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BOTANICAL BRIEFING

Cell Cycle Control in *Arabidopsis*

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Although the basic mechanism of cell cycle control is conserved among eukaryotes, its regulation differs in each type of organism. Plants have unique developmental features that distinguish them from other eukaryotes. These include the absence of cell migration, the formation of organs throughout the entire life-span from specialized regions called meristems, and the potency of non-dividing cells to re-enter the cell cycle. The study of plant cell cycle control genes is expected to contribute to the understanding of these unique developmental phenomena. The principal regulators of the eukaryotic cell cycle, the cyclin-dependent kinases (CDKs) and cyclins, are conserved in plants. This review focuses on cell cycle regulation in the plant *Arabidopsis thaliana*. While expression of one *Arabidopsis* CDK gene, *Cdc2aAt*, was positively correlated with the competence of cells to divide, expression of a mitotic-like cyclin, *cyc1At*, was almost exclusively confined to dividing cells. The expression of the *Arabidopsis* δ-type cyclins appears to be an early stage in the response of plant cells to external and internal stimuli.

Key words: *Arabidopsis thaliana* (L.) Heynh., cell cycle, CDK, cyclin, plant development, plant hormone.

INTRODUCTION

Progression through the eukaryotic cell cycle is dependent upon specific serine/threonine protein kinases that associate with regulatory subunits called cyclins, and are therefore referred to as cyclin-dependent kinases (CDKs) (reviewed by Pines, 1994). The activity of the CDK-cyclin complexes is modulated by CDK phosphorylation and dephosphorylation, and by association of the complexes with inhibitory proteins termed CKIs. CDK and cyclin homologues have been identified in several plant species (reviewed by Jacobs, 1995), however, no CKIs or kinases and phosphatases that modify CDK have been identified so far in plants. This review deals with our present knowledge of cell cycle regulation in *Arabidopsis thaliana*, which is widely used as a model to study various developmental and molecular events in plants.

CLONING AND CLASSIFICATION OF THE *ARABIDOPSIS* CELL CYCLE GENES

The Arabidopsis *CDKs*

Two CDKs have been identified so far in *Arabidopsis* (Ferreira *et al*., 1991; Hirayama *et al*., 1991; Imajuku *et al*., 1992). These two proteins, nominated Cdc2aAt and Cdc2bAt, share 56% identity at the amino acid level, and are approximately 64 and 54% identical to other eukaryotic CDKs, respectively. Cdc2aAt possesses the motif PSTAIRE that is conserved among other eukaryotic CDK proteins, whereas this motif is modified to PPTALRE in the Cdc2bAt protein. The three residues Thr14, Tyr15, and threonine at position 161 or 167, whose phosphorylation and dephosphorylation in yeast and animals can modulate CDK activity at the G_2/M transition (Lewin, 1990), are conserved within Cdc2aAt. In Cdc2bAt, Thr14 and Tyr15 are conserved, and the other putatively phosphorylated threonine residue is located at position 169. It is currently not known whether any of these residues are phosphorylated *in vivo* in plants. The CDK's distinctive Ser277, phosphorylation of which peaks during the G_1 phase and drops on entering the S phase (Krek and Nigg, 1991), is not conserved in either Cdc2aAt or Cdc2bAt.

Cdc2aAt, but not Cdc2bAt, was able to partially complement yeast CDK mutants (Ferreira *et al*., 1991; Hirayama *et al*., 1991), demonstrating that this protein is a functional homologue of yeast CDKs. Distinct CDK homologues from alfalfa (*Medicago sativa*), maize (*Zea mays*), rice (*Oryza sativa*), and soybean (*Glycine max* L.) were also able to partially complement yeast CDK mutants (Hirt *et al*., 1991; Colasanti, Tyers and Sundaresan, 1991; Hashimoto *et al*., 1992; Miao, Hong and Verma, 1993). However, similar to human and *Drosophila* CDKs, the plant homologues were not able to completely restore the wild-type yeast phenotype.

The Arabidopsis *cyclins*

All the cyclins share a highly homologous region of approximately 150 amino acids, termed the cyclin box (Nugent *et al*., 1991), that is required for their interaction with the CDK catalytic subunit (Lees and Harlow, 1993). Using PCR and oligonucleotides corresponding to this conserved region, Hemerly *et al*. (1992) and Ferreira *et al*. (1994*a*) have identified five cyclin genes in *Arabidopsis*, that

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were nominated *cyc1At*, *cyc2aAt*, *cyc2bAt*, *cyc3aAt* and *cyc3bAt*. The deduced amino acid sequence of each pair of genes within class 2 or 3 is approximately 74% identical. Cyclins are classified into different groups according to sequence similarity and timing of expression during the cell cycle. Sequence analyses indicated that these *Arabidopsis* cyclins show highest similarity to either the A or B group of animal mitotic cyclins, but cannot be exclusively assigned to either class. In fact, plant mitotic-like cyclins are more related to each other than to animal or yeast cyclins, and can be grouped into three different classes, of which one is slightly more homologous to animal A-type cyclins, while the other two are more related to animal B-type cyclins (Ferreira *et al*., 1994*a*; Renaudin *et al*., 1994).

In animals, both A- and B-type cyclins are required for entry into mitosis, and A-type cyclins also play a role during the S phase (reviewed by King, Jackson and Kirschner, 1994). *cyc1At* was able to induce maturation of *Xenopus* oocytes (Hemerly *et al*., 1992), indicating that it is a functional homologue of animal mitotic cyclins (functional tests were not performed for the other *Arabidopsis* mitotic-like cyclins). Among the other cloned plant cyclins, an ability to induce maturation of *Xenopus* oocytes has also been reported for soybean and maize cyclins (Hata *et al*., 1991; Renaudin *et al*., 1994).

The oscillatory appearance of cyclins during the cell cycle is partly regulated at the level of protein stability. Animal A- and B-type cyclins contain a conserved sequence termed the 'destruction box' near their amino terminus, which mediates their ubiquitin-dependent proteolysis during anaphase (Glotzer, Murray and Kirschner, 1991). PEST sequences found in many, but not all, G_1 cyclins, are responsible for their rapid turn-over rate (reviewed in Reed, 1991). Motifs that resemble the mitotic destruction box are located near the amino terminus of the *Arabidopsis* cyclin 1 and 2 classes. The cyclin 2 class also contains PEST sequences, that could not be identified in any of the other two classes.

The human G_1 cyclins that belong to the C, D, and E groups were identified by their ability to rescue yeast G_1 cyclin mutants (Lew, Dulić, and Reed, 1991). Soni *et al*. (1995) used a similar strategy in *Arabidopsis*, and identified a fourth class of plant cyclins, nominated δ-type. These cyclins exhibit highest similarity to mammalian D-type cyclins, which are implicated in the exit from the quiescent state (Quelle *et al*., 1993). Each of the three identified *Arabidopsis* δ-type cyclins shares approximately 30% sequence identity with the other two. Several potential PEST sequences were identified in these cyclins. Mammalian D-type cyclins contain an N-terminal sequence motif LXCXE that was originally identified in certain viral oncoproteins, and is strongly implicated in binding to the retinoblastoma protein pRb (reviewed in Wiman, 1993). All the identified *Arabidopsis* δ-type cyclins contain such an N-terminal LXCXE motif, that could not be identified in any other cloned plant cyclin. This suggests that pRb homologues, known to be important regulators of cell proliferation and differentiation but previously identified only in mammals, may be present in plants.

Day and Reddy (1994) obtained PCR fragments of three additional *Arabidopsis* cyclins. The resulting clones have only been sequenced over the cyclin box regions, and classification of these cyclins awaits further sequence data of full-length clones.

DEVELOPMENTAL REGULATION OF THE *ARABIDOPSIS* CDKs AND CYCLINS EXPRESSION

Spatial and temporal regulation

cdc2aAt transcript was identified, in decreasing order of abundance, in roots, stem, callus, flower, and leaf of mature *Arabidopsis* plants (Ferreira *et al*., 1991). The expression of the *cdc2aAt* gene during plant development was studied by *in situ* hybridization (Martinez *et al*., 1992; Hemerly *et al*., 1993) and in transgenic *Arabidopsis* plants transformed with the *cdc2aAt* promoter fused to the β-glucuronidase (*gus*) reporter gene (*uidA* from *Escherichia coli*) (Hemerly *et al*., 1993). The expression pattern observed was similar (see below), suggesting that *cdc2aAt* mRNA abundance is regulated at the transcriptional level. *cdc2aAt* expression was observed in the shoot and root apical meristems. High levels of expression were found in developing first leaves and in the root–shoot junction, from which adventitious roots initiate. Fully expanded leaves exhibited an almost undetectable level of expression. *cdc2aAt* expression was temporally delayed in the shoot apical meristem of etiolated seedlings compared to light-grown plants. This meristem does not divide in etiolated seedlings, indicating that the activation of *cdc2aAt* expression might correlate with the acquisition of the competence to divide rather than with actual cell division. *cdc2aAt* expression was also found in lateral root meristems, the parenchyma of the vascular cylinder, and in the pericycle cells (Fig. 1A). The pericycle is a differentiated tissue surrounding the vascular cylinder, which retains a potential to divide, and is responsible for lateral root formation and root thickening. Expression in pericycle cells was low in older parts of the root. A correlation between the abundance of the CDK transcript and the proliferative state of the tissue has also been demonstrated in maize and alfalfa (Colasanti *et al*., 1991; Hirt *et al*., 1991).

Expression of one of the *Arabidopsis* mitotic-like cyclins, namely *cyc1At*, was studied in detail by whole-mount *in situ* hybridization and in transgenic *Arabidopsis* plants expressing the *cyc1At* promoter fused to *gus* (Ferreira *et al*., 1994*a*, *b*). It was found that this cyclin is expressed primarily in the shoot and root apical meristems. Neither *cdc2aAt* nor *cyc1At* were expressed in the root cap or the root quiescent centre, which have low mitotic activity. Similarly, in the *Arabidopsis* shoot apical meristem, and during flower and embryo formation, expression of both genes corresponds to the pattern of [^{3}H]-thymidine incorporation. Expression of *cyc1At* could not be detected in the shoot apical meristem of etiolated seedlings, that does not divide. Expression of *cyc1At* in the root pericycle cells was confined to zones of lateral root initiation (Fig. 1B), even before morphological changes were noticed.

Thus, in contrast to *cdc2aAt*, which was expressed also in tissues with a high potential to divide, such as the shoot apical meristem of etiolated seedlings and the root pericycle

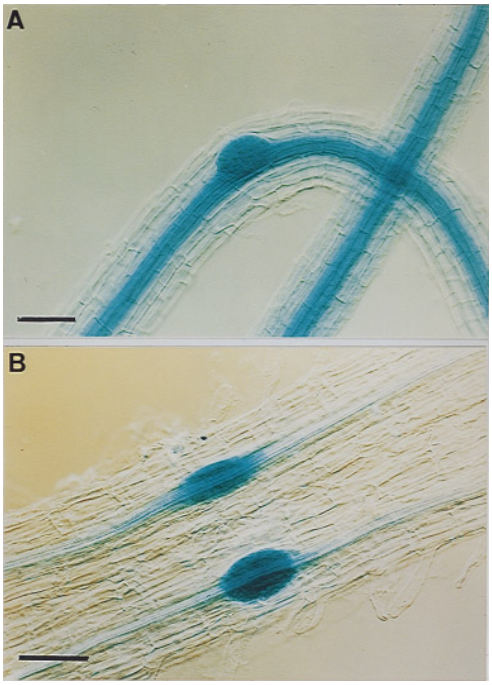

FIG. 1. Histochemical analyses of *gus* expression in roots of transgenic *Arabidopsis* plants, expressing either the *cdc2aAt* promoter-*gus* fusion (A) or the *cyc1At* promoter-*gus* fusion (B). While the *cdc2aAt* promoter drives relatively uniform *gus* expression in the pericycle, the *cyc1At* promoter-related expression in the pericycle is confined to the zones of lateral root initiation. Bars = 100 μm.

cells, *cyc1At* expression seems to be restricted to dividing cells. Furthermore, in wounded *Arabidopsis* leaves, *cdc2aAt* expression was induced in less than 30 min around damaged surfaces, although no significant cell proliferation had occurred there. This induction may represent an increased division competence within the cells in this area. *cyc1At* expression could not be observed in such wounded areas.

Transcripts of the *Arabidopsis* δ-type cyclins were identified in all *Arabidopsis* tissues with differing abundance (Soni *et al.*, 1995). The expression of these cyclins was analysed in detail in *Arabidopsis* cell suspension cultures (see below).

Regulation by plant hormones

Cell cycle genes are candidates to play a role in mediating the effect of various growth regulators. When roots of *Arabidopsis* plants transformed with the *cdc2aAt* promoter-*gus* construct (Hemerly *et al.*, 1993) were treated with the auxin indole-3-acetic acid (IAA), which increased the frequency of lateral root initiation, no change was observed in the spatial specificity of expression in the pericycle and new initials. After treatment with cytokinins, increased GUS activity was detected in the pericycle and parenchyma cells in the upper part of the main root. [^{3}H]-thymidine incorporation in cells with induced GUS activity indicated that this was correlated with DNA synthesis. *Gus* expression in *Arabidopsis* plants transformed with the *cyc1At* promoter-*gus* construct were also analysed during auxin-induced lateral root formation (Ferreira *et al.*, 1994*b*). *De novo gus* expression was observed in sites where new root primordia were being formed.

Plant hormones are required for de-differentiation and induction of cell division in leaf mesophyll protoplasts. When such protoplasts, derived from tobacco plants expressing the *cdc2aAt* promoter-*gus* fusion (Hemerly *et al.*, 1993) were treated with the appropriate concentrations of auxin and cytokinin to stimulate division, an induction of GUS activity was detected. Cultivation in the presence of either auxin or cytokinin alone was insufficient to induce cell division, but nevertheless cytokinin alone, and to a lesser extent auxin alone, induced *cdc2aAt* expression. Thus, the induction by these hormones of the competence to divide was accompanied by *cdc2aAt* expression, even in the lack of actual divisions. In contrast, in similar protoplasts derived from tobacco plants expressing the *cyc1At* promoter-*gus* fusion (Ferreira *et al.*, 1994*b*), *gus* expression was induced only by treatment with the appropriate combination of auxin and cytokinin that allowed cell division. These data imply that when quiescent plant cells are reinduced to divide by plant hormones, *cdc2aAt* and *cyc1At* transcription is activated. Chung and Parish (1995) have suggested the presence of several putative hormone-responsive elements and transcription factor binding-sites within the *cdc2aAt* promoter.

The effect of plant hormones on the expression of the δ-type cyclins was studied when *Arabidopsis* cell suspension cultures that were previously depleted of the required hormones (auxin and cytokinin) and carbon source (sucrose), were resupplemented with various combinations of these compounds (Soni *et al.*, 1995). Cyclin δ3 expression was induced by cytokinin alone, and even more strongly by cytokinin plus sucrose, although the absence of auxin did not allow these cells to enter the S phase. The presence of auxin reduced the extent of this induction. In contrast, induction of the cyclin δ2 transcript was solely dependent on sucrose resupplementation. Thus it seems that in contrast to the mitotic-like cyclin *cyc1At*, expression of the *Arabidopsis* δ-type cyclins is not restricted to dividing cells, and therefore may be an early stage in the response of plant cells to mitogenic stimuli, similar to the function of mammalian D-type cyclins.

Cell-cycle regulation

Whereas in yeast the levels of CDK mRNA and protein are constant during the cell cycle (Durkacz, Carr and Nurse, 1986), in animal cells their levels fluctuate (McGowan, Russell and Reed, 1990). Arrest of *Arabidopsis* cell suspension cultures in early S phase or metaphase using hydroxyurea or colchicine, respectively, caused only a small decrease in *cdc2aAt* transcript level (Hemerly *et al.*, 1993), indicating that this gene is expressed at similar levels in both phases. Double-target *in situ* hybridizations have demonstrated that expression of *cdc2aAt* does not fluctuate during the cell cycle (Fobert *et al.*, 1994). A similar conclusion was drawn for alfalfa (Magyar *et al.*, 1993). When leaf mesophyll protoplasts derived from tobacco plants expressing the

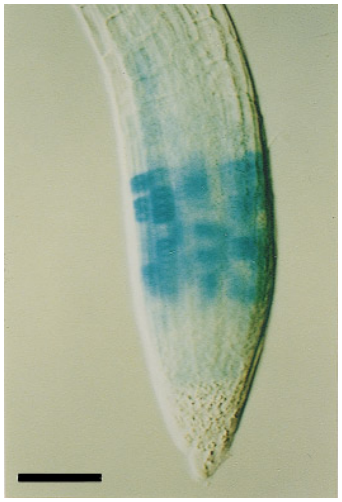

FIG. 2. Patchy pattern of *gus* expression in the root apical meristem of transgenic *Arabidopsis* plants expressing the *cyc1At* promoter-*gus* fusion. Bar = 100 μm.

cdc2aAt promoter–*gus* fusion were reinduced to divide by plant hormones, *gus* induction could not be blocked by hydroxyurea. Thus it seems that in quiescent cells, induction of divisions by hormones activate *cdc2aAt* transcription at or before early S phase, and that this activation is not dependent upon progression through the cell cycle.

Cyclin levels in eukaryotes are known to oscillate during the cell cycle. In agreement with this, and in contrast to the observation with the *cdc2aAt* gene, the steady-state transcript levels of *Arabidopsis* mitotic-like cyclin genes were found to vary between distinct cell cycle phases (Ferreira *et al*., 1994*a*). Whole-mount *in situ* hybridization of *Arabidopsis* main root tips treated with oryzalin, which inhibits microtubule polymerization and arrests cells at metaphase, revealed accumulation of the *cyc1At* transcript, whereas transcripts of the class 2 and 3 cyclins were barely visible. In contrast, treatment with hydroxyurea, which blocks DNA replication and arrest cells at early S phase, led to elimination of the *cyc1At* transcript, whereas the transcript levels of the class 2 and 3 cyclins were similar to untreated plants. Similar results were observed in the main root tips of plants transformed with the *cyc1At* promoter fused to *gus* (Ferreira *et al*., 1994*b*). The auxin induction of *gus* expression in founder cells of lateral roots was not blocked by pre-treatment with oryzalin, which blocked cell division, indicating that induction of *cyc1At* precedes metaphase and completion of the first round of division. In contrast, hydroxyurea pre-treatment eliminated *gus* induction, suggesting that *cyc1At* is induced after early S phase. Hybridization with the *cyc1At* probe of nuclei derived from *Arabidopsis* cell suspension cultures, which were sorted by flow cytometry on the basis of DNA content, has indicated that this gene is expressed in cells with double DNA content. Altogether, these data clearly indicate that *cyc1At* is induced between early G_2 and metaphase. The class 2 and 3 cyclins seem to be expressed earlier than *cyc1At* during the cell cycle. The root apical meristems of plants transformed with the *cyc1At* promoter fused to *gus* show a patchy pattern of *gus* expression—some cells have a more intense staining than their neighbours, presumably due to their being at the cell-cycle stage at which *cyc1At* is expressed (Fig. 2). Similar observations were made in *Antirrhinum* and soybean (Fobert *et al*., 1994; Kouchi, Sekine and Hata, 1995). Such patchy pattern was not observed in plants expressing the *cdc2aAt–gus* fusion.

To determine the timing of expression of the *Arabidopsis* δ-type cyclins during the cell cycle, *Arabidopsis* cell suspension cultures were treated with hydroxyurea (Soni *et al*., 1995). Upon release from this block, induction of δ3 cyclin slightly preceded the induction of histone H4 gene expression in the S phase, and remained high until the end of the experiment at the end of the S phase. During this time there was no significant induction of the *cyc1At* gene. Because transcript levels of the δ1 and δ2 cyclins were low in either hydroxyurea- or colchicine-treated cells, and remained low after release from the hydroxyurea block, it was suggested that these cyclins are expressed during the G_1 phase, before the point at which hydroxyurea blocks cell cycle progression.

The above data suggest that, similar to other eukaryotes, the expression of the various *Arabidopsis* cyclins is regulated during the cell cycle, and that they may fulfil different functions. Differential expression during the cell cycle was also demonstrated for alfalfa, *Antirrhinum*, and soybean cyclins (Hirt *at al*., 1992; Fobert *et al*., 1994; Kouchi *et al*., 1995).

A model for the concerted operation of the Arabidopsis *cell cycle genes*

In higher eukaryotes CDK expression correlates positively with cell proliferation (Lee *et al*., 1988; Krek and Nigg, 1989). The study of the *Arabidopsis cdc2aAt* gene demonstrated that this gene is mainly expressed in proliferating tissues, suggesting the involvement of a long-term developmental transcriptional control. However, *cdc2aAt* expression was not restricted to dividing cells. Its expression could also be found in non-dividing cells with a high competence to divide, such as cytokinin-treated leaf protoplasts, root pericycle cells, shoot apical meristems of etiolated seedlings, and wounded areas. These data indicate that *cdc2aAt* expression is involved in determining the competence for proliferation, but additional signals are required for cell division to occur. One possible signal could be the presence of an activating cyclin subunit. Consistent with this possibility, it was found that in contrast to *cdc2aAt*, the *Arabidopsis cyc1At* cyclin gene is almost exclusively expressed in actively dividing cells. This can imply that the control of cell proliferation in some developmental programs may involve transcriptional regulation of this cyclin, and possibly of other cyclins. In plant cells that are arrested at the G_0 or G_1 phase, commitment to a new round of division is probably occurring before the cell cycle phase at which mitotic cyclins such as *cyc1At* are expressed. The *Arabidopsis* δ-type cyclins are candidates to play a role in such commitment, and in coupling plant mitogenic stimuli and cell proliferation. Furthermore, it

seems that in contrast to *cyc1At*, expression of the δ-type cyclins is not restricted to dividing cells and, therefore, may be an earlier stage in the response of plant cells to external and internal stimuli. However, cyclin expression is almost certainly not the sole factor in determining cell division.

PERSPECTIVE

Most of our present knowledge about cell cycle regulation in *Arabidopsis* is concerned with the transcription regulation of representative CDK and cyclin genes, namely *cdc2aAt*, *cyc1At*, and the δ-type cyclins. Expression analysis of other *Arabidopsis* cell cycle genes is currently going on in our laboratory. However, it is well documented that, at least in yeast and animals, cell cycle progression is to a great extent regulated at the post-transcriptional level. CDK undergoes both activating and inhibitory phosphorylation and dephosphorylation events, requiring specific upstream kinases and phosphatases. Some of the amino acids in CDK that are phosphorylated in yeast and animals are also conserved in plants; however, there is no evidence that these residues are actually undergoing any modification, and no kinases or phosphatases that modify CDK have been identified so far in plants. It is also not known whether plants, similar to other eukaryotes, possess cell cycle inhibitors (CKIs). The activity of CDKs is also dependent on association with cyclins, whose abundance is partially regulated at the level of protein stability. Despite the identification of potential destruction boxes and PEST motifs in plant cyclin sequences, it is still not known whether they influence the protein turnover during the cell cycle. It is also essential to demonstrate which *Arabidopsis* CDK interacts with which cyclin during which point of the cell cycle. This interaction was studied in yeast and animals (van den Heuvel and Harlow, 1993), and is thought to determine the sub-cellular localization and substrate specificity of the CDK/cyclin complexes. Until now, partial characterization of plant phase-specific CDK complexes has only been performed in alfalfa (Magyar *et al.*, 1993). Finally, it is essential to identify putative substrates of activated CDK/cyclin complexes in plants. Co-localization of CDK with the preprophase band of microtubules, which predicts the future division site, was demonstrated in onion and maize, but a causal relationship has not been established (Mineyuki, Yamashita and Nagahama, 1991; Colasanti *et al.*, 1993).

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