The phosphorylation-specific peptidyl prolyl cis\textbackslash trans isomerase (PPIase) Pin1 in humans and its homologues in yeast and animal species play an important role in cell cycle regulation. These PPIases consist of an NH\textsubscript{2}-terminal WW domain that binds to specific phosphoserine- or phosphothreonine-proline motifs present in a subset of phosphoproteins and a COOH-terminal PPIase domain that specifically isomerizes the phosphorylated serine/threonine-proline peptide bonds. Here, we describe the isolation of MdPin1, a Pin1 homologue from the plant species apple (Malus domestica) and show that it has the same phosphorylation-specific substrate specificity and can be inhibited by juglone \textit{in vitro}, as is the case for Pin1. A search in the plant expressed sequence tag data bases reveals that the Pin1-type PPIases are present in various plants, and there are multiple genes in one organism, such as soybean (Glycine max) and tomato (Lycopersicon esculentum). Furthermore, all these plant Pin1-type PPIases, including AtPin1 in \textit{Arabidopsis thaliana}, do not have a WW domain, but all contain a four-amino acid insertion next to the phospho-specific recognition site of the active site. Interestingly, like Pin1, both MdPin1 and AtPin1 are able to rescue the lethal mitotic phenotype of a temperature-sensitive mutation in the Pin1 homologue \textit{S. cerevisiae}. However, deleting the extra four amino acid residues abolished the ability of AtPin1 to rescue the yeast mutation under non-overexpression conditions, indicating that these extra amino acids may be important for mediating the substrate interaction of plant enzymes. Finally, expression of MdPin1 is tightly associated with cell division both during apple fruit development \textit{in vivo} and during cell cultures \textit{in vitro}. These results have demonstrated that phosphorylation-specific PPIases are highly conserved functionally in yeast, animal, and plant species. Furthermore, the experiments suggest that although plant Pin1-type enzymes do not have a WW domain, they may fulfill the same functions as Pin1 and its homologues do in other organisms.

Peptidyl prolyl cis\textbackslash trans isomerasers (PPIases)\textsuperscript{1} catalyze the energetically unfavorable and intrinsically slow process of \textit{cis}/\textit{trans} isomerization of peptide bonds amino-terminal to a proline (1–3). The PPIases are ubiquitous enzymes and have been shown to be involved in protein folding, protein translocation through biological membranes, and signal transduction (1, 4, 5). Three structurally distinct families of PPIases have been identified so far. Cyclophilins and FK506-binding proteins are two well characterized families of PPIases, which are targets for the immunosuppressive drugs cyclosporin A and FK506, respectively (2, 6, 7). However, inhibition of the PPIase activity of cyclophilins and FK506-binding proteins is not required for the immunosuppressive property of cyclosporin A and FK506 (8). Furthermore, despite their high conservation in various organisms, no member of the cyclophilin or FK506-binding protein families seems to be essential, because a yeast strain with deletions in all these PPIases is still viable (9). Thus, evidence for the biological importance of the isomerase activity in these two PPIase families has been limited.

Recently, a third family of PPIases, with the \textit{Escherichia coli} parvulin being the prototype, has been described. Among this family the highly conserved Pin1-type PPIases (10, 11), which are the only PPIases that seem to be essential for cell survival, at least in budding yeast and HeLa cells. The Pin1-type PPIases, such as the human Pin1 (11), \textit{Saccharomyces cerevisiae} ESI1/PTF1 (10, 12, 13), Drosophila Dodo (14), Neurospora crassa Sap1 (15), \textit{Xenopus} Pin1 (16), \textit{Aspergillus nidulans} Pin1 (17), and others, consist of an amino-terminal WW domain and a carboxyl-terminal PPIase domain. WW domains, which are characterized by two invariant tryptophans, are present in a variety of signaling and regulatory proteins and were originally identified as protein interaction modules that bind to proline-rich regions in protein targets (18, 19). The WW domain of Pin1 has recently been shown to function as a phosphoprotein-binding module that binds to specific phosphoserine- and phosphothreonine-proline (pSer\textbackslash pThr\textbackslash Pro) motifs (20). Because these motifs are targets for some proline-directed kinases in the cell cycle, activation of these kinases is thought to create the binding sites for the WW domain of Pin1 (21). The PPIase domain of the Pin1-type proteins shows some homology to the \textit{E. coli} parvulin (22) and the human parvulin homologue, hPar14 (23). However, in contrast to other parvulin-type PPIases, all members of the Pin1-type PPIases exhibit a very distinct substrate specificity. They specifically isomerize only phosphorylated Ser\textbackslash Thr\textbackslash Pro bonds, but not their nonphosphorylated counterparts (24, 25). As proposed earlier (26), the phosphorylation-specific eukaryotic PPIases have been desig-
nated Pin1-type PPIases for clarity throughout this article. Significantly, phosphorylation on Ser/Thr-Pro motifs further restrains the already slow cis/trans prolyl isomerization of peptide bonds (25, 27). Moreover, phosphorylation also renders pSer/pThr-Pro peptide bonds resistant to the catalytic action of other PPIases characterized so far (25). Therefore, phosphorylation on Ser/Thr-Pro leads to a distinct structural feature that cannot be achieved by phosphorylation on other Ser/Thr sites that are not followed by a proline, and Pin1-type PPIases are unique isomerases that specifically isomerize phosphorylated Ser/Thr-Pro bonds (21).

As an essential mitotic regulator in budding yeast and HeLa cells, Pin1 binds to a defined subset of phosphoproteins, many of which are also recognized by the mitosis- and phospho-specific monoclonal antibody MPM-2, like Cdc25, tau, and the COOH-terminal domain of RNA polymerase II (25, 28–30). Furthermore, Pin1 regulates the functions of its binding proteins, including inhibiting the mitosis-promoting activity of Cdc25C (28) and restoring the biological activity of Alzheimer-associated tau protein (29). Depletion or mutations of Pin1 induce premature mitotic entry and mitotic arrest in yeast, HeLa cells, and Xenopus egg extracts (11, 13, 16, 17, 28). Pin1 is also required for the replication checkpoint in Xenopus extracts (16). Furthermore, in neurons of Alzheimer’s disease patients, depletion of Pin1 is associated with hyperphosphorylated tau and neuronal loss (29). In addition, ESS1/PTF1 seems to play an important role in mRNA maturation, because its mutation leads to a defect in 3′-end formation of pre-mRNAs (13). Recently, a link between ESS1/PTF1 and the general transcription machinery via its interaction with the phosphorylated COOH-terminal domain of RNA polymerase II has been described (30). Recent results from our laboratory indicate that the phosphatase PP2A is conformation-specific, dephosphorylating only the trans isomer of its specific pSer/Thr-Pro targets. Interestingly, the dephosphorylation of a protein or peptide by PP2A can be dramatically influenced by the PPIase activity of Pin1, which apparently converts cis pSer/Thr-Pro bonds in the substrate into PP2A-accessible trans peptide bonds (31). Furthermore, a function in protein folding has been suggested for the N. crassa protein Sp1, because it is able to accelerate the refolding of urea-denatured dihydrofolate reductase in vitro (15).

Recently, a new member of the Pin1-type PPIases, AtPin1, from the plant Arabidopsis thaliana has been reported (26). AtPin1 lacks an NH₂-terminal WW domain but has significant homology to the PPIase domain of Pin1 and as such a strong substrate specificity toward phosphoserine-proline in a two-dimensional NMR spectroscopy PPIase assay (26). However, it has not been described whether AtPin1 has any role in cell cycle progression.

Here we describe the isolation and functional characterization of a Malus domestica Pin1 homologue, MdPin1, which has the characteristic Pin1-type PPIase domain but lacks the NH₂-terminal WW domain. In the standard protease-coupled PPIase assay, MdPin1 exhibits the same phosphorylation-specific substrate specificity and can be inhibited by juglone, as is the case for human Pin1. A search in the plant EST databases reveals that all plant Pin1-type PPIases do not contain a WW domain, but all contain a four-amino acid insertion next to the phospho-specific recognition site of the active site. Interestingly, like Pin1, both MdPin1 and AtPin1 are able to rescue the lethal mitotic phenotype of a temperature-sensitive mutation in the Pin1 homologue ESS1/PTF1 gene in S. cerevisiae. In contrast, deleting the extra four amino acid residues abolishes the ability of AtPin1 to rescue the yeast mutation. Finally, Northern blot analysis indicates that expression of MdPin1 is tightly associated with cell division of the plant cell in growing apple fruit in vivo and in suspension cell cultures in vitro. These results have demonstrated for the first time that the function of phosphorylation-specific prolyl isomerases is highly conserved in plants and have further suggested that although plant Pin1-type enzymes do not have a WW domain, they may fulfill the same functions as Pin1 and its homologues do in other organisms.

**EXPERIMENTAL PROCEDURES**

**Cloning MdPin1 using PCR**—The template cDNA was synthesized from poly(A) mRNA extracted from apple (M. domestica cv. Granny Smith) fruitlets harvested 2 days after pollination plus gibberellin treatment as described by Dong et al. (32). DNA fragments were amplified from the cDNA using the Expand High Fidelity PCR system (Roche Molecular Biochemicals) with the two degenerative primers MADS-4 (5′-CGCTTGAATTTCTATGGCGNNGGNAARAT) and MADS-5 (5′-CGCTCGAGGATCAGTTRTTARTRCNCCYYC(M = A/C)). The primers were designed according to the conserved amino acid sequences MARGKI in the MADS-box domain and GGDDYNV in the COOH-terminal region of an alignment of the three MADS-box genes APETALA2, pMADS1, and Deficiens. The underlined EcoRI and BamHI sites were used for cloning the PCR product. The conditions for the PCR reaction were as follows: initial denaturation at 94 °C for 4 min; then 35 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 3 min; and a final extension of 5 min at 72 °C. The amplified DNA fragments of ~600 bp were cloned into the Bluescript SK vector (Stratagene, San Diego, CA) using EcoRI and BamHI and sequenced. Using the sequence information, the gene-specific primer PPI-1 (5′-GGGATCCGAGGAATGCGCTGGAAGG) was designed. The 3′ region of the MdPin1 gene was amplified using PPI-1, and the 3′ rapid amplification of cDNA ends primer (5′-GGAGGAGACTAGTCTCGAG), which anneals to the adapter used in the cDNA library synthesis. The PCR conditions were the same as above. The amplified fragments were cloned into the pGEM-T EASY vector (Promega) and sequenced. A PCR-based mutagenesis technique was used to join the 5′ and 3′ ends of the MdPin1 cDNA to obtain a full-length cDNA. AtPin1 was amplified from A. thaliana using PCR based on a published sequence (26), and its deletion mutant was made using a PCR-based mutagenesis technique and confirmed by sequencing, as described previously (28, 29).

**Northern Hybridization**—Total RNA was extracted from young (1, 2, 3, and 4 weeks following hand pollination) and mature apple fruit using the in vivo extraction methods of Chang et al. (33) and from cell suspension cultures using Trizol (Life Technologies, Inc.). The cell suspension cultures were made using a PCR-based mutagenesis technique and confirmed by sequencing, as described previously (28, 29).

**Peptidyl-Prolyl cis/trans Isomerase Activity Assay**—Recombinant MdPin1 protein was produced by subcloning the MdPin1 cDNA into the pGEX-2T vector. PPIase activity was assayed by monitoring the absorbance of released 4-nitroanilide at 390 nm at 4 °C in triplicate, as described (25). Briefly, enzymes were pre-incubated with substrate in a buffer containing 35 mM HEPES, pH 7.8, 1 mg/ml bovine serum albumin for 5 min. The reaction was initiated by the addition of the protease stock solution. Measurement of the bimolecular rate constant kₜ/Kₚ for the PPIase-catalyzed isomerization was performed by a protocol modified from Fischer et al. (7). Because [S]₀ ≪ Kₚ was valid, absorbance data points were fitted to a first-order kinetics. The kₜ/Kₚ values were calculated according to the equation Kₚ/kₜ = (kₜ/Kₚ) * cat/obs, where kₜ is the first-order rate constant for spontaneous cis/trans isomerization, and kₚ is the pseudo-first-order rate constant for cis/ trans isomerization in the presence of PPIase. Inhibition of PPIase activity by juglone was performed by pre-incubating enzymes with different concentrations of juglone for 4 h, followed by PPIase assay, as described previously (36).

**Expression of MdPin1 in the S. cerevisiae Strain YPM2**—The coding
The full-length cDNA of MdPin1 was cloned into the vector pBC100, which allows for galactose-induced expression of hemagglutinin-tagged proteins, as described previously (11). YPM2 cells were grown overnight in YAPD medium (2% Difco Bacto-peptone, 1% Difco yeast extract, 2% glucose) at 23 °C and then transformed with MdPin1/pBC100 using the polyethylene glycol method (20, 31). The transformed cells were plated on minimal medium containing 2% glucose but lacking the amino acid leucine to select for transformants and grown at 23 °C for 5–7 days. Colonies were then restreaked at least two times and incubated under the same conditions. For the spotting analysis, single yeast colonies were suspended in TE buffer 10 mM Tris-HCl, pH 8.0, 1 mM EDTA. The same procedure was used to isolate YPM2 cells expressing human Pin1, its PPIase domain AtPin1, or its deletion mutant. As controls, cells carrying either the pBC100 vector without insert or the human Pin1, its PPIase domain (Fig. 2). The Pin1 WW domain is a pSer/pThr-Pro-binding module (20), and the PPIase domain catalyzes sequence-specific and phosphorylation-dependent prolyl isomerization (25). MdPin1, however, lacks the WW domain and consists only of the Pin1-type PPIase domain in the MdPin1 cDNA is probably not due to a partial sequence, because an in-frame stop codon was identified in the untranslated region. Furthermore, homologues of Pin1-type PPIases, which also lack the NH2-terminal WW domain, have been identified in the plants Arabidopsis thaliana (41) (Fig. 2) and tomato (Lycoeris esculenta) (41). MdPin1 has an amino acid sequence identity of 79% to the A. thaliana homologue and of 55% to the tomato homologue (41). These observations suggest that multiple Pin1-type PPIases in plants—An alignment of the MdPin1 amino acid sequence with homologues of Pin1 from selected organisms is shown in Fig. 2. Pin1 and ESS1 play significant roles in the cell cycle and consist of two functional domains, the WW domain and the PPIase domain. The Pin1 WW domain is a pSer/pThr-Pro-binding module (20), and the PPIase domain catalyzes sequence-specific and phosphorylation-dependent prolyl isomerization (25). MdPin1, however, lacks the WW domain and consists only of the Pin1-type PPIase domain (Fig. 2). The absence of the NH2-terminal WW domain in the MdPin1 cDNA is probably not due to a partial sequence, because an in-frame stop codon was identified in the 5′ untranslated region. Furthermore, homologues of Pin1-type PPIases, which also lack the NH2-terminal WW domain, have recently been identified in the plants A. thaliana (26) (Fig. 2) and Digitalis lanata (41). MdPin1 has an amino acid sequence identity of 79% to the A. thaliana homologue and of 55% to the PPIase domain of Pin1. A search in the plant EST data bases reveals that the Pin1-type PPIases are present in various plants. Furthermore, there are multiple genes in one organism, such as soybean (Glycine max) and tomato (Lycoeris esculenta), which were also noted by Metzner et al. (41). These observations suggest that multiple Pin1-type proteins might also be present in mammalian cells. Interestingly, we also
found that all plant enzymes do not have a WW domain but contain a four-amino acid insertion next to the phospho-specific recognition site of the active site (Fig. 2). These four amino acid residues are important for the in vivo function of proteins, as shown below (see Fig. 4).

Phosphorylation-specific PPIase Activity of MdPin1 and Its Inhibition by Juglone—Because of the high amino acid homology of MdPin1 to the PPIase domain of human Pin1, which includes the highly conserved amino acids Lys-63, Arg-68, and Arg-69 that have been shown to be critical for the unique substrate specificity of Pin1 (24), we wanted to know if MdPin1 also shows a phosphorylation specificity in a PPIase assay. The results in Table I show that MdPin1 exhibited the same substrate specificity in the standard protease-coupled PPIase assay as Pin1. Namely, MdPin1 prefers acidic residues like glutamate NH2-terminal to the proline. Furthermore, like Pin1, MdPin1 showed an even higher isomerase activity, when a pSer-Pro-containing peptide was used as a substrate, with a specific activity similar to that of Pin1 (Table I). In addition, because juglone has been shown to inhibit the enzymatic activity of many enzymes, including Pin1-type PPIases, by covalently inactivating their active site cysteine residue (36), we examined the effect of juglone on MdPin1. Not surprisingly, juglone inhibited the PPIase activity of MdPin1 in a concentration-dependent manner (Fig. 3), with a potency similar to that of inhibiting Pin1 or parvulin, as shown previously (36). These results show that MdPin1, although it lacks an NH2-terminal WW domain, belongs to the Pin1-type subfamily of PPIases, which require a phosphorylated amino acid NH2-terminal to the proline in their substrate to efficiently catalyze the isomerase reaction.

**Table I**

Comparison of the substrate specificity of MdPin1, AtPin1, and hPin1

| Substrate                        | MdPin1 | AtPin1 | hPin1 |
|----------------------------------|--------|--------|-------|
| succinyl-Ala-Ala-Pro-Phe-p-nitroanilide | 3      | 6      | 7     |
| succinyl-Ala-Glu-Pro-Phe-p-nitroanilide | 1614   | 1796   | 2550  |
| acetyl-Ala-Ala-pSer-Pro-Phe-p-nitroanilide | 3096   | 2746   | 5200  |

Functional Rescue of the Essential ESS1/PTF1 in Yeast by MdPin1 or AtPin1—In light of the same phosphorylation-specific substrate specificity of Pin1 and MdPin1, we asked whether MdPin1 could perform the function of Pin1 in vivo. To answer this question, we examined whether expression of MdPin1 in the yeast strain YPM2 (12, 13), which carries a tem-
100 nM MdPin1 was incubated with different concentrations of juglone, and the PPIase activity toward the peptide succinyl-Ala-Glu-Pro-Phe-\(p\)-nitroanilide was measured using the protease-coupled PPIase assay. The curves were fitted to a first-order reaction kinetics, with the maximal absorbance at 390 nm being defined as 1.0. B, concentration-dependent inhibition of MdPin1 by juglone. The \(k_{\text{m}}/K_{\text{m}}\) of a PPIase reaction without juglone was set at 100%. The relative PPIase activity is plotted against the amount of juglone used in the reaction.

**Fig. 4. Functional rescue of the yeast ESS1/PTF1 mutation by plant Pin1s and the critical role of the four-amino acid insertion in the PPIase domain of plant Pin1s.** A, functional rescue of the yeast ESS1/PTF1 mutation by MdPin1. YPM2 cells transformed with the MdPin1/pBC100 and hPin1-PPI/pBC100 expression constructs were spotted in a series 10\(^{-x}\) dilution onto agar plates with glucose, glucose/galactose, or galactose as carbon source. The plates were incubated at 23 °C (permissive) or 30 °C (restrictive). Galactose allows for overexpression of the transgene. B, functional rescue of the yeast ESS1/PTF1 mutation by AtPin1 and its deletion mutant. YPM2 cells transformed with Pin1, AtPin1, and AtPin1Δ expression constructs were spotted onto agar plates with glucose/galactose or galactose as carbon source. The plates were incubated at the permissive or restrictive temperature. AtPin1Δ contains a deletion of four amino acid residues that are present in plant Pin1s but not other Pin1s with a WW domain, as shown in Fig. 2. C, similar expression of transgenes in yeast cells. YPM2 strains stably transformed with various Pin1 constructs were subjected to immunoblotting analysis using 12CA5 antibody specific to the hemagglutinin tag inserted at the NH\(_2\) terminus of all transgenes. HA, hemagglutinin.
As a probe, [32P]dCTP-labeled MdPin1 cDNA was used. 

Cultures after 1, 2, 4, 5, 7, and 11 days of growth. RNA extracted from fruitlets in different stages of their development (1–4 weeks and mature (Ma) fruit). B, RNA extracted from cell suspension cultures after 1, 2, 4, 5, 7, and 11 days of growth.

YPM2 strain, it was expressed at a level similar to that of wild type AtPin1 protein (Fig. 4C). However, AtPin1Δ failed to support the growth of YPM2 cells at partially induced conditions (Glu/Gal media) but was able to fully support cell growth at fully induced conditions (Gal media) (Fig. 4B), a phenotype almost identical to that of the Pin1 PPIase domain alone (Fig. 4A). These results indicate that these extra four amino acids in AtPin1 are apparently able to substitute for the function of the WW domain, at least in this yeast functional assay.

Cell Division Regulated Expression of MdPin1 in Plants—Because MdPin1 is able to rescue the temperature-sensitive phenotype of YPM2, the above results suggest that MdPin1 may also be involved in the regulation of cell division in apples. To examine this possibility, we analyzed the expression of MdPin1 in apple fruit tissues at progressive developmental stages and cell suspension cultures to determine whether the expression of MdPin1 is correlated to plant cell division. Northern blot analysis revealed that MdPin1 was expressed in 1–3-week-old fruit but not in 4-week-old or mature fruit (Fig. 5A).

Previous research into apple fruit development suggested that fruit growth in the first 3–4 weeks resulted primarily from cell division, whereas the subsequent growth is mainly based on cell enlargement (37). It was also recently shown that the major cell division period in “Braeburn” apple fruit occurs in the first 3–4 weeks after pollination (38). In this respect, the Northern data indicates that the MdPin1 expression is associated with cell division in apple fruit development. To further support this observation, we examined the MdPin1 expression in cultured apple cells. Significant levels of MdPin1 transcripts were detected in 2–5-day-old cultures but not in 7–11-day-old cultures (Fig. 5B). It is important to note that under our conditions, the growth in the suspension cell cultures reaches a stationary level after 7 days (data not shown), i.e., cell division stops. Similar to the experiments using the apple fruit, MdPin1 expression in suspension-cultured cells was only detected in dividing cells. These data therefore further support the conclusion that MdPin1 expression is tightly associated with cell division of the plant cell.

**DISCUSSION**

This paper describes the isolation and characterization of MdPin1, a new member of the Pin1-type PPIases from *M. domestica*, and also demonstrates the functional conservation of phosphorylation-specific PPIases in plants. MdPin1 consists only of a PPIase domain, which shows a high homology to other members of the Pin1-type PPIases and exhibits phosphorylation-specific PPIase activity similar to Pin1. Interestingly, there are multiple Pin1-type PPIases present in plant EST data bases, even within one organism, and all do not have a WW domain but contain a four-amino acid insertion next to the phospho-specific recognition site of the active site in their PPIase domains. Importantly, both MdPin1 and AtPin1 are able to rescue the temperature-sensitive mutant phenotype of the yeast *ESS1/PTF1* gene under partially induced conditions, as is the case for Pin1. However, deleting the extra four amino acid residues abolished the ability of AtPin1 to rescue the yeast mutation under the same conditions, indicating the critical role of these extra amino acids for the plant enzyme in vivo. Finally, expression of MdPin1 is tightly associated with cell division during apple fruit development in vivo and during cell cultures in vitro. These results have demonstrated that phosphorylation-specific PPIases are highly conserved functionally from plants to humans and also suggest that despite the absence of the WW domain, plant Pin1-type enzymes may fulfill the same functions as Pin1 and its homologues do in other organisms.

MdPin1 does not have a WW domain at the NH2 terminus, in contrast to human Pin1 and its homologues from various other organisms (Fig. 2). Furthermore, two other Pin1-type PPIases from plants, AtPin1 (26) and DIPin1 (DIPar13) (41), also lack the WW domain. The WW domain of Pin1 specifically interacts with phosphoserine- or phosphothreonine-proline motifs and is normally essential for human Pin1 to perform its biological function (20). Furthermore, Pin1 also binds Alzheimer’s associated phosphorylated tau protein via its WW domain and restores its biological activity, which also requires the PPIase activity of Pin1 (29). Moreover, neither the WW domain nor the PPIase domain alone is sufficient to rescue the yeast mitotic phenotype under normal expression conditions. However, when overexpressed, only the PPIase domain, but not the WW domain, can rescue the yeast muta-

These results indicate that the WW domain of Pin1 is normally required for targeting Pin1 to its substrates, the PPIase domain performs the essential function (31). These results indicate that although the WW domain of Pin1 is normally required for targeting Pin1 to its substrates, the PPIase domain performs the essential function (31). Although neither MdPin1 nor AtPin1 contains a WW domain (Fig. 2), both enzymes are able to carry out the essential mitotic function in yeast (Fig. 4). These results further support the notion that it is the PPIase domain that carries out the essential function of Pin1 in vivo.

MdPin1 seems to play a role in the plant cell cycle similar to that of hPin1 and its homologues in other eukaryotic cells. Our results have shown that the presence of MdPin1 transcripts in apple cells is tightly associated with cell division. Developing fruits show MdPin1 expression only for up to 3 weeks, when the division of the fruit cells stops (Fig. 5A), and the continuing growth of the fruits is achieved primarily by cell enlargement (37). This result is consistent with the absence of a mitotic regulator like MdPin1, whose RNA transcripts are no longer detected at this later time point. Further support for this model comes from our studies using apple suspension cell cultures. After transferring the cells to fresh medium, cell growth starts, and the cell number increases gradually because of cell division. After 7 days the culture reaches a stationary state without cell division. As can be seen in the Northern blots of these cultures (Fig. 5B), the appearance of the MdPin1 transcript exactly reflects the growth stages. MdPin1 mRNA can be de-
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