and Ma, H. (2004) Genome-wide analysis of the cyclin family in Arabidopsis and comparative phylogenetic analysis of plant cyclin-like proteins. *Plant Physiol.* **135**, 1084–1099.

Ye, Z.-H. (2002) Vascular tissue differentiation and pattern formation in plants. *Annu. Rev. Plant Biol.* **53**, 183–202.

