THE ROYAL SOCIETY

Published online 9 May 2002

Plant D-type cyclins and the control of G1 progression

E. Ann Oakenfull, Catherine Riou-Khamlichi† and James A. H. Murray*

Institute of Biotechnology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QT, UK

The basic pattern of controls that operate during the G1 phase of the plant cell cycle shows much closer similarity to animals than to the yeasts and other fungi. The activity of D-type cyclin (CycD) kinases is induced in response to stimulatory signals, and these phosphorylate the plant homologue of the retinoblastoma tumour susceptibility (Rb) protein. It is likely that Rb phosphorylation results in the activation of genes under the control of E2F transcription factors, including those required for S phase entry. As the initial triggers of the cascade, attention has focused on the CycDs, and a family of 10 genes is present in *Arabidopsis*, divided into three major and three minor groups. Analysis to date suggests that these groups are functionally distinct.

Keywords: plant D-type cyclins; control of G1; plant cell cycle

1. INTRODUCTION: CELL DIVISION IN PLANT DEVELOPMENT

The general developmental pattern in plants is one of indeterminate growth and iterative organogenesis, characterized by continued cell division in certain meristematic regions. Different patterns are observed in the root and shoot. In the root, the continued growth of the tip is driven by production and expansion of new cells in the root meristem and the immediately distal region, and new lateral roots are initiated independently of the primary root meristem by reactivation of pericycle cells in non-dividing regions of the root. Each new lateral root therefore requires the building of a new meristem as a consequence of the proliferation of the reactivated cell. In the SAM, a different pattern is observed. As in the root, the division of cells within the meristem provides a driving force for growth, but the iterative production of organs is closely associated with the meristem. The apical lateral organs, primarily leaves and flowers, are therefore derived from a population of founder cells set aside directly from the SAM itself, and do not result from the reactivation of previously differentiated cells. Indeed, axilliary meristems that lie between the lateral organs and the stem, and may be activated on the relaxation of apical dominance, are also set aside from the SAM as each leaf forms, and are not created *de novo*. In addition to these general patterns of development occurring at the two principal meristems are other differentiation events producing specific specialized cells.

These general comments illustrate both that cell division is of central importance to the functioning of meristems and hence to the overall development of plants, and also that the relationships between cell proliferation and cellular differentiation are different in root and shoot. Regulation of these processes, and the relationship of cell division controls to developmental state, are therefore likely to be complex and plastic in plants.

In this review, we focus on the regulation of the G1 phase of the cell cycle, and particularly on the role of CycDs and the pathway in which they act. We review the evidence for differential roles of CycDs in plant cell-cycle control and development, and discuss their possible modes of regulation.

2. CONSERVED ASPECTS OF THE CELL CYCLE

In plants as in all eukaryotes, the four basic phases of the mitotic cell cycle are conserved. In addition to the coupled cycles where DNA replication (S phase) is followed by G2 and M and hence gives rise to daughter cells, alternative cycles also occur in certain developmental situations. Endoreduplication or endocycles are apparent in many plants, and involve repeated S phases without an intervening M, resulting in an increase in ploidy levels within a single nucleus. Endocycles are characteristic of certain differentiated cells, and indeed in *Arabidopsis*, extensive endoreduplication occurs in trichomes and other leaf cell types (Galbraith *et al.* 1991), and may be associated with increasing nuclear volume to service large expanded cells. A further type of mitotic cycle occurs during endosperm development, where a 'normal' cycle is uncoupled from cytokinesis to produce a syncytium containing multiple nuclei of normal ploidy level (Berger 1999). However, few details are known concerning the regulation of these 'unusual' cycles, and here we focus on the conventional mitotic cycles that are involved in meristematic cell division activity.

The cell cycle is regulated at multiple points, but major controls operate at the G1–S and G2–M phase boundary. These transitions represent the onset of DNA replication and M respectively. In addition, there is a point during

* Author for correspondence (j.murray@biotech.cam.ac.uk).
† Present address: Institut de Biotechnologie, EA 3176 Avenue A. Thomas, 87199 Limoges cedex, France.

One contribution of 12 to a Discussion Meeting Issue 'The plant cell: between genome and plant'.

© 2002 The Royal Society

mid-to-late G1 that represents irreversible commitment to the cycle, known in yeasts as START and mammalian cells as the R point. It is likely that an equivalent point of commitment also exists in plant cells. START is defined as the point after which cells are no longer responsive to mating pheromone. Yeast cells treated with pheromone respond before START by arresting their cell cycle and differentiating a specific cell morphology associated with mating. Cells likewise treated after START are unable to respond until they have completed the cycle they have already initiated (reviewed in Pines (1995)). Analogously, the mammalian R point is that after which cells no longer require the presence of serum growth factors to complete a whole cell cycle. These definitions make clear not only the concept of a point of no return for a cell during G1, but also that before this point alternative decisions of cell fate (such as differentiation) are possible, before commitment is made. It is therefore probable that multiple decisions must be integrated during G1 before commitment, comprising information from extra- and intracellular sources. G1 controls will therefore involve responses to growth, environmental and developmental cues.

At the molecular level, cell-cycle transitions in all eukaryotes are controlled by a specific type of serine/threonine protein kinases known as CDKs. These are subunits of a catalytic domain, the CDK itself, and a regulatory subunit or cyclin, which is responsible both for the activation of the CDK complex and for determining its substrate specificity. In yeasts, there is a single CDK involved in central cell-cycle control, known as Cdc28 in budding yeast (*Saccharomyces cerevisiae*) (Mendenhall & Hodge 1998). Multiple cyclins are present in yeasts, and the association of different cyclins with the CDK creates different kinase specificities. The timing of these activities during the cell cycle is also controlled by cyclin association, as cyclins are transcribed only during specific time windows and are also highly unstable proteins whose destruction is controlled in a cell-cycle-dependent manner. For example, the G1 commitment point START in budding yeast is controlled by a rise in the levels of CDK (Cdc28) activity associated with the transcriptional and translational regulation of the yeast G1 cyclins Cln1, Cln2 and Cln3.

However, CDK activity is not regulated solely by cyclin association but also by post-translational modification of the CDK subunits and by the association of further regulatory proteins (figure 1). The catalytic cleft of the CDK is largely blocked by a loop of the protein, until a threonine residue around position T164 is phosphorylated by a CAK. This is generally considered to be a cell-cycle-regulating event in animal cells, although the picture is less clear in plants (Meijer & Murray 2000). There are also differences between individual CDKs as to whether CAK phosphorylation has a role in CDK complex assembly. However, further phosphorylation events at the N-terminus of the CDK (T14 and Y15) have key roles in cell cycle control. Phosphorylation of these residues likewise blocks the activation of the CDK–cyclin complex (CAK phosphorylation notwithstanding). This is the function of the Wee1-related kinases (so called because their mutation in fission yeast results in premature entry into M). Removal of the phosphates from these residues results in CDK complex activation, and this is carried out by the Cdc25 phosphatase.

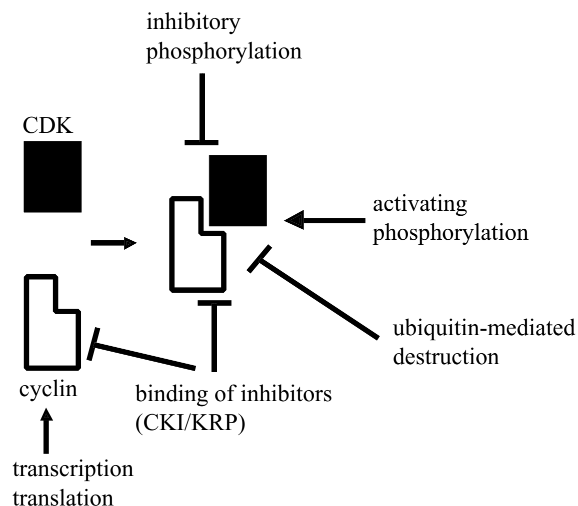

Figure 1. Levels of regulation of CDK complexes. Initial specificity and timing of CDK complex activity is regulated by the cyclin subunit, and further regulation of the CDK subunit can also occur by post-translational modification of the CDK subunits and by association of further regulatory proteins, for example: by ubiquitin-mediated degradation of the cyclin subunit; activating phosphorylation by CAKs; inhibitory phosphorylation by inhibiting kinases; and inhibition by the binding of proteins such as the CKI and KRP.

The regulation by Wee1 and Cdc25 was defined by their role in the entry into M of the fission yeast. However, these mechanisms are conserved in vertebrates, where there are different Cdc25s that operate to activate CDK activity at both the G1–S and G2–M boundaries. In plants, the regulatory mechanism appears to exist and a Wee1-like kinase has been cloned from maize (Sun *et al.* 1999). No clear Cdc25 homologue has been identified in the *Arabidopsis* genome or in that from other plants, but expression of the yeast Cdc25 gene has profound effects in tobacco cells (McKibbin *et al.* 1998), suggesting that the regulatory mechanism probably exists and that another protein phosphatase with lower homology to Cdc25 may be playing this role.

Two classes of proteins are also known to be involved in forming stable associations with CDK complexes. The Cks proteins are small proteins, generally 9–13 kDa, which appear to act either as scaffolding components or to modulate the target specificity of the complex. In yeast, the Cks protein is known as Suc1, and the human protein as Cks1. The structure of a human CDK–cyclin–Cks ternary complex has been solved (Bourne *et al.* 1996). In *Arabidopsis*, there are two Cks genes and the expression pattern of one has been reported by *in situ* hybridization (Jacqmard *et al.* 1999; Stals *et al.* 2000).

CKIs bind to CDK complexes or their components and inhibit their activity. They appear to play important roles in controlling cell-cycle regulation, particularly in response to inhibitory signals. For example in yeast, the CKI Far1 inhibits Cdc28-Cln activity in response to mating signals. The G1–S transition itself in yeast is controlled by a further CKI (Sic1), which blocks S-phase-specific kinase activity until Sic1 is itself phosphorylated by G1 CDKs and is consequently destroyed. In mammals, G1 progression is inhibited by the $p16^{INK4}$ type of CKI charac-

terized by ankyrin repeats, which is a specific inhibitor of CycD kinases (see below). A second and unrelated class of CKIs in mammals is represented by p27^{KIP1}, which is responsible for cell-cycle arrest in response to contact inhibition, and the protein p21. This is a general modulator of CDK activity that appears to be a normal component of certain active CDK complexes when present at a low concentration, but inhibits their activity at higher concentrations. p21 is induced by the DNA damage sensor and general 'gatekeeper' p53, which controls both cell-cycle arrest and apoptosis (Levine 1997; Rich *et al.* 2000; Vousden 2000).

3. CELL-CYCLE OVERVIEW: YEASTS AND HIGHER EUKARYOTES

A general theme that can be seen in all eukaryotes is the presence of three broad groups of cyclins that control different parts of the cell cycle, generally active in G1, S–M and G2–M. In yeast, the G1 cyclins are known as Cln cyclins, but higher eukaryotes (animals and plants) share CycDs as the major G1 control. In the S phase, new cyclins and hence kinase activities are present. In budding yeast these are the B-type cyclins Clb5 and Clb6, but again animals and plants have a group known as A-type cyclins that appear to play important roles in the S phase and are destroyed in early M. Further B-type cyclins are involved in the G2–M transition in yeast (Clb1–4), and B-type cyclins are also involved in this role in animals and plants. We can therefore see broad lines of conservation in the general types of molecules involved but differences in their uses in various groups of organism.

Higher eukaryotes have elaborated the CDKs in specific directions. The single yeast CDKs (Cdc28 in budding yeast and Cdc2 in fission yeast) are characterized by the amino acid sequence PSTAIRE (single-letter code) in their cyclin-binding domain. Direct homologues of the yeast CDKs are found in all eukaryotes (known as CDK1/cdc2 in mammals, and CDKA/cdc2 in plants). In animals CDK1 interacts primarily with B-type cyclins (but also A-types) to control the G2–M transition, whereas in plants the equivalent protein CDKA interacts with CYCA, CYCB and CYCD cyclins. By contrast, mammals have specialized variant CDKs (CDK4 and CDK6) that interact with CycDs, and a further CDK (CDK2) that partners cyclins A and E. Plants have their own novel CDK group (CDKB), characterized by the consensus sequence PPT(A/T)LRE, that are involved in S–M phase control (Joubes *et al.* 2000) and are unique among CDKs because they show cell-cycle regulation of their expression of both RNA and protein (Fobert *et al.* 1996; Magyar *et al.* 1997; Segers *et al.* 1998).

The consensus outline overview of the plant cell cycle is shown in figure 2 and involves the sequential activity of D-, A- and B-type cyclins in combination with CDKA and CDKB (see Mironov *et al.* (1999) for a more detailed discussion). CDKA has a role throughout the cell cycle and associates with all types of cyclins. Its transcript level and protein abundance are constant through the cell cycle. However, its kinase activity is increased at the G1–S transition, remains high in S phase, and shows a further peak at G2–M in both tobacco and *Arabidopsis* cells (Reichheld *et al.* 1999; Sorrell *et al.* 2001; Menges & Murray 2002).

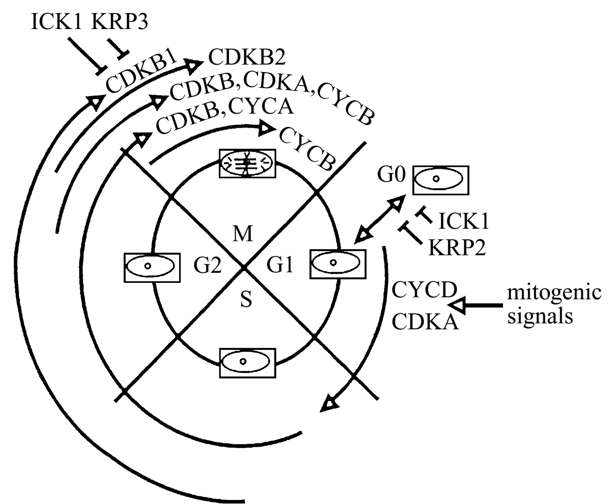

Figure 2. Plant cell-cycle overview. The plant cell cycle involves the sequential activity of D-, A- and B-type cyclins in combination with CDKA (PSTAIRE) and CDKB (CDKB1, PPT(A/T)LRE; CDKB2, P(S/P)TTLRE). The arrows indicate the timing of expression of each of these genes/proteins. Roles of the CKIs (ICK1, KRP2 and KRP3) are proposed based on expression timing (Menges & Murray 2002).

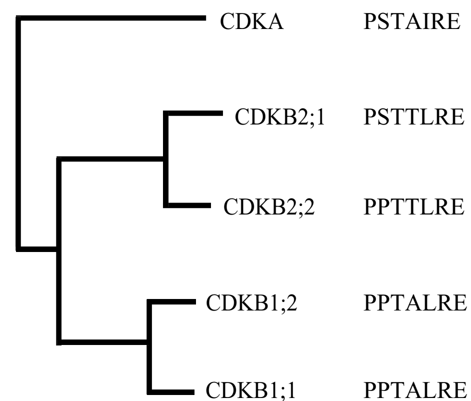

Figure 3. The cell-cycle-related CDKs of *Arabidopsis*. A protein sequence alignment of *Arabidopsis* CDKA (X57839), CDKB1;1 (X57840), CDKB1;2 (Q9ZVI4), CDKB2;1 (Q9C527) and CDKB2;2 (Q9SYP4) was generated with ClustalX (Thompson *et al.* 1997) using the default alignment and tree construction parameters. The two types of CDK (A and B) form separate groups and two subgroups within the CDKB are found, which appear to have different expression timing (Menges & Murray 2002). The CDK nomenclature is as described in Sorrell *et al.* (2001).

These results suggest roles for CDKA kinase activity at all major cell-cycle transitions.

Analysis of the CDK genes of the *Arabidopsis* genome likely to encode cell-cycle-regulating proteins (figure 3) shows that *Arabidopsis* has a single CDKA protein, and four proteins of the CDKB type. These divide into two pairs of proteins, which are likely to have different expression timing. The CDKB1 proteins have the sequence PPTALRE and are expressed from the beginning of the S phase until M. The CDKB2 proteins have the sequence P(S/P)TTLRE and are expressed in a later and narrower window only from late G2–early M until late M (Menges & Murray 2002), although in both cases the

hybridization analysis used would not distinguish between the pair of closely related genes in each group. To our knowledge the *in vivo* cyclin partners of CDKB proteins have not been determined, although we have demonstrated that CDKB1 is not a kinase partner of CycDs (Healy *et al.* 2001).

The presence of the CDKB1 and CDKB2 types of CDK and their difference in expression timing appears to be a common feature of all plants that have been examined (Joubes *et al.* 2000; Sorrell *et al.* 2001), including *Antirrhinum* (Fobert *et al.* 1994) and alfalfa (Magyar *et al.* 1997) (see Joubes *et al.* (2000) to relate original with current nomenclature).

4. G1 AND G1–S CONTROLS

Since several comprehensive reviews of the plant cell cycle have been published recently (Segers *et al.* 1998; Huntley & Murray 1999; Mironov *et al.* 1999; Inze 2000; Dewitte & Murray 2002), we will concentrate here on the control of the G1 phase and the G1–S transition, and relate the regulation in yeast and mammals to that observed in plants.

As already mentioned, G1 control in yeast is mediated by Cdc28–Cln activity. G1 progression is initiated by Cln3 translation, which is responsive to the growth state of the cell, and in particular the rate of protein synthesis. The transcription of Cln1 and Cln2 is then activated, controlled by the SBF transcription factor complex, and a positive feedback loop results from the activation of SBF by CDK activity. S-phase genes are under the control of a second complex, MluI cell-cycle box binding factor, which shares a component (Swi6) with SBF (reviewed in Pines (1995)). The critical event for entering the S phase is the destruction of the Sic1 CKI, which blocks the activity of cyclin B kinases Cdc28–Clb5/6. Once activated these kinases trigger the entry into S phase.

Although higher eukaryotes share the same theme of G1-specific CDK activity, and the ultimate role of transcriptional activation in S-phase entry, the other proteins involved are not homologues of those used in yeast. The components in both animals and plants are the CycDs, whose associated kinase activity is targeted to the Rb protein, resulting in the activation of E2F transcription factors (figure 4).

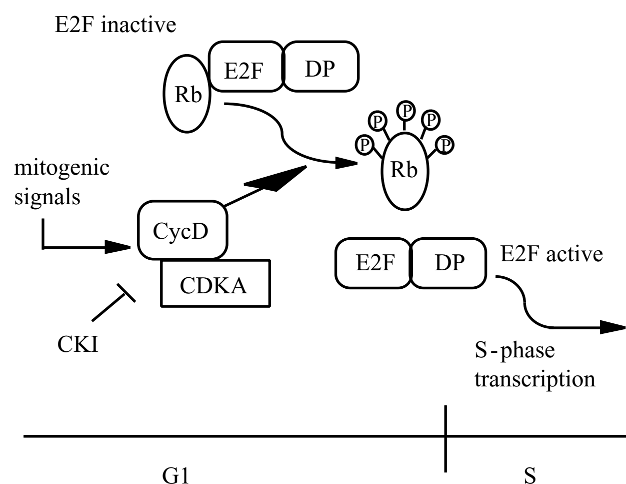

Figure 4. General model of G1 and G1–S phase regulation in higher eukaryotes. In plants and animals the same components are found to regulate the transition from G1–S phase: the CycDs and their associated CDKs respond positively to mitogenic signals (and negatively to CKIs) and target their kinase activity to the Rb protein which results in the activation of E2F transcription factors.

(a) *CycD*

Three types of CycDs were originally isolated from *Arabidopsis* by complementing a yeast strain deficient in Cln cyclins (Soni *et al.* 1995). Further *Arabidopsis* CycDs were isolated by De Veylder *et al.* (1999) and Swaminathan *et al.* (2000). The former was described as *CycD4*, although more recent analysis suggests it probably forms part of the *CycD2* group (figure 5), and the latter is a second *CycD3* gene. Analysis of the completed genome sequence of *Arabidopsis* reveals a total of 10 genes related to *CycD*. There is a single *CycD1* gene, three genes in the *CycD2* group (if *CycD4* (*CycD2;2*) and a closely related gene (*CycD2;3*) are included), and three *CycD3* genes. In addition, there are three cyclin genes that do not lie in these groups and may form separate CycD classes with single members (figure 5). Nothing is published on the role or expression of these genes. This analysis suggests that there may be at least six separate CycD groups, which may well have distinct functional roles.

A defining structural characteristic of both animal and plant CycDs is the presence of the amino acid motif LxCxE (where x represents any amino acid), near the N-terminus of the protein, which is responsible for binding to Rb. In the mid–late G1 phase of the cell cycle in both plants and animals, cyclin D–CDK complexes bind Rb proteins through the cyclin D LxCxE motif, thereby targeting the phosphorylation activity of the complex to Rb. Up to this point, Rb proteins are bound to transcription factors known as the E2Fs. Phosphorylation of Rb releases E2Fs allowing them to activate the transition of the cell from G1 phase into S phase (figure 4). In human cells, one of the genes known to be under the control of E2F is cyclin E, which in association with CDK2 completes the phosphorylation of Rb, allowing complete activation of E2F (see reviews by Mittnacht (1998) and Hengstschlager *et al.* (1999)). Further levels of control are provided by CKIs that modulate cyclin D kinase assembly and activity (see above).

CycDs are rate-limiting components for this pathway of progression through G1. Their expression in mammals is under the control of external signals such as serum growth factors, and they therefore are responsible for triggering the cell cycle in response to such mitogenic signals. Their expression is not strongly cell-cycle regulated, but rather is activated by growth factors and disappears rapidly when the mitogenic signal is removed (Sewing *et al.* 1993; Sherr 1993), in contrast to the strongly cell-cycle-regulated A- and B-type cyclins. Similarly in plants, CycD levels and activity respond to signals such as hormones and carbohydrate levels that are important in influencing decisions by plant cells to divide.

Consistent with their responsiveness to the presence of extracellular signals, and their role in mediating such signals in cell-cycle control, most CycD proteins are unstable. Human CycD can be degraded rapidly by a mechanism that involves phosphorylation of the protein

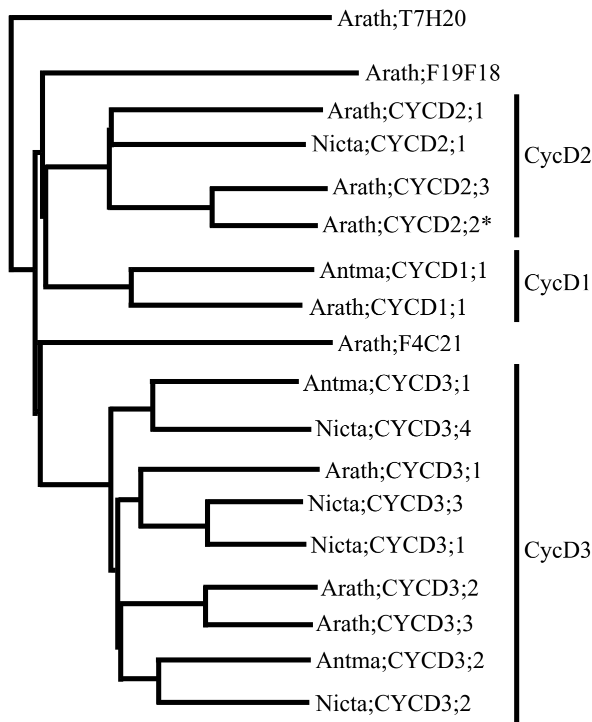

Figure 5. The plant CycD family. An alignment of plant CycD protein sequences was made with ClustalX, using the default alignment and tree construction parameters. There appear to be three larger groups and three minor groups of plant CycDs. The cyclin nomenclature is according to Renaudin *et al.* (1996). Arath;T7H20 (AJ245415); Arath;F19F18 (Q9SZF6); Arath;CYCD2;1 (X83370); Nicta;CYCD2;1 (AJ011892); Arath;CYCD2;3 (CAB89399); Arath;CYCD2;2 (AJ131636); Antma;CYCD1;1 (AJ250396); Arath;CYCD1;1 (X83369); Arath;F4C21 (AAD14455); Antma;CYCD3;1 (AJ250397); Nicta;CYCD3;4 (M. Sekine, personal communication); Arath;CYCD3;1 (X83371); Nicta;CYCD3;3 (AB015222); Nicta;CYCD3;1 (AJ011893); Arath;CYCD3;2 (BAB09645); Arath;CYCD3;3 (CAB62115); Antma;CYCD3;2 (AJ250398); Nicta;CYCD3;2 (AJ011894). Asterisk, CYCD2;2 is also known as CYCD4;1.

on T286 and subsequent ubiquitin-mediated proteolysis (Diehl & Sherr 1997; Diehl *et al.* 1997). This mechanism is likely to be common to all or most CycDs, as T286 or its equivalent are conserved in various organisms including plants. This threonine residue is located within a larger so-called PEST domain; such motifs are characterized as hydrophilic sequences containing at least one proline and at least one acidic residue, and a serine or threonine bounded by basic residues (Rogers *et al.* 1986; Rechsteiner & Rogers 1996). This motif is also found in other mammalian CycDs (and in the unstable yeast Cln cyclins; reviewed by Mendenhall & Hodge (1998)). Whether the PESTs are wider signals for proteolysis, or whether T286 alone is sufficient to confer rapid turnover, is unclear (figure 6).

In *Drosophila* and mammals, the CDK partner of CycD is a variant CDK known as CDK4. However, there is no evidence for a CDK4 homologue in plants, and indeed there is now substantial evidence that the CDKA class are the partners of the plant CycDs in the control of the G1–S transition. Tobacco CYCD3 has been shown to associate with CDKA *in vitro* (Nakagami *et al.* 1999), and

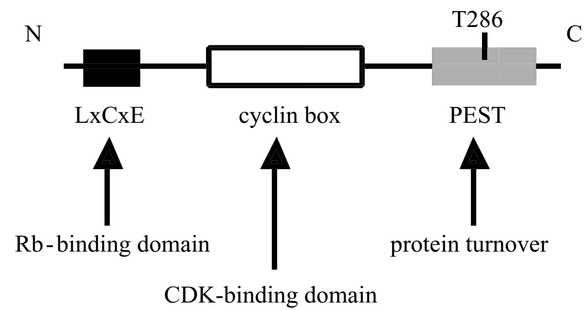

Figure 6. The structure of CycDs. The conserved domains within CycDs are the LxCxE (single-letter amino acid codes), Rb-binding domain, the cyclin box (the CDK-binding domain) and the PEST region which could be a signal for proteolysis, or the amino acid T286 alone may be sufficient for the rapid turnover of the CycDs.

Arabidopsis CYCD2 and CYCD3 co-immunoprecipitates with CDKA and not CDKB1 in cell extracts (Healy *et al.* 2001). CDKA is also the only CDK so far detected as being expressed in G1 cells re-entering the cell cycle (Sorrell *et al.* 2001), although it is possible that other untested plant CDKs could also be partners for other CycDs.

A further significant difference between plant and animal CycDs is found in their substrate specificity. Human CycD kinases phosphorylate only the Rb protein, and histone H1 (the normal *in vitro* assay substrate for CDKs) is a very poor substrate for CDK4–cyclin D. Plant CDKA–CYCD kinases phosphorylate histone H1 both *in vitro* and as immunoprecipitates from cell extracts (Cockcroft *et al.* 2000; Healy *et al.* 2001). Experiments with tobacco Rb have shown direct phosphorylation of plant Rb protein by a plant CYCD–CDK complex assembled in insect cells (Nakagami *et al.* 1999), but phosphorylation of Rb using kinase activity immunoprecipitated from plant cells has not yet been demonstrated.

(b) *Rb protein*

The Rb protein interacts with a large number of substrates, and appears to act as a scaffold protein for building intermolecular interactions. Its particular role in the cell cycle appears to involve its recruitment to gene promoters carrying E2F-binding sites by E2F, which remains bound to DNA throughout the cell cycle. Rb both interacts directly with RNA polymerases and also recruits histone deacetylases to render chromatin less transcriptionally active. It is also believed to interact with chromatin assembly complexes that may play further roles in modulating gene activity at the chromatin level. Rb is therefore regarded as being recruited to E2F-containing promoters, and this association is lost as a consequence of Rb phosphorylation at G1–S (de Jager & Murray 1999; Harbour & Dean 2000).

In the past five years several Rb protein homologues have been identified in maize (Grafi *et al.* 1996; Ach *et al.* 1997) and in tobacco (Nakagami *et al.* 1999), and shown to interact with G1 cyclin proteins as observed in animals. A single Rb protein is present in the *Arabidopsis* genome (Inzé *et al.* 1999). ZmRb proteins interact with *Arabidopsis* CycDs *in vitro*, with the interaction depending on the

LxCxE motif that is also required for human CycDs to interact with Rb proteins (Ach *et al.* 1997; Huntley *et al.* 1998). The conserved interactions between the components of the G1–S pathway extends to the extent that ZmRb1 protein binds to human and *Drosophila* E2F and can inhibit the transcriptional activation ability of human E2F (Huntley *et al.* 1998). Interestingly, when Rb levels were examined in developing maize leaves, higher levels of ZmRb protein expression are found in differentiated cells near the leaf tip than in the proliferating region near the base (Huntley *et al.* 1998). ZmRb protein may therefore be serving a similar function of being necessary for cell-cycle exit as observed in mice cells (Clarke *et al.* 1992; Jacks *et al.* 1992; Lee *et al.* 1992, 1994; Zacksenhaus *et al.* 1996).

(c) *E2Fs*

E2F factors are heterodimers of two subunits, the E2F itself and the DP. DPs share homology with E2F in their DNA-binding domains, and the DNA-binding activity of the E2F heterodimer has contributions from both its E2F and DP components.

E2F genes have been isolated from several plant species including wheat, tobacco and carrot Rb (Miller & Nasmyth 1984; Ramirez-Parra *et al.* 1999; Sekine *et al.* 1999). In each case a single gene has been reported from each species (although undoubtedly further E2F genes are present in the genomes of these plants), which share conserved domains with human E2Fs and interact with Rb, are upregulated at the G1–S phase transition, and in both carrot and tobacco the protein products of the E2F genes have been shown to have trans-activational activity (Miller & Nasmyth 1984; Ramirez-Parra *et al.* 1999; Sekine *et al.* 1999). Further functional analysis in tobacco has indicated that E2F elements play a role in the upregulation of the ribonucleotide reductase gene (which codes for a key enzyme in the DNA synthesis pathway) at the G1–S phase transition. This provides further evidence that E2F elements are active in plant cell-cycle regulation of gene transription (Chaboute *et al.* 2000).

In *Arabidopsis*, three E2F genes have been cloned by conventional approaches (de Jager *et al.* 2001). In addition, genome searches reveal that the full extent of the E2F family in *Arabidopsis* includes the three E2F genes, two further genes lying in a plant-specific group referred to as ELPs, and two DP genes (figure 7; de Jager *et al.* 2001).

The sequence of the three E2F genes from *Arabidopsis* (AtE2F1–3) indicates that AtE2F1 and 3 are most closely related to the other plant E2Fs (figure 7), while AtE2F2 is the least closely related to the other plant E2Fs. AtE2F2 appears to share some characteristics with human E2F6 and *Drosophila* dE2F2, which are unusual in lacking transcriptional activation potential (de Jager *et al.* 2001). AtE2F1 and 3 were found to activate transcription in yeast cells and bind to ZmRb, while AtE2F2 could neither activate transcription nor bind Rb (de Jager *et al.* 2001). In addition all AtE2Fs accumulated in partially synchronized *Arabidopsis* cells re-entering the cell cycle during G1, again suggesting their involvement in the control of the G1–S phase transition. AtE2F1 was also found to bind *in vitro* to a sequence of a consensus E2F site present in the *Arabidopsis* CDC6 gene promoter, indicating that the E2F may

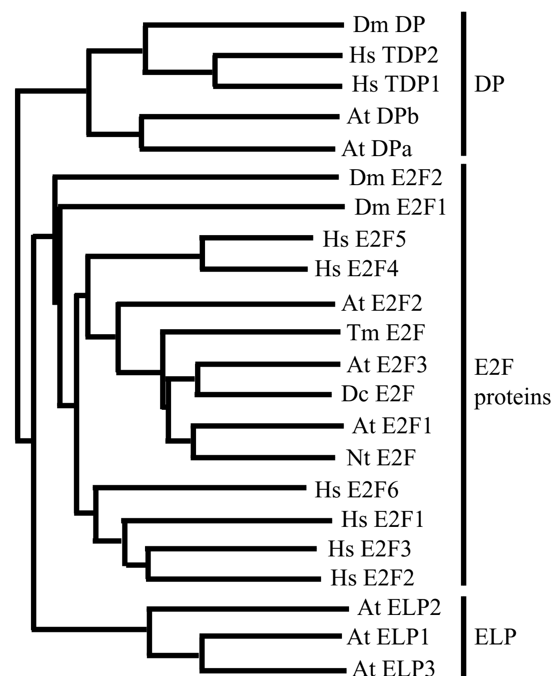

Figure 7. The E2F gene family. An alignment of E2F proteins, ELP and DP sequences was constructed using ClustalX (Thompson *et al.* 1997) with the default alignment and tree construction parameters. The nomenclature is as used in de Jager *et al.* (2001). (Dm, *Drosophila melanogaster*; Hs, *Homo sapiens*; At, *Arabidopsis thaliana*; Tm, *Triticum* sp.; Dc, *Daucus carota*.) Dm E2F2 (AB016824); Dm E2F1 (X78421); Hs E2F6 (AF059292); Hs E2F1 (Q01094); Hs E2F2 (Q14209); Hs E2F3 (Q15000); At E2F2 (Q16254); Tm E2F (AJ238590); Nt E2F (AB025347); At E2F1 (AF242580); Dc E2F (AJ251586); At E2F3 (AF242582); Hs E2F4 (Q16254); Hs E2F5 (Q15329); At ELP2 (Q9STS2); At ELP3 (Q9LFQ9); At ELP1 (Q9SRI0); At DPa (Q9LZE7); At DPb (Q9LZ55); Dm DP (Q9V6M0); Hs TDP1 (Q14186); Hs TDP2 (Q14188).

account for its cell-cycle regulation. E2F consensus sites are also present in a number of G1 and G1–S-related genes in *Arabidopsis*, including the CycD3 promoter (Gutierrez 1998; de Jager *et al.* 2001).

Recently, the first three plant DP genes have been isolated: two in *Arabidopsis* (Magyar *et al.* 2000) and one in wheat (Ramirez-Parra & Gutierrez 2000). Preliminary characterization of these genes indicates that they have similar domain organization to their animal homologues. The *Arabidopsis* DP genes are expressed in actively dividing cells with the highest expression levels in early S phase, and they heterodimerize *in vitro* with *Arabidopsis* E2F-related proteins (Magyar *et al.* 2000). The wheat DP gene is expressed ubiquitously and the purified protein stimulates E2F–DNA complex formation *in vitro* (Ramirez-Parra & Gutierrez 2000).

(d) *CDK inhibitors*

In mammalian cells, CKIs play a major role in controlling G1 progression (Harper & Elledge 1996). The $p16^{INK4}$ inhibitor is specific for CDK4, inhibiting both its association with CycD and the kinase activity of the complex. High levels of $p16^{INK4}$ therefore increase free CycD levels, which then associate with other CDKs such as

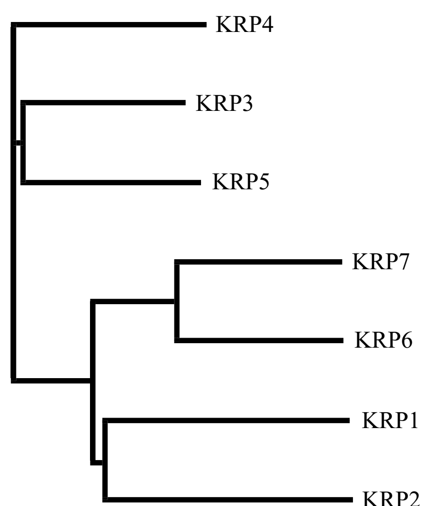

Figure 8. The CKI/KRP genes of *Arabidopsis*. The *Arabidopsis* CKI/KRP sequences were aligned using ClustalX (Thompson *et al.* 1997) with the default alignment and tree construction parameters. This preliminary analysis of the relationships between the KRPs indicates that they may separate into two main groups (KRP3, 4 and 5 versus KRP1, 2, 6 and 7). The nomenclature is as described in De Veylder *et al.* (2001). KRP1/ICK1 (U94772); KRP2/ICK2 (AJ251851); KRP3 (AJ301554); KRP4 (AJ301555); KRP5 (AJ301556); KRP6 (AJ301557); KRP7 (AJ301558).

CDK1 and CDK2. However, these complexes are catalytically inactive, therefore further reducing the overall CDK activity present in cells. The $p27^{KIP1}$ and p21 inhibitors also play roles in contact inhibition and DNA damage response as mentioned above.

In plants, the roles of CDK inhibitors in controlling cell-cycle and G1–S progression are not fully elucidated. Plant CKIs have been named ICK (Wang *et al.* 1997, 1998) or KRP (Stals *et al.* 2000; De Veylder *et al.* 2001). They have limited homology to the p21/p27 type of mammalian CKI at their C-termini, and this is the region where different plant ICKs share homology, the rest of the proteins being divergent. In *Arabidopsis*, seven ICKs are present in the genome (Stals *et al.* 2000; De Veylder *et al.* 2001) (figure 8). ICK1 was found to bind both CDKA and CYCD3 in yeast two-hybrid assays, as well as inhibit CDK activity *in vitro*, suggesting that it could have roles in G1 and might act in inhibiting both assembly and activity of complexes (Wang *et al.* 1998). The plant hormone ABA was also found to induce levels of ICK1 mRNA dramatically (Wang *et al.* 1998). Overexpression of ICK1 in plants inhibits cell division, resulting in highly lobed leaves and causing a severe dwarfing effect (Wang *et al.* 2000). More detailed analysis of the growth and division effects of ICK2/KRP2 during leaf development show that its overexpression slows cell division, but does not affect the developmental window during which division takes place (De Veylder *et al.* 2001).

The roles of different ICK/KRP genes at different times in the cell cycle are so far unclear. However, expression analysis of ICK1/KRP1, KRP2 and KRP3 in synchronized cells shows that each has a distinct expression timing, suggesting distinct roles in controlling cell-cycle transitions (Menges & Murray 2002).

5. EXPRESSION AND ACTIVITY OF PLANT CycDs

The majority of CycDs do not show strongly cell-cycle-dependent mRNA regulation but may show tissue-specific expression. *CycD1* is expressed at low or undetectable levels in liquid cultured cells, whereas *CycD2* and *D3* have been shown to have constant levels of mRNA from day 1 of the growth cycle to early stationary phase on day 7 (Riou-Khamlichi *et al.* 2000). This indicates that the expression of these cyclins is not dependent on the active cell division or the cells being in a particular part of the growth cycle. This is in contrast to the expression of histone H4, which is expressed only in cells in S phase and was present in higher levels in exponential cells and at low levels in day-7 cells (Riou-Khamlichi *et al.* 2000). However, *CycD3* mRNA levels were found to be strongly dependent on the continued presence of carbon source in the medium. Either exhaustion of carbon source or subculturing cells into medium lacking sucrose led to a rapid loss of *CycD3* transcripts (Riou-Khamlichi *et al.* 2000), protein and kinase activity (Healy *et al.* 2001).

Tobacco *CycD3; 2* has also been shown to be expressed at a constant level after induction in G1 (Sorrell *et al.* 1999), whereas a somewhat higher abundance in mitotic cells was seen for *CycD2;1* and *D3;1*. It is possible that this greater expression of CycDs is due to a process similar to one in the G2–M of mammals, where Rb is further phosphorylated before being dephosphorylated later in M phase. Alternatively, this mitotic enhanced expression could be a unique feature in BY-2 tobacco cells, which have been grown in culture for the past 30 years. A similar increase in cyclin D1 in G2–M phase was observed in human HeLa tumour cell lines but not in other cell lines or in primary cultures (Motokura *et al.* 1992), and it was suggested that this was due to inadvertent selection of the HeLa cells over prolonged cell culture, causing an alteration of the *CycD1;1* expression (Sewing *et al.* 1993).

In animals, the CycDs are stimulated in response to serum growth factors. Plant CycDs are also responsive to growth-regulating substances, including plant hormones (Davies 1995) and sucrose. Sucrose is the major transported carbon source in plants, and as such it is a likely candidate as a signalling molecule in the regulation of genes controlling the cell division cycle. Early experiments indicated that addition of sucrose to sucrose-starved *Arabidopsis* cell cultures induces *CycD2* and *CycD4* mRNA expression (Soni *et al.* 1995; De Veylder *et al.* 1999). A subsequent more detailed study demonstrated sucrose to have a large effect on the G1 phase expression of *Arabidopsis CycD2* and *D3* (Riou-Khamlichi *et al.* 2000). Specifically, in a cell culture starved of sucrose (and therefore in a quiescent state), *CycD2* mRNA levels increased within 30 min of the addition of sucrose, i.e. in early G1 phase. *CycD3* mRNA levels take a little longer to increase (4 h after the addition of sucrose), occurring at the time of late G1 phase near the boundary with the S phase (Riou-Khamlichi *et al.* 2000).

The induction of *CycD2* and *D3* gene expression by sucrose is a direct response to the sucrose rather than an indirect result of the cells growing and dividing. This was shown by the use of the protein synthesis inhibitor, CHX, which can block cell-cycle progression and *de novo* protein synthesis. By addition to the cell culture of concentrations

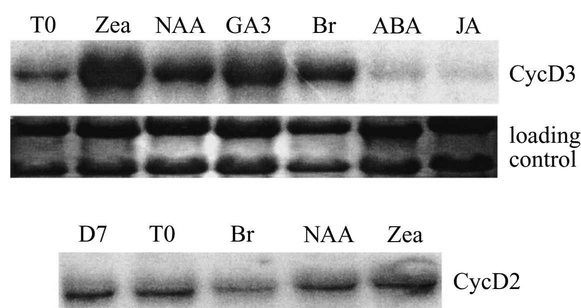

Figure 9. Differential hormone regulation of CycD2 and CycD3 expression. Several hormones were added to *Arabidopsis* cell suspension cultures and the RNA levels of *CycD3* and *CycD2* were examined after 4 h. The hormones cytokinin (Zea, zeatin), auxin (NAA, naphthaleneacetic acid) gibberellin (GA3) and brassinolide (Br) all induced *CycD3* levels, with cytokinin having the greatest effect. None of cytokinin, auxin or Br had any effect on *CycD2* expression levels. The growth-inhibiting hormone ABA and JA both downregulated the levels of *CycD3*. (D7, RNA levels when cells are in stationary phase; T0, RNA levels at time of hormone addition.)

of CHX suitable for blocking *de novo* protein synthesis as well as cell-cycle progression, sucrose was still able to induce *CycD2* and *CycD3* expression (Riou-Khamlichi *et al.* 2000). These results are consistent with plant CycDs being effectors of exogenous signals, such as nutrient availability, in cell-cycle control.

The mechanism by which *CycD2* and *CycD3* are induced by exogenous signals involves protein phosphatases. This was illustrated when the application of phosphatase inhibitors to cell cultures inhibited the induction of these cyclins (Riou-Khamlichi *et al.* 2000). Interestingly, *CycD2* mRNA accumulation was less sensitive to the effect of these inhibitors than *CycD3*, suggesting that different protein phosphatase pathways may be involved in their induction.

Other evidence for differential regulation of *Arabidopsis CycD2* and *CycD3* is their response to the hormone cytokinin. *CycD3* expression can be induced by the addition of cytokinin to the cell culture and expression levels decrease after the removal of cytokinin, whereas *CycD2* expression remains unaffected by either the addition or the removal of this hormone (figure 9). However, the induction of *CycD3* by cytokinin is dependent on the presence of sucrose, and both *CycD3* and *CycD2* can be induced by sucrose alone, without cytokinin (Riou-Khamlichi *et al.* 1999, 2000). Therefore sucrose appears to be upstream and dominant to cytokinin in the regulation of *CycD3* expression.

Further analysis suggests that CycD3 may have wider roles in responding to plant hormones. Hu *et al.* (2000) have shown that brassinosteroids induce *CycD3* gene expression, and indeed several hormones which could be considered 'mitogenic' all induce an accumulation of *CycD3* mRNA within 4 h of treatment, although not to the same extent as observed with cytokinins (figure 9). This includes cytokinin, auxin, gibberellin and brassinolide. There is no effect on *CycD2* mRNA levels in the same experiment. Interestingly, the growth-inhibiting hormones ABA and JA resulted in a clear downregulation of *CycD3* mRNA levels (figure 9). Whether *CycD3* is responding directly to these hormones or as a consequence of interlinking of hormone response pathways is not known.

Consistent with the stimulation of *CycD3* expression by cytokinin, constitutive expression of *CycD3* in transgenic *Arabidopsis* plants enabled the induction and maintenance of callus from leaf explants without the exogenous cytokinin (Riou-Khamlichi *et al.* 1999), whereas normally both auxin and cytokinin would be required. This suggestion is consistent with the work of Houssa *et al.* (1990, 1994), who found that the application of cytokinin led to the activation of latent DNA replication origins. If CYCD3 kinase activity is involved in the activation of DNA replication, then induction by cytokinin could explain these results. Furthermore, the effect of cytokinin on replication origins can be blocked by the addition of ABA (Jacqmard *et al.* 1994), which has also been shown to be a strong inhibitor of *CycD3* expression.

Recently observations of protein expression and kinase activity have added to the evidence that *Arabidopsis* CYCD2 and CYCD3 function in separate pathways, although they interact with the same CDK partner; CDKA (Healy *et al.* 2001). CYCD3 protein levels and kinase activity closely mirror its mRNA levels, and CYCD3 protein rapidly disappears on sucrose removal (*ca.* 90% decline within 1 h). By contrast, CYCD2 protein abundance and associated kinase activity does not parallel its mRNA levels, and CYCD2 appears to be a rather stable protein, surviving at least 24 h of sucrose starvation (Healy *et al.* 2001). It also appears that CYCD2 kinase activity is regulated by the sequestration of CYCD2 protein in a form that is also inaccessible to immunoprecipitation with antibodies against either CYCD2 or CDKA. These immunoprecipitation results also suggest that it is unlikely that the protein CYCD2 is complexed with CDKA at these times (Healy *et al.* 2001).

In summary, CycD2 and D3 appear to act during G1 phase to mediate exogenous signals and allow the cell to enter the cell cycle. The evidence suggests that they have distinct roles, as their kinase activity appears at different times in cells re-entering the cell cycle, and they are regulated by separate pathways (figure 10). *CycD3* is primarily transcriptionally regulated, whereas *CycD2* appears to be regulated post-translationally or by protein associations.

6. G1 CONTROLS, CELL DIFFERENTIATION AND PLANT DEVELOPMENT

The importance of the control of G1–S is illustrated well in humans where almost all tumours show a loss of control of the Rb regulation pathway (Sherr 1996). Also, during embryogenesis Rb is highly expressed in cells undergoing specific differentiation (Szekely *et al.* 1992), and loss of Rb has been shown in knockout mice to result in the death of the embryos (Clarke *et al.* 1992; Jacks *et al.* 1992; Lee *et al.* 1992, 1994; Zacksenhaus *et al.* 1996). Interestingly, death in Rb^{-}/Rb^{-} embryos occurs in mid-embryogenesis and is associated not with a failure of cell-cycle control but with the terminal differentiation of cells of the haemopoietic, neural and muscle lineages (Zacksenhaus *et al.* 1996). This has led to the suggestion that Rb and the G1 control pathway links decisions

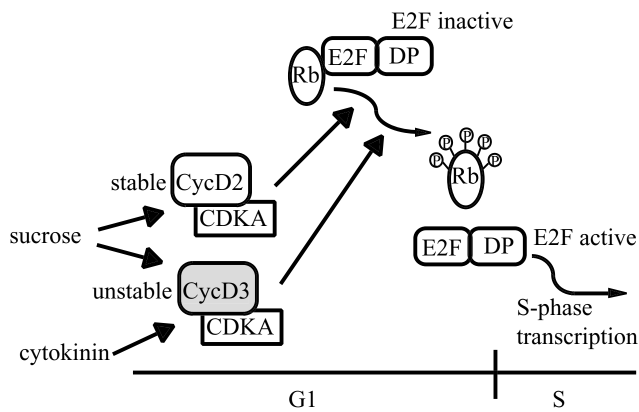

Figure 10. Summary of CycD2 and CycD3 activity. CYCD2 and CYCD3 both activate E2F protein complexes but apparently by separate pathways. Both CYCD2 and CYCD3 interact with CDKA and both are stimulated by sucrose. CYCD3 expression and kinase activity is also stimulated by addition of cytokinin, whereas CYCD2 is unaffected by this hormone. After sucrose removal CYCD3 protein disappears rapidly while CYCD2 protein is relatively stable and appears to be regulated post-translationally or by protein associations. (See text for details.)

involved in cell proliferation and differentiation, and that certain types of differentiation event require cell-cycle exit.

Evidence that CycDs and the Rb pathway are intimately involved in plant development has come from several sources. First, some *CycD* genes are expressed in a tissue-specific manner. In other words, they are not expressed in all cells that are dividing. This is most clearly seen in the expression patterns of two *CycD3* genes of *Antirrhinum*. *CycD3b* appears to be expressed in all dividing cells, whereas *CycD3a* is absent from the shoot meristem and is expressed primarily in developing primordia that will form lateral organs (Gaudin *et al.* 2000) (see figure 5 for the relationship of these genes to those of *Arabidopsis*). Preliminary analysis suggests that *CycD3* of *Arabidopsis* (i.e. *CycD3;1*) is expressed in lateral organs in a similar pattern to *CycD3a* of *Antirrhinum*.

As discussed in § 1, plant growth and development are probably both controlled in the meristems of the root and shoot. These are also the locations with the greatest cell division activity and therefore it seems likely that alteration in cell-cycle regulation could have effects on downstream developmental processes. Investigations of these effects have begun with transgenic tobacco plants that overexpress the *Arabidopsis CycD2* gene (Cockcroft *et al.* 2000) and *Arabidopsis* plants that overexpress the *Arabidopsis CycD3* gene (Riou-Khamlichi *et al.* 1999). Overexpression of these two genes produced strikingly different results. *CycD2* overexpression created an increase in overall growth rates of the tobacco plant but did not affect development or patterning (Cockcroft *et al.* 2000). The cell and meristem sizes were normal, but accelerated development was seen at all stages of growth from the seed to the adult plant. The accelerated rate was found to be due to a decreased length of the G1 phase. It should be noted that the same effect has not so far been observed in *Arabidopsis* plants overexpressing the same gene, and indeed *CycD2* overexpression in *Arabidopsis* causes no readily obvious phenotypes (C. E. Cockcroft, B. den Boer and J. A. H. Murray, unpublished data).

By contrast, CycD3 overexpression triggered morphological differences in the SAM and delays in leaf senescence, as well as allowing cytokinin-independent proliferation of calli from leaf explants (as mentioned in § 5) (Riou-Khamlichi *et al.* 1999). Development of the plant was retarded but flowering occurred at the same developmental stage as in the wild-type, i.e. when the same number of rosette leaves were present. The leaves produced were curled about their medial axis due to an increase in the number of cells on the upper epidermis. The cells have been observed to be smaller and less differentiated than wild-type cells, and the normal layered structure of the mesophyll into palisade and spongy layers is absent, being replaced by more numerous and smaller cells (Dewitte & Murray 2002). Morphological changes have also been seen with overexpression of *Arabidopsis CycD1;1* in *Arabidopsis* plants (R. P. Huntley and J. A. H. Murray, unpublished results). These results again indicate that *CycD2* and *CycD3* function in separate pathways. Increased *CycD2* levels appear to promote cell division in a manner that is still influenced by pattern control, whereas *CycD3* acts like an oncogene to drive cell division largely independently of development and pattern control. The overexpression of *CycD3* is therefore not analogous to the effect of overexpression of the inhibitor of CDK activity, KRP2. As mentioned, in these plants cell division is inhibited, but the time window during which proliferation occurs is not altered. By contrast, *CycD3* results in continued mitotic activity in mature leaves, and therefore extends the proliferation window. In addition, *CycD3* expression appears to result in an inhibition of cellular differentiation, suggesting that the CycD–Rb pathway is closely involved in cell differentiation as well as proliferation (Dewitte & Murray 2002).

(a) *Future directions*

One of the major questions facing plant cell-cycle research is why are there such large numbers of genes encoding cell-cycle regulators in plants. More specifically, do the proteins encoded by these genes have distinct biochemical functions? Or do multiple genes, each with distinct expression, provide a convenient way for the plant to create complex regulatory patterns through the summation of expression activity for all the genes encoding a particular protein activity? This would represent an alternative solution to that used by *Drosophila* to generate complex developmental and cell-specific control, where genes like *STRING*, encoding the cdc25 phosphatase, have large and highly complex promoters. Certainly, there are a number of examples of genes in *Arabidopsis*, where duplicate genes exist, one expressed in a widespread and rather constitutive manner, and the other showing meristematic or tissue-specific expression (e.g. genes encoding the initiation factor eIF4E; Rodriguez *et al.* 1998). The tissue-specific expression of some plant cell-cycle genes (e.g. Gaudin *et al.* 2000) would also support the latter suggestion, as does the reputed lack of phenotype for a number of cell-cycle gene knockouts. A full answer will require detailed genetic analysis of combinatorial mutants and the developmental expression of whole families of cell-cycle genes.

Examples of the types of approaches that are starting to be applied in higher throughput modes to link genome information with cellular and developmental function are the identification of T-DNA or transposon insertion mutants (Parinov & Sundaresan 2000; Bouche & Bouchez 2001), targeted overexpression analysis (Haseloff *et al.* 1997; Haseloff & Siemering 1998) and techniques for downregulation of gene expression (Waterhouse *et al.* 1998). Such strategies allow the phenotypes associated with the up- and downregulation of large numbers of genes to be assessed and then analysed by genome-wide expression profiling of RNA and protein (Richmond & Somerville 2000). When coupled with the developmental phenotypic analysis of such plants, genome-wide profiling provides a picture of the response of the RNA and protein profiles that result from the perturbation to the overall system. From such analysis, a picture not only of the biochemical pathways involved but also of the dynamic interactions within and between pathways may be constructed.

A major limitation in the ability to exploit genomic information for cell-cycle research in *Arabidopsis* has been the lack of synchronized cell systems that allow the analysis of populations of cells largely at the same point in the cell cycle. Although such cultures and techniques exist from some species, notably the well-known tobacco BY-2 system (Nagata *et al.* 1992), which has been extensively used for analysis of CDKs (Sorrell *et al.* 2001) and cyclins (Reichheld *et al.* 1996; Sorrell *et al.* 1999), no such comparable system has been developed for *Arabidopsis* (Richmond & Somerville 2000). This appears to be due both to the nature of available cell cultures and to specific techniques necessary. Recently, we have developed cell cultures of *Arabidopsis* that can be synchronized to high levels with the inhibitor aphidicolin, resulting in 80% of cells in S phase and 92% in G2 (Menges & Murray 2002).

(b) *Concluding remarks*

The recognizable similarities between the molecules involved in G1–S transition in plants and animals, compared with the similarity of only the cyclin and CDK molecules involved with yeast, may be indicative of a system that was developed to allow more complex organisms to form. In complex organisms such as plants and animals, the control of the cell cycle not only coordinates cell proliferation but also has to integrate differentiation of cells into the many different types required to produce complex tissues. However, because of the differences in the life and developmental strategies of plants and animals, this basic pathway is utilized in different ways to coordinate the development and growth of these diverse organisms.

A major goal in plant biology is to achieve an integrated understanding of how cell division is controlled at the molecular, biochemical, cellular, developmental and whole plant levels. The complexity already apparent from current studies, coupled with the large number of genes involved that have been identified following the sequencing of the *Arabidopsis* genome, makes clear the scale of this task. The unravelling of the network of controls will require combined techniques of reverse genetics, molecular analysis, genetic analysis and the use of synchronized cell systems, combined with genome-wide profiling techniques to understand how alterations in one component affect the other aspects of cell division and its control. As we have discussed here, common characteristics are apparent in the basic pathways of cell-cycle control between different eukaryotic groups. It will be of particular interest to establish the extent to which system-wide dynamic responses to perturbations, about which we know little as yet, also show certain conserved features. This will be revealed with studies of global genome responses to overexpression or loss of specific cell-cycle regulators.

We thank the BBSRC, EC and Aventis CropScience for support of plant cell-cycle research in our laboratory. We thank Sarah de Jager for the alignments and trees shown in figures 3, 5, 7 and 8.

REFERENCES

Ach, R. A., Durfee, T., Miller, A. B., Zambryski, P. C., Hanley-Bowdoin, L. & Gruissem, W. 1997 *RRB1* and *RRB2* encode maize retinoblastoma-related proteins that interact with a plant D-type cyclin and geminivirus replication protein. *Mol. Cell Biol.* **17**, 5077–5086.

Berger, F. 1999 Endosperm development. *Curr. Opin. Plant Biol.* **2**, 28–32.

Bouche, N. & Bouchez, D. 2001 *Arabidopsis* gene knockout: phenotypes wanted. *Curr. Opin. Plant Biol.* **4**, 111–117.

Bourne, Y., Watson, M. H., Hickey, M. J., Holmes, W., Rocque, W., Reed, S. I. & Tainer, J. A. 1996 Crystal structure and mutational analysis of the human CDK2 kinase complex with cell cycle-regulatory protein CksHs1. *Cell* **84**, 863–874.

Chaboute, M. E., Clement, B., Sekine, M., Philipps, G. & Chaubet-Gigot, N. 2000 Cell cycle regulation of the tobacco ribonucleotide reductase small subunit gene is mediated by E2F-like elements. *Plant Cell* **12**, 1987–2000.

Clarke, A. R., Maandag, E. R., van Roon, M., van der Lugt, N. M., van der Valk, M., Hooper, M. L., Berns, A. & te Riele, H. 1992 Requirement for a functional Rb-1 gene in murine development. *Nature* **359**, 328–330.

Cockcroft, C. E., den Boer, B. G., Healy, J. M. S. & Murray, J. A. H. 2000 Cyclin D control of growth rate in plants. *Nature* **405**, 575–579.

Davies, P. J. 1995 *Plant hormones: physiology, biochemistry and molecular biology*. Dordrecht: Kluwer and Academic.

de Jager, S. M. & Murray, J. A. H. 1999 Retinoblastoma proteins in plants. *Plant Mol. Biol.* **41**, 295–299.

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

De Veylder, L., de Almeida Engler, J., Burssens, S., Manevski, A., Lescure, B., Van Montagu, M., Engler, G. & Inze, 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., Krols, L., Terras, F., Landrieu, I., Van Der Schueren, E., Maes, S., Naudts, M. & Inze, D. 2001 Functional analysis of cyclin-dependent kinase inhibitors of *Arabidopsis*. *Plant Cell* **13**, 1653–1668.

Dewitte, W. & Murray, J. A. H. 2002 Regulation of cell division in plants. In *Meristematic tissues in plant growth and development* (ed. M. T. McManus & B. Veit), pp. 254–278. Sheffield, UK: Sheffield Academic Press.

Diehl, J. A. & Sherr, C. J. 1997 A dominant-negative cyclin D1 mutant prevents nuclear import of cyclin-dependent kinase 4 (CDK4) and its phosphorylation by CDK-activating kinase. *Mol. Cell Biol.* **17**, 7362–7374.

Diehl, J. A., Zindy, F. & Sherr, C. J. 1997 Inhibition of cyclin D1 phosphorylation on threonine-286 prevents its rapid degradation via the ubiquitin-proteasome pathway. *Genes Dev.* **11**, 957–972.

Fobert, P. R., Coen, E. S., Murphy, G. J. P. & Doonan, J. H. 1994 Patterns of cell division revealed by transcriptional regulation of genes during the cell cycle in plants. *EMBO J.* **13**, 616–624.

Fobert, P. R., Gaudin, V., Lunness, P., Coen, E. S. & 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.

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

Gaudin, V., Lunness, P. A., Fobert, P. R., Towers, M., Riou-Khamlichi, C., Murray, J. A. H., Coen, E. & Doonan, J. H. 2000 The expression of D-cyclin genes defines distinct developmental zones in snapdragon apical meristems and is locally regulated by the Cycloidea gene. *Plant Physiol.* **122**, 1137–1148.

Grafi, G., Burnett, R. J., Helentjaris, T., Larkins, B. A., DeCaprio, J. A., Sellers, W. R. & Kaelin, W. G. 1996 A maize cDNA encoding a member of the retinoblastoma protein family: involvement in endoreduplication. *Proc. Natl Acad. Sci. USA* **93**, 8962–8967.

Gutierrez, C. 1998 The retinoblastoma pathway in plant cell cycle and development. *Curr. Opin. Plant Biol.* **1**, 492–497.

Harbour, J. W. & Dean, D. C. 2000 Chromatin remodeling and Rb activity. *Curr. Opin. Cell Biol.* **12**, 685–689.

Harper, J. W. & Elledge, S. J. 1996 CDK inhibitors in development and cancer. *Curr. Opin. Genet. Dev.* **6**, 56–64.

Haseloff, J. & Siemering, K. R. 1998 The uses of GFP in plants. In *Green fluorescent protein: properties, applications and protocols* (ed. M. Chalfie & S. Kain). New York: Wiley.

Haseloff, J., Siemering, K. R., Prasher, D. C. & Hodge, S. 1997 Removal of a cryptic intron and subcellular localization of green fluorescent protein are required to mark transgenic *Arabidopsis* plants brightly. *Proc. Natl Acad. Sci. USA* **94**, 2122–2127.

Healy, J. M. S., Menges, M., Doonan, J. H. & Murray, J. A. H. 2001 The *Arabidopsis* D-type cyclins CycD2 and CycD3 both interact *in vivo* with the PSTAIRE cyclin-dependent kinase Cdc2a but are differentially controlled. *J. Biol. Chem.* **276**, 7041–7047.

Hengstschlager, M., Braun, K., Soucek, T., Miloloza, A. & Hengstschlager-Ottnad, E. 1999 Cyclin-dependent kinases at the G1-S transition of the mammalian cell cycle. *Mutat. Res.* **436**, 1–9.

Houssa, C., Jacqmard, A. & Bernier, G. 1990 Activation of replicon origins as a possible target for cytokinins in shoot meristems of *Sinapis*. *Planta* **181**, 324–326.

Houssa, C., Bernier, G., Pieltain, A., Kinet, J.-M. & Jacqmard, A. 1994 Activation of latent DNA-replication origins: a universal effect of cytokinins. *Planta* **193**, 247–250.

Hu, Y., Bao, F. & Li, J. 2000 Promotive effect of brassinosteroids on cell division involves a distinct CycD3-induction pathway in *Arabidopsis*. *Plant J.* **24**, 693–701.

Huntley, R. P. & Murray, J. A. H. 1999 The plant cell cycle. *Curr. Opin. Plant Biol.* **2**, 440–446.

Huntley, R. P. (and 14 others) 1998 The maize retinoblastoma protein homologue ZmRb-1 is regulated during leaf development and displays conserved interactions with G1/S regulators and plant cyclin D (CycD) proteins. *Plant Mol. Biol.* **37**, 155–169.

Inzé, D., Gutiérrez, C. & Chua, N.-H. 1999 Trends in plant cell cycle research. *Plant Cell* **11**, 991–994.

Inze, D. 2000 The plant cell cycle. *Plant Mol. Biol.* **43**, 545–786. (Special Issue)

Jacks, T., Fazeli, A., Schmitt, E. M., Bronson, R. T., Goodell, M. A. & Weinberg, R. A. 1992 Effects of an rb mutation in the mouse. *Nature* **359**, 295–300.

Jacqmard, A., Houssa, C. & Bernier, G. 1994 Regulation of the cell cycle by cytokinins. In *Cytokinins: chemistry, activity and function* (ed. D. W. S. Mok & M. C. Mok), pp. 197–215. Boca Raton, FL: CRC Press.

Jacqmard, A., De Veylder, L., Segers, G., de Almeida Engler, J., Bernier, G., Van Montagu, M. & Inze, 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.

Joubes, J., Chevalier, C., Dudits, D., Heberle-Bors, E., Inze, D., Umeda, M. & Renaudi, J. P. 2000 CDK-related protein kinases in plants. *Plant Mol. Biol.* **43**, 607–620.

Lee, E. Y. H. P., Chang, C.-Y., Hu, N., Wang, Y.-C. J., Lai, C.-C., Herrup, K., Lee, W.-H. & Bradley, A. 1992 Mice deficient for Rb are non-viable and show defects in neurogenesis and haematopoiesis. *Nature* **359**, 288–294.

Lee, E. Y. H. P., Hu, N., Yuan, S.-S. F., Cox, L. A., Bradley, A., Lee, W.-H. & Herrup, K. 1994 Dual roles of the retinoblastoma protein in cell cycle regulation and neuron differentiation. *Genes Dev.* **8**, 2008–2021.

Levine, A. J. 1997 p53, the cellular gatekeeper for growth and division. *Cell* **88**, 323–331.

McKibbin, R. S., Halford, N. G. & Francis, D. 1998 Expression of fission yeast cdc25 alters the frequency of lateral root formation in transgenic tobacco. *Plant Mol. Biol.* **36**, 601–612.

Magyar, Z. (and 12 others) 1997 Cell cycle phase specificity of putative cyclin-dependent kinase variants in synchronized alfalfa cells. *Plant Cell* **9**, 223–235.

Magyar, Z., Atanassova, A., De Veylder, L., Rombauts, S. & Inze, D. 2000 Characterization of two distinct DP-related genes from *Arabidopsis thaliana*. *FEBS Lett.* **486**, 79–87.

Meijer, M. & Murray, J. A. H. 2000 The role and regulation of D-type cyclins in the plant cell cycle. *Plant Mol. Biol.* **43**, 621–633.

Mendenhall, M. D. & Hodge, A. E. 1998 Regulation of Cdc28 cyclin-dependent protein kinase activity during the cell cycle of the yeast *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* **62**, 1191–1243.

Menges, M. & Murray, J. A. H. 2002 Synchronous *Arabidopsis* suspension cultures for analysis of cell cycle gene activity. *Plant J.* (In the press.)

Miller, A. M. & Nasmyth, K. A. 1984 Role of DNA replication in the repression of silent mating type loci in yeast. *Nature* **312**, 247–251.

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

Mittnacht, S. 1998 Control of pRB phosphorylation. *Curr. Opin. Genet. Dev.* **8**, 21–27.

Motokura, T., Keyomarsi, K., Kronenberg, H. M. & Arnold, A. 1992 Cloning and characterization of human cyclin D3, a cDNA closely related in sequence to the PRAD1/cyclin D1 proto-oncogene. *J. Biol. Chem.* **267**, 20 412–20 415.

Nagata, T., Nemoto, Y. & Hasezawa, S. 1992 Tobacco BY-2 cell line as the 'HeLa' cell in the cell biology of higher plants. *Int. Rev. Cytol.* **132**, 1–30.

Nakagami, H., Sekine, M., Murakami, H. & Shinmyo, A. 1999 Tobacco retinoblastoma-related protein phosphorylated by a distinct cyclin-dependent kinase complex with Cdc2/cyclin D *in vitro*. *Plant J.* **18**, 243–252.

Parinov, S. & Sundaresan, V. 2000 Functional genomics in *Arabidopsis*: large-scale insertional mutagenesis complements the genome sequencing project. *Curr. Opin. Biotechnol.* **11**, 157–161.

Pines, J. 1995 Cyclins and cyclin-dependent kinases: theme and variations. *Adv. Cancer Res.* **66**, 181–212.

Ramirez-Parra, E. & Gutierrez, C. 2000 Characterization of wheat DP, a heterodimerization partner of the plant E2F transcription factor which stimulates E2F-DNA binding. *FEBS Lett.* **486**, 73–78.

Ramirez-Parra, E., Xie, Q., Boniotti, M. B. & Gutierrez, C. 1999 The cloning of plant E2F, a retinoblastoma-binding protein, reveals unique and conserved features with animal G_1/S regulators. *Nucleic Acids Res.* **27**, 3527–3533.

Rechsteiner, M. & Rogers, S. W. 1996 PEST sequences and regulation by proteolysis. *Trends Biochem. Sci.* **21**, 267–271.

Reichheld, J.-P., Chaubet, N., Shen, W. H., Renaudin, J. P. & Gigot, C. 1996 Multiple A-type cyclins express sequentially during the cell cycle in *Nicotiana tabacum* BY2 cells. *Proc. Natl Acad. Sci. USA* **93**, 13 819–13 824.

Reichheld, J.-P., Vernoux, T., Lardon, F., van Montagu, M. & Inze, D. 1999 Specific checkpoints regulate plant cell cycle progression in response to oxidative stress. *Plant J.* **17**, 647–656.

Renaudin, J.-P. (and 13 others) 1996 Plant cyclins: a unified nomenclature for plant A-, B- and D-type cyclins based on sequence organization. *Plant Mol. Biol.* **32**, 1003–1018.

Rich, T., Allen, R. L. & Wyllie, A. H. 2000 Defying death after DNA damage. *Nature* **407**, 777–783.

Richmond, T. & Somerville, S. 2000 Chasing the dream: plant EST microarrays. *Curr. Opin. Plant Biol.* **3**, 108–116.

Riou-Khamlichi, C., Huntley, R., Jacqmard, A. & Murray, J. A. H. 1999 Cytokinin activation of *Arabidopsis* cell division through a D-type cyclin. *Science* **283**, 1541–1544.

Riou-Khamlichi, C., Menges, M., Healy, J. M. S. & Murray, J. A. H. 2000 Sugar control of the plant cell cycle: differential regulation of *Arabidopsis* D-type cyclin gene expression. *Mol. Cell Biol.* **20**, 4513–4521.

Rodriguez, C. M., Freire, M. A., Camilleri, C. & Ribaglia, C. 1998 The *Arabidopsis thaliana* cDNAs coding for eIF4E and eIF(iso)4^E are not functionally equivalent for yeast complementation and are differentially expressed during plant development. *Plant J.* **13**, 465–473.

Rogers, S., Wells, R. & Rechsteiner, M. 1986 Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. *Science* **234**, 364–368.

Segers, G., Rouzé, P., van Montagu, M. & Inzé, D. 1998 Cyclin-dependent kinases in plants. In *Plant cell proliferation and its regulation in growth and development* (ed. J. A. Bryant & D. Chiatante), pp. 1–19. Chichester: Wiley.

Sekine, M., Ito, M., Uemukai, K., Maeda, Y., Nakagami, H. & Shinmyo, A. 1999 Isolation and characterization of the E2F-like gene in plants. *FEBS Lett.* **460**, 117–122.

Sewing, A., Bürger, C., Brüsselbach, S., Schalk, C., Lucibello, F. C. & Müller, R. 1993 Human cyclin D1 encodes a labile nuclear protein whose synthesis is directly induced by growth factors and suppressed by cyclic AMP. *J. Cell Sci.* **104**, 545–555.

Sherr, C. J. 1993 Mammalian G_1 cyclins. *Cell* **73**, 1059–1065.

Sherr, C. J. 1996 Cancer cell cycles. *Science* **274**, 1672–1677.

Soni, R., Carmichael, J. P., Shah, Z. H. & Murray, J. A. H. 1995 A family of cyclin D homologs from plants differentially controlled by growth regulators and containing the conserved retinoblastoma protein interaction motif. *Plant Cell* **7**, 85–103.

Sorrell, D. A., Combettes, B., Chaubet-Gigot, N., Gigot, C. & Murray, J. A. H. 1999 Distinct cyclin D genes show mitotic accumulation or constant levels of transcripts in tobacco bright yellow-2 cells. *Plant Physiol.* **119**, 343–352.

Sorrell, D. A. (and 10 others) 2001 Cell cycle regulation of cyclin-dependent kinases in tobacco cultivar bright yellow-2 cells. *Plant Physiol.* **126**, 1214–1223.

Stals, H., Casteels, P., Van Montagu, M. & Inze, D. 2000 Regulation of cyclin-dependent kinases in *Arabidopsis thaliana*. *Plant Mol. Biol.* **43**, 583–593.

Sun, Y., Dilkes, B. P., Zhang, C., Dante, R. A., Carneiro, N. P., Lowe, K. S., Jung, R., Gordon-Kamm, W. J. & 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.

Swaminathan, K., Yang, Y., Grotz, N., Campisi, L. & Jack, T. 2000 An enhancer trap line associated with a D-class cyclin gene in *Arabidopsis*. *Plant Physiol.* **124**, 1658–1667.

Szekely, L., Jiang, W. Q., Bulicjakus, F., Rosen, A., Ringertz, N., Klein, G. & Wiman, K. G. 1992 Cell type and differentiation dependent heterogeneity in retinoblastoma protein expression in SCID mouse fetuses. *Cell Growth Differ.* **3**, 149–156.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. 1997 The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25**, 4876–4882.

Vousden, K. H. 2000 p53: death star. *Cell* **103**, 691–694.

Wang, H., Fowke, L. C. & Crosby, W. L. 1997 A plant cyclin-dependent kinase inhibitor gene. *Nature* **386**, 451–452.

Wang, H., Qi, Q., Schorr, P., Cutler, A. J., Crosby, W. L. & Fowke, L. C. 1998 ICK1, a cyclin-dependent protein kinase inhibitor from *Arabidopsis thaliana* interacts with both Cdc2a and CycD3, and its expression is induced by abscisic acid. *Plant J.* **15**, 501–510.

Wang, H., Zhou, Y., Gilmer, S., Whitwill, S. & Fowke, L. C. 2000 Expression of the plant cyclin-dependent kinase inhibitor ICK1 affects cell division, plant growth and morphology. *Plant J.* **24**, 613–623.

Waterhouse, P. M., Graham, M. W. & Wang, M. B. 1998 Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. *Proc. Natl Acad. Sci. USA* **95**, 13 959–13 964.

Zacksenhaus, E., Jiang, Z., Chung, D., Marth, J. D., Phillips, R. A. & Gallie, B. L. 1996 pRb controls proliferation, differentiation, and death of skeletal muscle cells and other lineages during embryogenesis. *Genes Dev.* **10**, 3051–3064.

GLOSSARY

ABA: abscisic acid
CAK: CDK activating kinase
CDK: cyclin-dependent kinase
CHX: cycloheximide
CKI: CDK inhibitor protein
CycD: D-type cyclin
DP: dimerization protein
ELP: E2F-like protein
ICK: inhibitors of CDK
JA: jasmonic acid
KRP: Kip-related protein
M: mitosis
PEST: domain named after the single-letter abbreviations for proline, glutamic acid, serine and threonine
R: restriction
Rb: retinoblastoma tumour susceptibility
SAM: shoot apical meristem
SBF: Swi4–Swi6-dependent cell-cycle box binding factor
STM: *SHOOTMERISTEMLESS*
T164: threonine 164
ZmRb: Maize Rb