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Developmental Expression of the Arabidopsis Cyclin Gene *cyc1At*

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In eukaryotes, the control of cell cycle progression is exercised by heteromeric protein kinase complexes composed of a cell cycle–dependent, kinase-related subunit (Cdc2) and a cyclin subunit. To explore the possibility that cyclin transcription plays a role in the developmental regulation of cell division, we examined the spatial and temporal expression of a cyclin gene (*cyc1At*) in Arabidopsis. In root and shoot apical meristems and during embryogenesis, *cyc1At* expression is almost exclusively confined to dividing cells. A cell-specific pattern of *cyc1At* expression was noticed in root meristems. We examined the effects of induction of cell division of differentiated cells on *cyc1At* expression. During lateral root formation, induction of *cyc1At* expression is a very early event and was detected before anatomical modifications were visible. Treatment of roots with oryzalin, which blocks cell division in metaphase, did not inhibit the auxin induction of *cyc1At*, suggesting that induction of *cyc1At* expression precedes the completion of the first division cycle after induction of lateral roots. In tobacco protoplasts, an increase in *cyc1At* expression was observed only when cell division was induced. Together, the results suggest that Cyc1At accumulation in Arabidopsis is transcriptionally regulated and might be one of the limiting factors for the activation of cell division.

INTRODUCTION

Cyclins constitute a group of related proteins that govern cell cycle progression in eukaryotes (reviewed in Pines and Hunter, 1991; Lew and Reed, 1992; Minshull, 1993; Sherr, 1993). First described as proteins that accumulate periodically during the early cleavage cycles of marine invertebrates (Evans et al., 1983), cyclins were next identified in eukaryotic species that are evolutionarily as distant as animals, yeast, and plants (Swenson et al., 1986; Minshull et al., 1989, 1990; Pines and Hunter, 1989; Richardson et al., 1989; Hata et al., 1991; Surana et al., 1991; Hemerly et al., 1992; Hirt et al., 1992; Fobert et al., 1994; Renaudin et al., 1994). It has been shown that the onset of events that regulate the cell cycle is determined by the activation of a series of kinase complexes that are cyclin–cyclin dependent. Cyclin-dependent kinases, the catalytic subunits of the kinase, are a set of serine/threonine protein kinases, homologous to the *Schizosaccharomyces pombe* cell cycle–dependent *cdc2*$^+$ gene product (Nurse and Bissett, 1981). Whereas levels of cyclin-dependent kinases do not oscillate during the cell cycle, periodic synthesis and degradation of cyclins determine, to a great extent, the periodic activation of the kinase.

Cyclin accumulation can be regulated at several levels. Transcription, translation control, and protein degradation all play a part in determining the amount of cyclin protein. In the embryonic cycles of marine invertebrates, cyclin levels and cell cycle progression are determined by cycles of translation of prestored mRNAs and protein degradation (Swenson et al., 1986; Pines and Hunt, 1987; Murray and Kirschner, 1989; Westendorf et al., 1989). In the first 13 syncytial cycles of Drosophila development, cyclins A, B, C, and E are translated from maternally derived transcripts (Lehner and O'Farrell, 1990; Whitfield et al., 1990; Léopold and O'Farrell, 1991; Richardson et al., 1993), and transcription starts in the 14th cycle. Nevertheless, fluctuations in cyclin protein levels are generally paralleled by oscillations of the correspondent mRNAs (Pines and Hunter, 1989; Lew et al., 1991), suggesting that transcriptional control may play an important role in governing cell cycle progression. In yeast, regulation of cyclin transcription is crucial for cell cycle progression (reviewed in Johnston, 1992; Nasmyth, 1993). Kinase activation at the G_1-to-S transition is dependent on transcription stimulation of the cyclin genes *CLN1* and *CLN2* (Nasmyth and Dirick, 1991; Ogas et al., 1991; Primig et al., 1992). There is also evidence that G_2 cyclins transcriptionally activate mitotic cyclins and repress G_1 cyclins (Amon et al., 1993).

Although the basic eukaryotic cell cycle machinery is conserved, the cell cycle regulators might respond to different controls. The existence of a variable cell cycle control may

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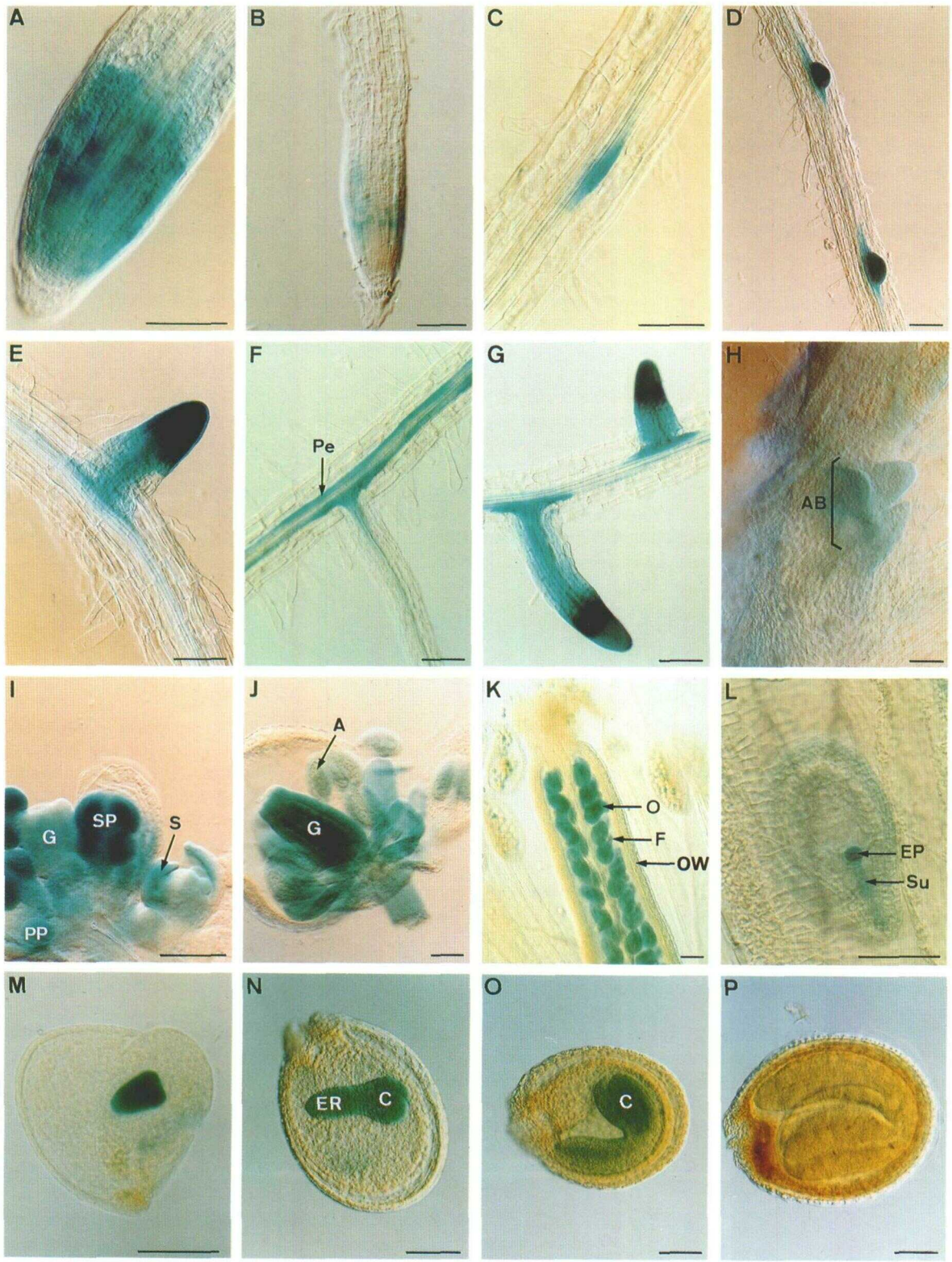

Figure 1. *cyc1At* Expression: Whole-Mount Histochemical Localization of GUS Activity.

be important to regulate different developmental programs inside the organisms. Plant developmental programs are distinct from those of animals (Steeves and Sussex, 1989; Lyndon, 1990). The particular developmental strategies of plants may have consequences for the regulation of cell division. Plant embryogenesis, in contrast to embryogenesis in animals, establishes only the basic blueprint of the organism. Most plant morphogenesis is achieved by continual iterative development at the apical meristems. Thus, throughout plant life, developmental controls must be perfectly integrated with the mechanisms regulating differential mitotic activity at the meristems to construct highly ordered structures. In contrast, animal morphogenesis is completed during embryo formation. Subsequent development consists simply of the renewal of the existing tissues.

Developmental studies have already suggested that the fundamental cell cycle apparatus is adaptable, and consequently, distinct strategies of cell cycle control can be used during development. Hence, one possibility is that in a particular developmental pathway most of the regulatory components will not be limiting and that the timing of cell division would be determined by the transcription of one rate-limiting factor (O'Farrell, 1992). In Drosophila, transcriptional regulation of *string*, the fly homolog of the *S. pombe cdc25*$^{+}$ phosphatase gene (Russell and Nurse, 1986; Dunphy and Kumagai, 1991), governs the pattern of cell division during early embryo development (Edgar and O'Farrell, 1989). In contrast, cyclins are the rate-limiting factor governing the first mitotic cycles in frog embryos (Murray and Kirschner, 1989).

In plants, previous analyses have shown that *cdc2a* expression is not always correlated with mitotic activity but rather with competence to divide (Martinez et al., 1992; Hemerly et al., 1993). Hence, transcription of *cdc2a* is most likely not rate determining for cell division during plant development. In contrast, RNA gel blot analysis revealed that steady state mRNA levels of a mitotic cyclin from Arabidopsis (Cyc1At) correlate with mitotic activity of the tissues (Hemerly et al., 1992). To investigate whether *cyc1At* transcription could be involved in the developmental control of cell division, we examined its spatial and temporal expression patterns in dividing cells. Our data suggest that Cyc1At accumulation in Arabidopsis is transcriptionally regulated. In addition, in almost all circumstances examined, *cyc1At* expression is confined to actively dividing meristematic regions. The results suggest that *cyc1At* transcriptional regulation might be one of the rate-limiting factors for the activation of cell division in some developmental programs.

RESULTS

Analysis of *cyc1At* Expression during Development

RNA gel blot analysis showed that the expression of the Arabidopsis *cyc1At* gene is positively correlated with cell proliferation, suggesting that it could be restricted to zones of active cell division (Hemerly et al., 1992). To gain further insight into the regulation of cyclin expression in plants, the promoter of *cyc1At* was isolated, fused to the β-glucuronidase reporter gene (*gus; uidA* from *Escherichia coli*), and introduced into Arabidopsis and tobacco plants. Preliminary inspection of the patterns of GUS activity in several transformants showed that *cyc1At* expression is indeed confined to dividing cells. The *cyc1At* promoter–*gus* chimeric gene in tobacco exhibited a developmental regulation similar to that in Arabidopsis (data not shown). Hence, detailed analyses of *cyc1At* expression during plant development were performed; these analyses focused on actively dividing tissues, such as meristems, and embryo

Figure 1. (continued).

(A) Root meristem from a 7-day-old seedling.
(B) Root meristem from a 3-week-old plant.
(C) Initiation of a lateral root.
(D) Developing lateral roots.
(E) Emerged lateral root.
(F) Root treated with 10^{-6} M indole-3-acetic acid.
(G) Lateral roots treated with 10^{-6} M indole-3-acetic acid.
(H) Tobacco axillary bud.
(I) Inflorescence.
(J) Flower bud.
(K) Flower at anthesis.
(L) Embryo at globular stage.
(M) Embryo at triangular stage.
(N) Embryo at late-heart/early-torpedo stage.
(O) Embryo at late-torpedo stage.
(P) Mature embryo.

Micrographs were taken using differential interference contrast optics of plant material cleared with lactophenol (Beeckman and Engler, 1994). A, anther; AB, axillary bud; C, cotyledon; EP, embryo proper; ER, embryonic root; F, funiculus; G, gynoecium; O, ovule; OW, ovary wall; Pe, pericycle; PP, petal primordium; S, sepal; SP, stamen primordium; Su, suspensor. Bars = 100 μm.

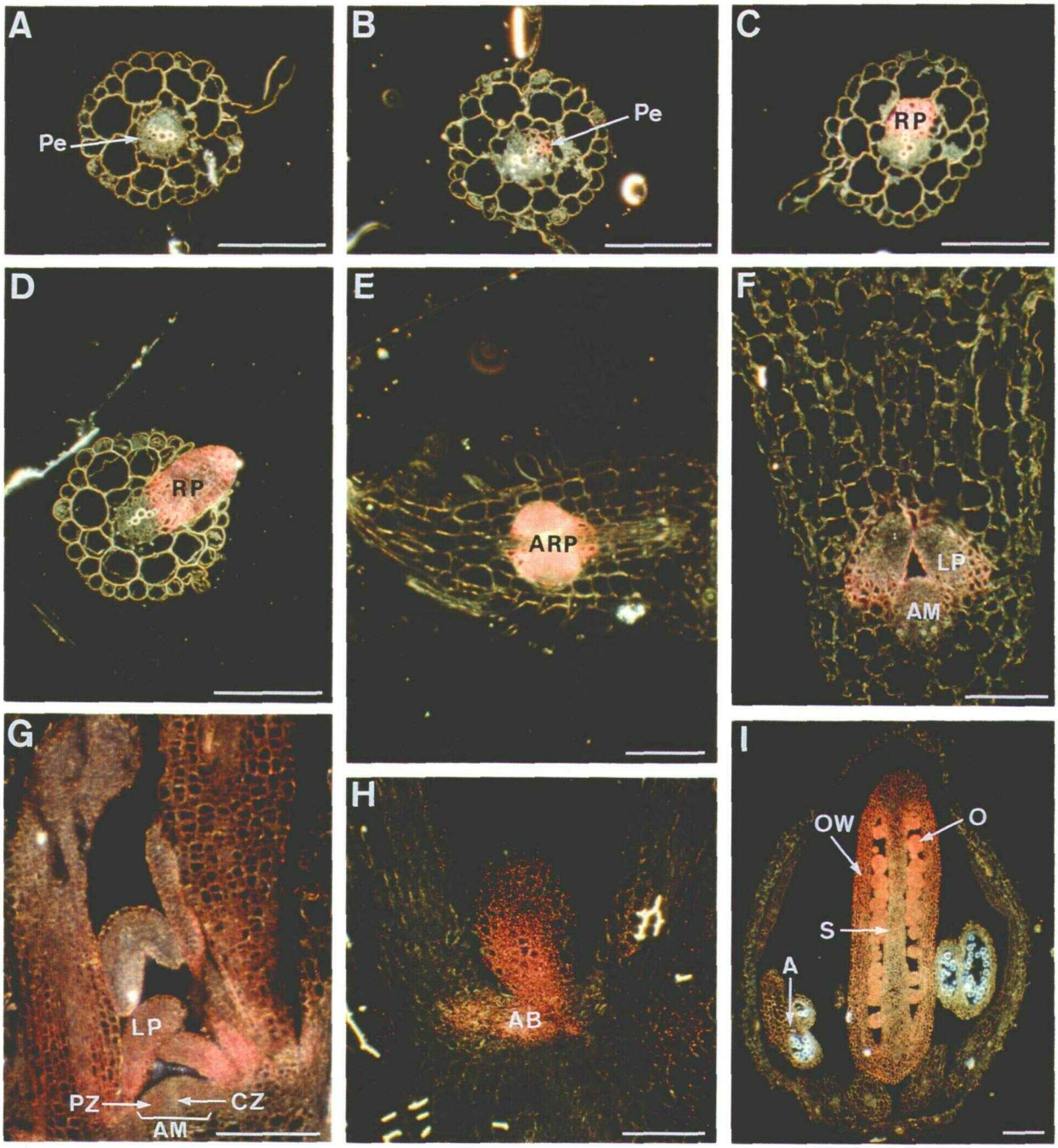

Figure 2. *cyc1At* Expression: Histochemical Localization of GUS Activity in Tissue Sections.

(A) Transverse section through a 2-week-old root.
(B) to **(D)** Transverse sections through a root at different stages of lateral root formation.
(E) Transverse section through an adventitious root.
(F) Longitudinal section through an apical meristem of a 3-day-old seedling.
(G) Longitudinal section through an apical meristem of a 12-day-old plant.
(H) Longitudinal section through an axillary bud.
(I) Longitudinal section through a flower before anthesis.
Micrographs were taken using dark-field optics. A, anther; AB, axillary bud; AM, apical meristem; ARP, adventitious root primordium; CZ, central zone; LP, leaf primordium; O, ovule; OW, ovary wall; Pe, pericycle; PZ, peripheral zone; RP, root primordium; S, septum. Bars = 100 μm.

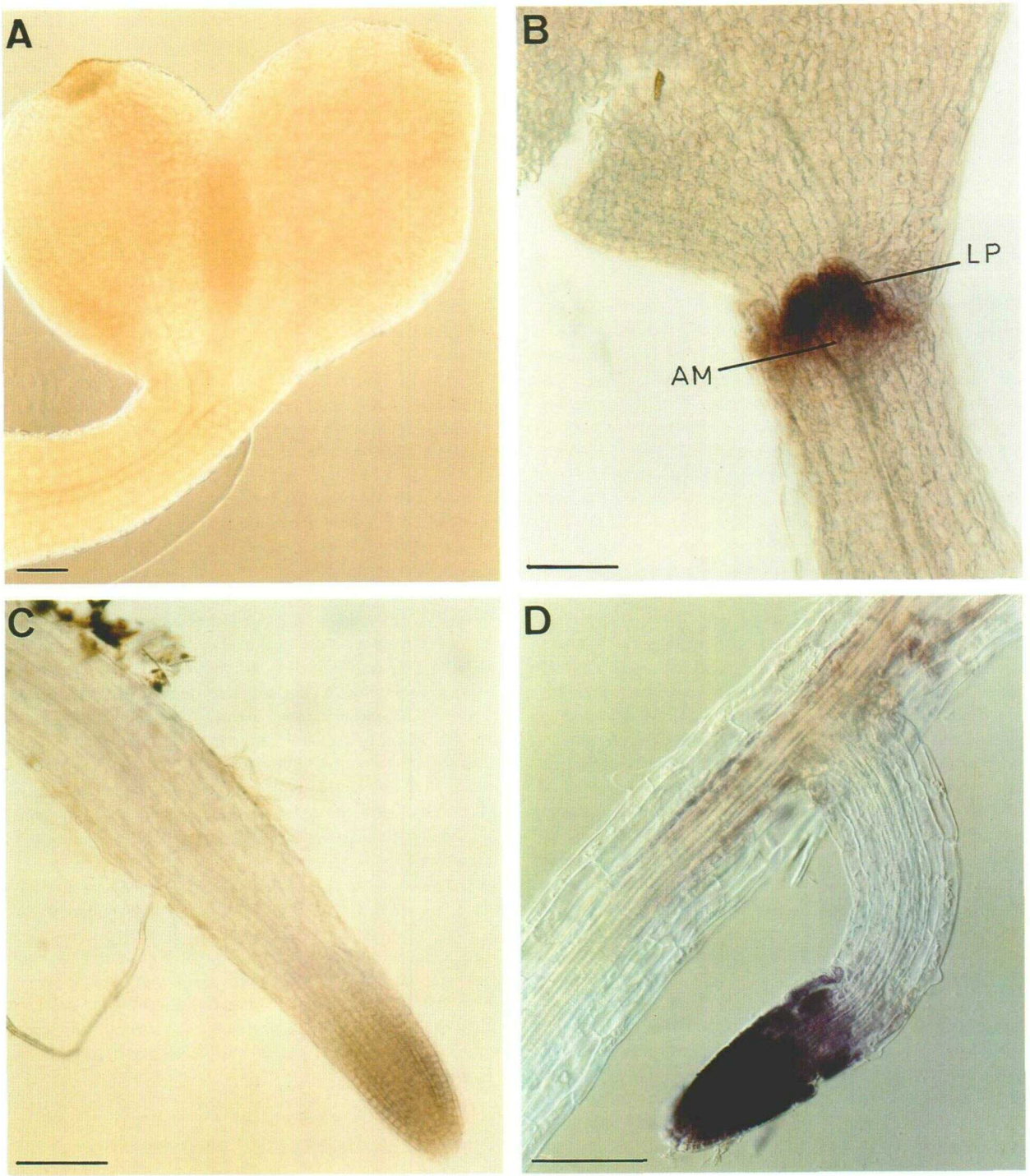

Figure 3. Whole-Mount in Situ Hybridizations in the Apical Shoot Meristem and Lateral Roots.

(A) Shoot apical meristem hybridized with the *cyc1At* sense probe.
(B) Shoot apical meristem hybridized with the *cyc1At* antisense probe.
(C) Lateral root hybridized with the *cyc1At* sense probe.
(D) Lateral root hybridized with the *cyc1At* antisense probe.
Micrographs in **(A)**, **(B)**, and **(C)** were taken using bright-field optics, and differential interference contrast optics were used for **(D)**. Dark stain represents RNA–RNA hybrids detected by alkaline phosphatase–labeled antibodies. AM, apical meristem; LP, leaf primordium. Bars = 100 μm.

formation. Figures 1 and 2 show the spatial and temporal patterns of *cyc1At* expression during plant development as revealed by histochemical GUS analyses. In addition, Figure 3 presents whole-mount in situ hybridization experiments using RNA probes generated from the *cyc1At* cDNA clone. These hybridizations were performed to ensure that the cloned *cyc1At* promoter region had all the necessary regulatory sequences to drive the accurate expression of *cyc1At*.

cyc1At Expression in Roots

During seed germination, as the radicle emerges, GUS activity was first detected in the root tip (data not shown). As the primary root grows, a cell-specific pattern of *cyc1At* expression was observed in the meristematic zone. Particular meristematic cells expressed *cyc1At* more strongly than other neighboring cells. This resulted in a patchy staining pattern (Figure 1A). In a more mature root, the proportion of cells exhibiting GUS activity decreased (Figure 1B), whereas GUS activity was absent from the quiescent center and the root cap of the primary roots (Figures 1A and 1B).

Lateral roots in higher plants originate from cells of the pericycle, opposite to the xylem poles. Pericycle cells seem to be partially differentiated (Esau, 1977). Figure 2A shows a transverse section through a mature region of the root where no GUS activity is found in pericycle cells prior to the initiation of lateral roots. Figure 1C shows that GUS activity is induced soon after lateral root initiation in the pericycle. Transverse sections of a primary root at the early stages of lateral root formation revealed that very few adjacent cells exhibit GUS activity, even before visible anatomical modifications of the pericycle can be observed (Figure 2B). Once a new meristem was formed, uniformly strong GUS staining was observed until the root primordium emerged (Figures 1D, 2C, and 2D). Figure 3D presents a whole-mount in situ hybridization confirming that *cyc1At* is strongly expressed in the apices of the newly formed lateral root. Expression was also visible in the vascular tissues, corresponding to cell divisions that will generate the phloem and xylem tissues and will connect the lateral root to the parent root (Esau, 1977; Figures 1E and 3D). Following further lateral root growth, a cell-specific pattern, similar to that observed in primary roots, developed (data not shown).

In a 4-day-old seedling, strong GUS activity was observed in a pair of symmetric adventitious roots formed in the transition zone between the primary root and the aerial part (Figure 2E). Two to 3 weeks after germination, weak and transient GUS activity was observed in the pericycle of the main root; these activities could be correlated with primary thickening (data not shown).

cyc1At Expression in Vegetative Apices and Floral Meristems and during Embryogenesis

In shoot apices, the localization and intensity of *cyc1At* expression were closely correlated with mitotic activity. GUS activity was detected in the bases of the first leaf primordia and uniformly throughout the shoot meristem (Figure 2F). A similar pattern of *cyc1At* expression was observed in whole-mount in situ hybridizations performed with 4-day-old seedlings (Figure 3B). Frequently, very little GUS activity was observed throughout the cotyledons, indicating that limited cell divisions occur after germination (data not shown). At later stages, strong GUS activity was continuously detected in the base of the leaf primordia, but in the meristem a more complex pattern of *cyc1At* expression developed. This pattern of expression correlated positively with the frequency of cell divisions. In the central zone of the apical meristem, where cells divide more slowly (Medford, 1992), GUS activity was very weak (Figure 2G). In the peripheral zone, where cells are actively dividing, GUS activity was stronger (Figure 2G).

Figure 2H shows that *cyc1At* is strongly expressed in axillary buds. In Arabidopsis, axillary buds in the inflorescence consist of actively dividing cells that continuously generate flowers or new inflorescences. To determine the status of *cyc1At* expression in dormant axillary buds, tobacco plants transformed with the chimeric *cyc1At* promoter–*gus* fusion were analyzed. Figure 1H shows that there is weak GUS activity in axillary buds, although cell division most likely is not occurring.

During the early stages of flower formation, weak GUS activity was observed throughout the meristem but mainly in the sepals (Figure 1I). After organ determination, an increase in GUS activity was first detected in the primordia of stamens and petals (Figure 1I). At a later stage, strong GUS activity was observed mainly in the gynoecium (Figure 1J). Before anthesis, GUS activity was restricted mainly to the gynoecium and was especially strong in the ovules and somewhat weaker in the septum and ovary wall (Figure 2I). Very weak GUS activity was found in the anthers (Figure 2I). During and immediately after pollination, GUS activity was found mainly in the ovules (Figure 1K).

cyc1At expression was detected during embryo formation in correlation with mitotic activity. At early globular stage, GUS activity was localized mainly in the embryo proper and in the suspensor (Figure 1L). During the triangular and early-heart stages, uniform GUS activity was found in the entire embryo (Figure 1M). At the torpedo stage, GUS activity became more localized in the growing cotyledons and developing embryonic root (Figures 1N and 1O). The mature embryo had no detectable GUS activity (Figure 1P).

Cell Cycle Regulation of *cyc1At* Expression

In general, cyclin mRNAs accumulate periodically through the cell cycle of somatic cells. In addition, cyclin transcription correlates well with the period of accumulation of the corresponding gene products (Lew and Reed, 1992; Nasmyth, 1993). Thus, determining the interval of the cell cycle in which a cyclin is expressed may give some clues to its function. Previous work has indicated that *cyc1At* mRNA preferentially accumulates in the G_2 phase of the cycle (Hemerly et al., 1992). To characterize

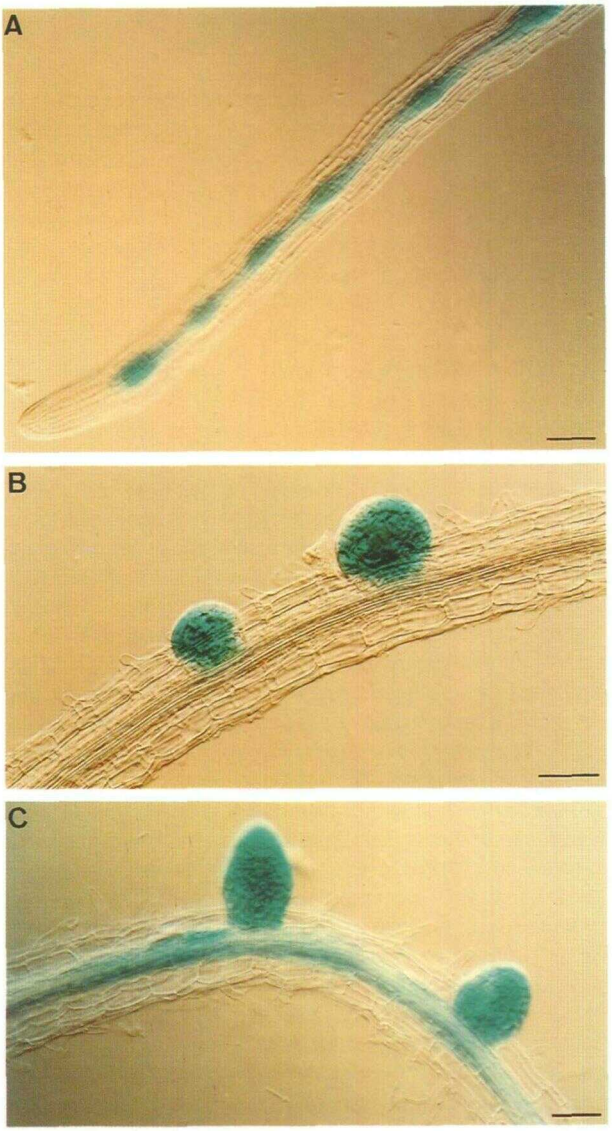

Figure 4. *cyc1At* Expression: Histochemical Localization of GUS Activity in the Pericycle and Lateral Root Primordia.

(A) Root treated with 10^{-6} M NAA.
(B) Root treated with 30 μM oryzalin.
(C) Root treated first with 30 μM oryzalin and subsequently with 10^{-6} M NAA.
Micrographs of lactophenol-cleared roots were taken using differential interference contrast optics. Bars = 100 μm.

further the phase of the cell cycle in which Cyc1At may function, intact roots of plants transformed with *cyc1At* promoter–*gus* were incubated with hydroxyurea, which arrests cells in the G_1-to-S transition, and with oryzalin, a dinitroaniline herbicide that causes microtubule polymerization (Morejohn et al., 1987; Hugdahl and Morejohn, 1993) and cell cycle arrest at metaphase (Verhoeven et al., 1990; Sree Ramulu et al., 1991; P.C.G. Ferreira, unpublished data). Figure 4B shows that GUS activity is increased in oryzalin-treated root primordia. In contrast, a drastic reduction in GUS activity was observed in hydroxyurea-treated plants (data not shown). Treatment with hydroxyurea and/or oryzalin did not affect *cdc2a* expression, indicating that the observed effects of these chemicals on *cyc1At* expression are specific (data not shown). These results were further confirmed by whole-mount in situ hybridizations with the *cyc1At* probe in roots of Arabidopsis incubated with hydroxyurea or oryzalin (data not shown). Thus, *cyc1At* appears to be expressed in an interval of the cycle between early G_2 and metaphase.

Studies on Hormonal Control of *cyc1At* Expression

The pattern of *cyc1At* expression observed in the roots indicated that cyclin transcription is induced de novo very early at the sites of lateral root formation. In contrast, *cdc2a* expression has been observed in nondividing pericycle cells (Hemerly et al., 1993). Together, these observations suggest that when cells of the pericycle are stimulated to divide, transcription of *cyc1At* and its consequent accumulation could be one of the limiting factors for the activation of $p34^{cdc2a}$. To examine this hypothesis further, we induced lateral root formation by auxin treatment of roots of intact plants transformed with *cyc1At* promoter–*gus*. Under the experimental conditions employed, new roots were visible 2 to 3 days after the incubation with 10^{-6} M indole-3-acetic acid. GUS activity was first noticed after 16 to 20 hr (data not shown). After 30 to 36 hr, newly induced GUS activity was found only in pericycle cells (Figure 1F), yet the pattern of GUS activity throughout the pericycle was not uniform. Regions of higher intensity were intercalated by others of weaker expression (Figure 1F). In general, sectors of strong GUS activity were also opposed in the pericycle by others of weaker GUS activity. Regions of higher *cyc1At* expression coincided with sites where new root primordia were being formed. A three-zone GUS staining pattern, similar to the one noted when roots of plants containing *cdc2a–gus* were treated with auxins (Hemerly et al., 1993), was observed in roots of auxin-treated *cyc1At* promoter–*gus* plants. However, the pattern was more variable: in some root tips only their extreme part was heavily stained, whereas in others the distal region had stronger GUS activity (Figure 1G).

To investigate further the control of *cyc1At* expression during the formation of lateral roots, intact roots of plants transformed with *cyc1At* promoter–*gus* were treated with an auxin, α-naphthalene acetic acid (NAA), and/or NAA together with either oryzalin or hydroxyurea. Figure 4A shows that in roots treated only with NAA, induction of GUS activity is restricted to the newly induced primordia. Lateral roots of plants treated only with oryzalin showed GUS activity exclusively in the swollen root primordia (Figure 4B). When cell division was blocked with oryzalin and then lateral roots were induced with NAA, GUS activity was also observed in the newly induced founder cells of the root and in most of the pericycle, indicating that induction of *cyc1At* precedes the completion of the first division cycle

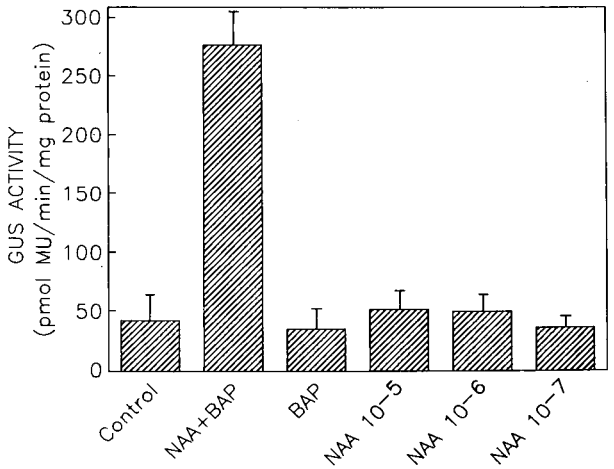

Figure 5. Fluorometric Detection of GUS Activity in Transgenic Tobacco Leaf Protoplasts.

Protoplasts from transgenic tobacco plants were cultured in medium without hormones (control) according to Hemerly et al. (1993) or containing 5 × 10^{-6} M NAA plus 10^{-6} M 6-benzylaminopurine (BAP), 10^{-6} M BAP, and 10^{-5}, 10^{-6}, and 10^{-7} M NAA, respectively. MU, 4-methylumbelliferone. The mean values of GUS activity represent four replicates of two independent experiments. Standard errors are shown.

after the induction of lateral roots from cells of the pericycle (Figure 4C). In contrast, if cell division was blocked with hydroxyurea and then plants transformed with *cyc1At* promoter–*gus* were treated with NAA, there was no induction of new root primordia or GUS activity (data not shown). This result is somewhat surprising in light of the data showing that cells from the pericycle of radish, a crucifer closely related to Arabidopsis, are differentiated in G_2 (Blakely and Evans, 1979). One would expect that if the cells of the pericycle were also in G_2, treatment with hydroxyurea, which blocks cells in G_1-to-S transition, would not block the induction of *cyc1At* expression, which is a mitotic cyclin.

Tobacco mesophyll protoplasts can be induced to dedifferentiate and be restimulated to divide by treatment with appropriate concentrations of auxin and cytokinin. To examine further the effects of auxin on *cyc1At* expression, protoplasts of tobacco plants carrying the chimeric *cyc1At* promoter–*gus* fusion were treated with different concentrations of auxins (NAA). The levels of *cyc1At* expression were evaluated by quantitative fluorometric GUS measurements. Figure 5 shows that a five- to sixfold increase in GUS activity was observed in protoplasts treated with NAA plus 6-benzylaminopurine at the appropriate concentrations to induce cell division. In contrast, protoplasts incubated with only 6-benzylaminopurine or NAA at different concentrations displayed levels of GUS activity comparable to those of controls incubated without hormones. The results show that induction of cyclin expression after hormone treatment is positively correlated with induction of cell division. However, *cyc1At* expression is not directly regulated by hormones; it is presumably a response to the induced physiological changes that will trigger actual cell division.

DISCUSSION

We examined the spatial and temporal patterns of *cyc1At* expression in meristematic zones by detailed histochemical GUS analyses of plants transformed with a chimeric *cyc1At* promoter–*gus* gene. The results showed that the Arabidopsis *cyc1At* gene is transcriptionally regulated and that this control could play a role in the spatial and temporal regulation of cell division during plant development.

cyc1At Expression Is Restricted to Dividing Cells

Previous analyses showed that there is a positive correlation between the spatial and temporal patterns of *cdc2a* expression and mitotic activity in Arabidopsis (Martinez et al., 1992; Hemerly et al., 1993). However, *cdc2a* transcripts are also found in several tissues where actual cell division is not occurring. There is an apparent correlation between levels of *cdc2a* mRNA and a competence of cells to divide (Hemerly et al., 1993). In contrast, the results obtained here indicate that *cyc1At* expression is almost exclusively confined to dividing cells. Thus, whereas in roots *cdc2a* expression is detected in nondividing parenchymal and pericycle cells (Martinez et al., 1992; Hemerly et al., 1993), *cyc1At* expression was not noticed in the parenchyma and was observed in the pericycle only transiently 2 to 3 weeks after germination or during formation of lateral roots. Histochemical analyses and fluorometric measurements showed that *cyc1At* expression is not influenced by wounding (data not shown), in contrast to what is observed with *cdc2a* (Hemerly et al., 1993). In addition, in etiolated seedlings, *cdc2a* expression is detected in the inactive apical meristem (Hemerly et al., 1993), whereas *cyc1At* expression is markedly absent (data not shown). Fluorometric GUS measurements revealed that mature leaves have quite high levels of *cdc2a* gene expression, which is in contrast to the almost undetectable enzymatic GUS activity in plants transformed with *cyc1At* promoter–*gus* (data not shown). Experiments with tobacco protoplasts showed that *cdc2a* expression can be induced by hormones even in the absence of cell division (Hemerly et al., 1993). In contrast, *cyc1At* expression is initiated only by hormone concentrations that will trigger cell division.

cyc1At Might Regulate the G_2-to-M Transition

Although expression analysis cannot establish final gene function, in most somatic cells oscillations in cyclin mRNA levels are followed by accumulation of the corresponding gene product. Thus, it is also reasonable to consider that the accumulation of Cyc1At protein parallels the *cyc1At* transcription patterns. The results of the treatments with hydroxyurea and oryzalin indicate that the patterns of *cyc1At* expression are similar to that observed for B-type cyclins (Pines and Hunter, 1989). Thus, *cyc1At* might be acting at the G_2-to-M transition. In yeast and animals, the cyclin function at this point is exerted by its

association with $p34^{cdc2}$, which is essential for kinase activation (Draetta et al., 1989; Minshull et al., 1990). So far, we have no formal proof of binding between the Cdc2a and Cyc1At proteins. However, preliminary data have shown that some high molecular weight chromatographic fractions purified from Arabidopsis cell suspensions and characterized by their capacity to phosphorylate histone H1 contained proteins recognized by antibodies raised against Cdc2a and Cyc1At (L. Bako and C. Koncz, personal communication), suggesting that they interact physically.

Cyc1At Levels Might Determine Mitotic Timing in Some Developmental Programs

In a cycling cell, the main controls of cell cycle progression are applied to guarantee the accuracy of the events that will result in the formation of two genetically identical daughter cells (Hartwell and Weinert, 1989). During development, a multiplicity of regulatory mechanisms appear to integrate the rates of cell division with the overall developmental programs followed by the organisms. The mitotic timing during development can be controlled by the abundance of a rate-determining factor (O'Farrell, 1992). The examination of the expression patterns of *cdc2a* in Arabidopsis indicated that the rates of cell division in the meristems are most likely not determined by accumulation of $p34^{cdc2a}$ (Martinez et al., 1992; Hemerly et al., 1993). In contrast, the tight association observed between mitotic activity and *cyc1At* expression suggests that transcription of *cyc1At* could be a limiting factor controlling the rates of cell division of some developmental programs.

Cell-specific *cyc1At* expression was observed in meristems of primary roots. A similar pattern was also observed by whole-mount in situ hybridizations with several Arabidopsis cyclin probes (Ferreira et al., 1994). In addition, disperse GUS activity in the flanks of the vegetative apical meristem was noticed. Analogous observations were recently reported after examination of the expression of two *cyc1At* homologous cyclins in inflorescence apices of snapdragon by in situ hybridization (Fobert et al., 1994). In addition, Fobert et al. (1994) observed that the histone H4 gene, which is transcribed mainly in S phase, and two *cdc2*-related genes exhibit comparable modes of expression. Transcriptional regulation of cyclin abundance during plant development could indicate that accumulation of cyclin during the G_2-to-M transition to threshold levels could control the timing of mitosis in the meristems (Fobert et al., 1994). In Xenopus, the timing of mitosis in the early cleavage cycles is dependent on the rate of cyclin accumulation (Murray and Kirschner, 1989). We observed that upon induction of lateral root formation from the pericycle, *cyc1At* expression is a very early event. Because these cells are most likely arrested in a G_2- differentiated state, transcription of *cyc1At* could be rate limiting for the de novo activation of cell division.

On the other hand, it is difficult to establish whether the differentially dispersed pattern of cyclin expression in dividing zones determines mitotic timing or reflects merely the proportion of cells in G_2 and the rates of cell division. One way the rates of cell division can be controlled in actively dividing meristems is through variation of the cell cycle length. An alteration in the type of cell cycle would also result in a different proportion of cells expressing *cyc1At*. In maize root tips, the length of the cell cycle tends to vary greatly, and this variation is generally a result of the time the cells spend in G_1 (Barlow, 1973). At the highest rate of cell division in the maize root meristem, a larger proportion of cells will be found in the S and G_2 phases of the cycle. A comparison of the patterns of *cyc1At* expression in different stages of root development is consistent with the possibility that multiple types of cell cycles exist in Arabidopsis root meristems. Although cell-specific GUS staining is observed in both young, rapidly dividing radicles and mature, more slowly growing root tips, a greater proportion of the cells are stained in the former (Figures 1A and 1B). During the formation of lateral roots in Arabidopsis, root primordia consisting of a few hundred cells are formed de novo in ~2 days (Figures 2B, 2C, and 2D). Although accurate measurements of cell cycle length in Arabidopsis roots remain to be done, this observation indicates that during lateral root formation, the rates of cell division are relatively high. Accordingly, GUS staining is observed in all cells of the newly formed meristem. The pattern of *cyc1At* expression during lateral root formation is also consistent with the possibility that the G_1 phase of the cycle is shortened. In general, we noticed that the highest levels of *cyc1At* expression can be detected in rapidly dividing cells, although we must consider that our results could have been partially influenced by *gus* mRNA stability and GUS enzyme stability and/or activity. Nevertheless, results similar to ours were obtained when the *cyc1At* RNA was used as a probe in in situ hybridizations of sectioned apical and inflorescence meristems of Arabidopsis (P.C.G. Ferreira, unpublished data; I. Gadisseur and A. Jacqmard, personal communication). A close correlation between *cyc1At* expression and mitotic activity is observed not only during the cell divisions of the iterative development, but also through the formative divisions of embryogenesis.

The sole exception to the rule that *cyc1At* expression is restricted to dividing cells was the observation of GUS activity in tobacco axillary buds. A dormant meristematic cell could be conceptually compared to pericycle cells, which do not exhibit *cyc1At* expression. However, the pericycle cells are partially differentiated and must dedifferentiate before starting to divide (Hashimoto et al., 1991). Cells in the axillary meristems are undifferentiated and remain dormant because of the dominance effect exerted by the shoot apex. Removal of the shoot apex normally results in the immediate development of the lateral branch. *cdc2a* is also expressed in lateral buds (A.S. Hemerly, unpublished data), and one speculative hypothesis for the expression of *cyc1At* in dormant buds is that apical dominance keeps preformed Cdc2a–Cyc1At complexes in an inactive state and withdrawal of the inhibitory influence exerted by the shoot apex activates the $p34^{cdc2a}$ kinase. Thus, in plants, as in other systems, different signals control the rate of cell division dependent on the developmental program involved. Whereas *cyc1At* transcription could limit the rate of cell division during the formation of lateral roots, it certainly does not in dormant tobacco buds, and other signals are needed.

During its life cycle, a plant must integrate diverse developmental programs to generate the patterns of cells, tissues, and organs of the plant body. There are indications that the basic cell cycle apparatus is flexible, resulting in an adaptable cell cycle that provides more possibilities of regulation to be used during development (O'Farrell et al., 1989; A.S. Hemerly, J. de Almeida Engler, C. Bergounioux, M. Van Montagu, G. Engler, D. Inzé, and P.C.G. Ferreira, manuscript in preparation). The results obtained here are consistent with the possibility that *cyc1At* expression could be determining the timing of mitosis in some developmental processes. Further investigation of the regulation of *cyc1At* transcription may help to uncover the mechanisms that coordinate the rates of cell division and developmental controls.

METHODS

Isolation of the *cyc1At* Gene and Construction of a Chimeric *cyc1At* Promoter–*gus* Gene

The *cyc1At* cDNA was used as a probe to screen a genomic library (λ Charon 35) prepared with total DNA of *Arabidopsis thaliana* ecotype Columbia (gift of D. Jofuku, University of California, Santa Cruz, CA). After restriction mapping of the candidates, a 3.0-kb HindIII fragment containing the regulatory promoter sequences was subcloned in pUC19, and both strands were sequenced by the dideoxy method of Sanger et al. (1977). Two oligonucleotides, one spanning the first methionine and introducing a NcoI site (5′-AGAAGTCACCATGGTA GTGT-3′) and the other 1.2 kb upstream and introducing a SalI site (5′-AAAGTTCGTCGACAGATGAC-3′), were used to amplify the promoter region by polymerase chain reaction. The resulting fragment was digested by SalI and NcoI and subcloned into these sites in the pGUS1 vector (Peleman et al., 1989) upstream from the β-glucuronidase gene (*gus*; *uidA* from *Escherichia coli*). A fragment containing the *cyc1At* promoter–*gus*-3′ octopine synthase gene was produced by digestion with SalI and SmaI and ligated into the SalI and ScaI sites of pGSV4 (Hérouart et al., 1994). The resulting plasmid, pGCYC1GUS, was mobilized into the *Agrobacterium tumefaciens* C58C1Rif^R (pGV2260) (Deblaere et al., 1985).

Transgenic Plants

Arabidopsis ecotype C24 plants transformed with pGCYC1GUS were obtained using the root transformation method (Valvekens et al., 1988). Twenty independent lines were examined in the R_2 generation, and no qualitative differences in GUS activity were observed in the pGCYC1GUS transformants. A representative line, containing one single locus of the pGCYC1GUS cassette, was chosen for further analysis. *Nicotiana tabacum* cv Petit Havana (SR1) was also transformed with pGCYC1GUS using the tobacco leaf disc protocol (Horsch et al., 1985). Analyses of GUS activity in transgenic tobacco plants were performed in primary transformants.

Histochemical GUS Assays

Histochemical assays of GUS activity were done essentially as described by Jefferson et al. (1987) and Hemerly et al. (1993), with minor modifications. Briefly, after 1-hr to overnight incubations of organs of transgenic plants or entire seedlings with 5-bromo-4-chloro-3-indolyl β-D-glucuronide (X-gluc; Biosynth, Staad, Switzerland), the reactions were stopped, and samples were fixed in 3% glutaraldehyde. For whole mounts, visualization of the reaction was enhanced by clearing the tissues with lactophenol (Beeckman and Engler, 1994). Cleared plant material was photographed using differential interference contrast optics. Thin sections of GUS-stained material were prepared according to Peleman et al. (1989).

Hormone, Hydroxyurea, and Oryzalin Treatments of Intact Plants

For hormone treatments of intact plants, 3-week-old, in vitro–grown plants were incubated for 30 to 72 hr in semisolid medium containing Murashige and Skoog salts (Flow Laboratories, McLean, VA), 0.3% agar, and 10^{-6} M indole-3-acetic acid or naphthalene acetic acid (NAA). Cell cycle arrest of root cells of intact *cyc1At* promoter–*gus* plants was induced by incubation for 24 to 48 hr in the same medium containing either 10 or 100 mM hydroxyurea or 30 μM oryzalin.

Whole-Mount in Situ Hybridization

A subclone of the *cyc1At* cDNA (Hemerly et al., 1992) used as a probe in whole-mount in situ hybridizations was generated as follows. The pcyc1At plasmid was restricted with XhoI. A fragment containing the complete pGEM7Z(+) vector (Promega, Madison, WI) sequences plus the 5′ nontranslated region and a part of the 5′ terminal coding region of *cyc1At* was eluted from an agarose gel and self-ligated. Whole-mount in situ hybridizations were done as described previously (Ludevid et al., 1992) and modified according to J. de Almeida Engler, M. Van Montagu, and G. Engler (manuscript in preparation).

Miscellaneous

DNA manipulations were performed as described by Sambrook et al. (1989). Tobacco protoplast manipulations and fluorometric assays were performed essentially as described by Hemerly et al. (1993).

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