

## Isolation and classification of a family of cyclin gene homologues in *Lupinus luteus*\*

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**The lupine (*Lupinus luteus* cv. Ventus) cDNA clones encoding homologues of cyclin (*CycB1;2*, *CycB1;3*, *CycB1;4*) have been isolated from cDNA library prepared from roots inoculated with *Bradyrhizobium lupini*. Comparison of the deduced amino-acid sequences of *CycB1;2*, *CycB1;3*, *CycB1;4* and previously described *CycB1;1* (Deckert *et al.* 1996, *Biochimie* 78, 90-94) showed that they share 46-65% of identical amino acids. The presence of conserved residues (Renaudin *et al.*, in *The Plant Cell Cycle*, in the press; Renaudin *et al.*, *Plant Mol. Biol.*, in the press) along with phylogenetic analysis of known plant cyclins revealed that the four lupine sequences belong to subgroup I of B-like mitotic cyclins.**

Although the primary concept of the cell cycle was based on observation made on plant cells [1], the molecular mechanisms regulating eukaryotic cell division have mostly been investigated in yeast and animal cells. A combination of biochemical and molecular genetic approaches have shown that cell division is regulated by a protein complex consisting of p34<sup>cdc2</sup> protein kinase (or related CDK) and cyclin [2, 3]. The p34<sup>cdc2</sup> protein kinase plays a catalytic role, whereas the cyclin moiety is a regulatory subunit. The p34<sup>cdc2</sup>/cyclin complex is required at two control points of the cell cycle: between G1 and S phase and between G2 and mitosis. The

pioneering study of Hata *et al.* [4] has shown that higher plants also contain the functional homologues of the cyclin gene. Since then, cyclin cDNA clones have been reported for many plant species (reviewed by [5-8]). A sequence comparison and functional study indicate that plant cDNAs, identified thus far, represent mitotic cyclins of type A and B [4, 9-14], G1 cyclins of type D [15, 16], cyclin expressed during S phase [12] and induced in G0 to G1 transition [17].

The two comprehensive reviews on plant cyclin structure, function and classification have been recently published [18, 19]. The plant cyclin nomenclature proposed by

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Renaudin *et al.* [19] and accepted by the Commission on Plant Gene Nomenclature has been used throughout this paper.

The progression of the cell cycle and the key molecules regulating cell division seem to be conserved throughout evolution, however, the development of higher plants differs in many significant aspects from other organisms. The cell division of higher plants is localized in meristematic tissues, such as the shoot and root apical meristems and cambium. The differentiated, non-dividing plant cells are capable of reentering the cell cycle as a result of developmental control and in response to plant-specific signals such as phytohormones, light, gravity, wounding and pathogenic or symbiotic interactions.

We have previously reported the isolation of the first full length cyclin cDNA clone (*CycB1;1*) from yellow lupine [20]. As a part of our study on regulation of cell-cycle genes in legume plants we report here the characterization of three more cyclin-related clones from *Lupinus luteus* and present the phylogenetic analysis of lupine cyclins.

## MATERIALS AND METHODS

The cyclin clones were isolated from Uni-ZAP<sup>TM</sup>XR (Stratagene) cDNA library made of poly(A)+RNA from *Lupinus luteus* (L) cv. Ventus roots, inoculated with *Bradyrhizobium lupini* (strain USDA 3045). About  $2.8 \times 10^5$  recombinants were transferred to nitrocellulose membranes and screened using *HincII/HindIII* fragment of soybean cyclin cDNA clone. The probe was labeled using the Boehringer Mannheim Random Primer Labeling kit and [ $\alpha$ -<sup>32</sup>P]ATP (NEN). The hybridization was carried out at 50°C in the solution recommended by Stratagene.

The fragments of cDNA were subcloned into pBluescript SK+/- (Stratagene) or pK18 [21] plasmid vectors and sequences were deter-

mined on both strands by the dideoxy chain-termination method and using TaqTrack Sequencing Systems (Promega). When required, oligonucleotides (18-mers) were synthesized according to sequence information and used directly as primers for further sequencing. The sequences of *CycB1;2*, *CycB1;3* and *CycB1;4* were submitted to GenBank and will appear under the accession numbers: U24193, U23194, U44857.

## RESULTS AND DISCUSSION

Recently we have reported the first lupine cyclin cDNA clone named according to the nomenclature proposed by Renaudin *et al.* [19] as *CycB1;1* [20]. The second full length cDNA clone, *CycB1;4*, encodes a protein composed of 475 amino acids and its predicted molecular mass is 52.8 kDa. The two other partial cDNA clones, designated as *CycB1;2* and *CycB1;3* are composed of 350 and 420 amino acids, respectively. Alignment of the four predicted lupine cyclins over their entire length (Fig. 1) showed the highest similarity of *CycB1;3* and *CycB1;4* (76% homology, 65% identity), whereas the *CycB1;1* and *CycB1;2* are the least related to each other (64% homology, 46% identity). Our data suggest that similarly to other plant species, lupine contains a family of cyclin-related genes.

The conserved sequence motif, *destruction box*, which is responsible for cell-cycle regulated degradation of cyclins, has been detected close to the amino terminus of *CycB1;4* (amino acids 35 to 43). The amino-acid composition of this motif, RKALGDIGN, indicates the similarity of *CycB1;4* to mitotic cyclin of type B [22]. The predicted amino-acid sequence of *CycB1;3* starts with truncated *destruction box* motif, DIGN (amino acids 1 to 4), at the N-terminus. The putative *CycB1;3* protein contains a PEST-like sequence (Pro-Glu-Ser-Thr) near the carboxyl

**Figure 1. Alignment of deduced amino-acid sequences of lupine cyclins.**

The *destruction box* is underlined twice, the *cyclin box* is bordered by the symbols > < and a putative PEST-like element of *CycB1;3* is underlined by a dotted line. Residues identical in all lupine cyclin sequences are indicated by asterisks. The plant specific sequence motifs [13, 18] are underlined. Conserved amino acids in A- and B-type cyclins (A-con and B-con) are shown below the lupine cyclins. The CLUSTAL program was used for alignment. The nucleotide sequences of *CycB1;1*, *CycB1;2*, *CycB1;3* and *CycB1;4* have been submitted to the GenBank with the respective accession numbers: U24192, U24193, U24194, U44857.

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          * * * * *
CycB1;1  -----MPSIAHSHVAAEPQHPKGENKQKNEGRNRRVLKDIGHLVVKQADPVAN--VP-KR-TKNFGCQLLANAQAAA
CycB1;2  -----
CycB1;3  -----DIGHL-----DRVKGV-EVKP-NRPITRSPCAQLLANAQVAA
CycB1;4  MASRPTVRLQQQARDEEVVGGGKQKKN-GAGDGKNRKALGDIGHL-----DHV-----KP-NRPVTRSPCAQLLANAQAAA
A-con          R ALGVI W
B-con          R ALGZI W

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          * * * * *
CycB1;1  --EKNEK-----QNTDVGNLAVVTKVEQTKKETEHEVI-----IISDDEEKEKQVVKGKQKQEKRLLVG 1
CycB1;2  -----VAPKPAEK-VTKPKPVEIVEISSGK--EVQ-KDKSANKKEQEMLS-P-K
CycB1;3  AVENKKEKLAIPNVGAKPNVVEGA-VAKRVAPKPAEK-VVEKPKPREAVEIS PHE--EVQ-KNKSVVKEKKEGGEN---K 1
CycB1;4  AAKNKKEKLAIPNVADPKPNVADGV-VAKRVAPKPAEK-VTAKPKAQEVIEINPAE--RAQ-KNKSVNKEKKEGGEN---K 1
A-con
B-con

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          * * * * *
CycB1;1  ERTQAFSAVLSARSKAA-CGLTHSQKIGIGDDIDVADIANELEAVEYLLDDIYKPKYKLTEDDGGV-HDYMPSPQPDIMIKR 2
CycB1;2  KKSQTLTSVLTARSKA--CGLTEN--QQQIIDIDAGDSRNELAAVEYIEDIYKPKYKLAENENRP-EQYMDSQPEINERM 1
CycB1;3  KKPQTLSSVLTAKKQSHVGLTKKP-QEQIIDIDANDSGNELAALEYIEDIYKPKYKLESEKPSHBYLDSQPEINERM 1
CycB1;4  KKSRTLTSVLTARSKAA-CGLTNKP-KEKIIDIDAGDSGNELAAVEYIEDIYKPKYKLAENENRP-EQYMDSQPDINEKMR 2
A-con          Y I Y R E K K Y M Q D I M R
B-con          D D P S E Y I Y Y E M R

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          * * * * *
CycB1;1  SILFDWLVIEVHKFELMQETLYLTLMIVDRFLSMKAVPRRELQLVGISSMLIACKYEEIWAPEVHD-FVCISDNAYVREN 2
CycB1;2  AILVDWLVLDVQTKFDLSLETLYLTIMIVDRFLAVNDVVERELQLVGVSAMLMASKYEEIWPPEVDSTFVCLTDGRAYTHEQ 2
CycB1;3  AILVDWLVLDVNRKFDLSLETLYLTIMIVDRFLAVKVVPRRELQLVGVSAMLVGSKYEEIWPPEVND-L-CAYQIERTLMN 2
CycB1;4  AILVDWLVLDVNRKFDLSLETLYLTIMIVDRFLAKTVPRRELQLVGVTOMLIGSKYEEIWPPEVDE-FVCLSDRAFIHEE 2
A-con          DWLVEV KEYKL ETL Y DRFLS V R KLQVQ A A KYEEIYPP V E F TDD Y Q
B-con          W Q F LLQET I DR V LQLVGVT A KYEEMY P D F D Y T

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          * * * * *
CycB1;1  ILIMEKTIILSKLEWTLVPTTYVFLVRF-KASTPYDKKIKIDMIFFLARLSIMDYPLVISYCPSMIAASAVYAARCILGRV 3
CycB1;2  ILAMEKSILGKNEWTLVPTTFVFLTRFIKASVP-DQELERNGHFLSELGMMHYA-TLVYCPSMVAVSTVFAARCTLNKT 2
CycB1;3  NTSDGIILGKLEWTLVPTTYVFLVRFIKASVP-DQELERNGSHFLSELGMMHYS-TLVYCPSMVAASAVFAARCTLNKT 3
CycB1;4  VLAMEKIIILGKLEWTLVPTTYVFLVRFIKASVP-DKELERNGARFLSELGMMHYG-TLVYCPSMIAASAVFAARCTLNKT 3
A-con          LRME L KL FD PT F K EL L Y PS A LA
B-con          I ME L L GRPL LHFLRA SK HT AKYLML PS IAA L L

```

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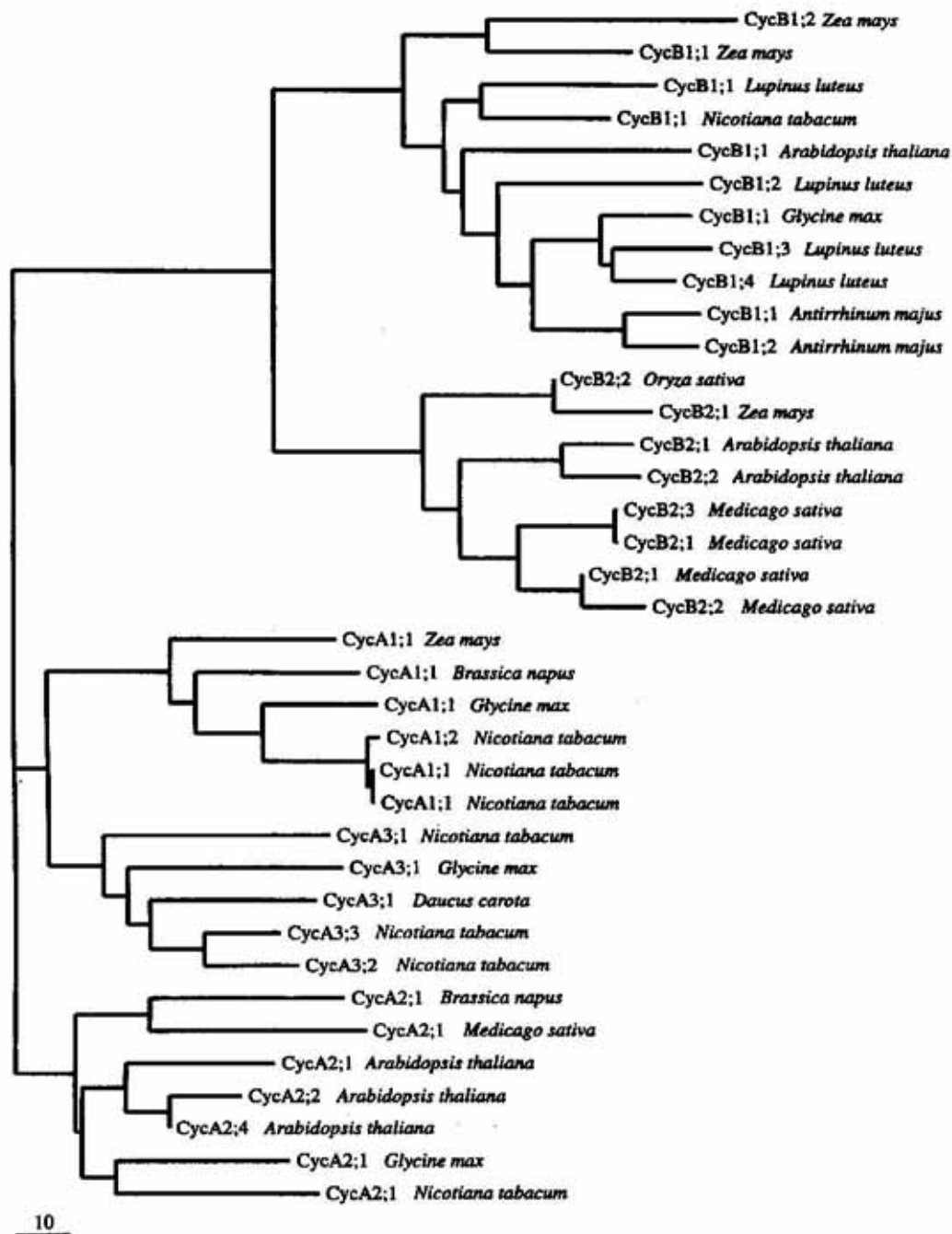
          * * * * *
CycB1;1  PYWTETLKHYTYGYYEQLRDCAKLMVNFHVSAPESKLRVAVYKFPSSLERGAVALVAPTENLLA----- 4
CycB1;2  PIWNETLQQTGYSEBQLMDCARLLVSFPLASVEWKLKVLRYKYSDPQRGAVSMHPPAKNLLPSSCG-SRV----- 3
CycB1;3  PFWNETLKLHTSYSEBQLMDCAKLLVSFSTIGGGKLVVHRKYSDPQKAVAVLPPAKYLMPESSSSGTRVSK----- 4
CycB1;4  PIWNETLKLHTGYSKQQLMDCARLLVSFHSIRGEKLVLYRYKYSDPERGAVAVLSPAKNLSSEGGIDLSVVKPTYNLH 4
A-con          W T Y L A QA KY Y
B-con          W Y Y E V KY

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CycB1;3  -----GLD-K--- 420
CycB1;4  LQLLLLFPLILFFSMSTNHC 475

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**Figure 2. Phylogenetic tree of the plant mitotic cyclins.**

Topology of the tree has been inferred from 213 positions of amino acid alignment, using the protein maximum parsimony algorithm; branch lengths have been recomputed using the Fitch–Margoliash method on the distance matrix obtained from the same protein sequence. Horizontal lengths reveal divergence. Sequences have been aligned by the CLUSTAL program, other computations have been performed with programs from Joe Felstein's PHYLIP package. The following cyclin sequences with accession numbers indicated in brackets were included: *CycB1;2-Zm* (U10078), *CycB1;1-Zm* (U10079), *CycB1;1-Ll* (U24192), *CycB1;1-Nt* (Z37978), *CycB1;1-At* (X62279), *CycB1;2-Ll* (U24193), *CycB1;1-Gm* (X62820), *CycB1;3-Ll* (U24194), *CycB1;4-Ll* (U44857), *CycB1;1-Am* (X76122), *CycB1;2-Am* (X76123), *CycB2;2-Os* (X82036), *CycB2;1-Zm* (U10076), *CycB2;1-At* (Z31400), *CycB2;2-At* (Z31401), *CycB2;3-Ms* (X78504), *CycB2;1-Ms* (X82039), *CycB2;1-Ms* (fragment — X68740), *CycB2;2-Ms* (X82040), *CycA1;1-Zm* (U10077), *CycA1;1-Bn* (L25406), *CycA1;1-Gm* (D50870), *CycA1;2-Nt* (X92967), *CycA1;1-Nt* (X92966), *CycA1;1-Nt* (fragment — D50735), *CycA3;1-Nt* (X92964), *CycA3;1-Gm* (D50868), *CycA3;1-Dc* (S49312), *CycA3;3-Nt* (X93467), *CycA3;2-Nt* (X92965), *CycA2;1-Bn* (L25405), *CycA2;1-Ms* (X85783), *CycA2;1-At* (Z31589), *CycA2;2-At* (Z31402), *CycA2;4-At* (U17889), *CycA2;1-Gm* (D50869), *CycA2;1-Nt* (D50736).

terminus. The PEST element is characteristic for G1 cyclins [23] lacking the *destruction box* and is common to rapidly degraded proteins [24]. The presence of two distinct sequence motifs, responsible for rapid protein degradation, the *destruction box* and PEST element, has been described for three other plant cyclins: *Arabidopsis thaliana* *CycA2;1* and *CycA2;2* [9] and *Brassica napus* *CycA1;1* [25]. The HRPITRSF-like motif, which seems to be conserved in plant B-type cyclins [18, 19], is located close to the *destruction box* of *CycB1;1*, *CycB1;3* and *CycB1;4*. The sequence motif characteristic for the B-type cyclin of subgroup I, KKXXXTL(S/T)-(S/T)VL(S/T)ARSKAACG, is well conserved in *CycB1;1*, *CycB1;2* and *CycB1;4* and partially changed in the *CycB1;3* clone. In spite of the overall similarities of all lupine cyclins, no homology was observed in the amino-terminal regions preceding the *destruction box* of *CycB1;1* and *CycB1;4* (Fig. 1).

The centrally located *cyclin box* contains conserved amino acids of both A- and B-type of mitotic cyclins in all lupine sequences (Fig. 1) and this feature has been reported for several plant cyclins [4, 9, 12–14].

Based on sequence homology, Renaudin *et al.* [20, 21] proposed that higher plants form three cyclin A subgroups and two cyclin B subgroups, which differ from classes reported for other organisms. The sequences of 37 plant cyclins were aligned by the CLUSTAL program, across 213 amino acids of the cyclin box. The constructed phylogenetic tree confirmed that plants have five subgroups of mitotic cyclins [18, 19] and revealed that all lupine sequences belong to B-like cyclins of subgroup I (Fig. 2), along with proteins from five other species, both mono- and dicotyledonous plants.

Although the four lupine cyclins described here belong to the same structural group, their function at particular stages of the cell cycle, regulation by plant-specific factors and during plant developmental processes remain to be established.

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