Isolation and characterization of two cyclin-like cDNAs from Arabidopsis

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Abstract

Cyclins are key regulators of a family of protein kinases called cyclin-dependent kinases (Cdks). Specific cyclins interact with specific Cdks to regulate the different transition points in the cell cycle. Six mitotic-like cyclins have previously been reported in Arabidopsis thaliana. Using polymerase chain reaction amplified cyclin-box sequences as probes, two new cyclin cDNAs are isolated from Arabidopsis. The deduced amino acid sequences of the isolated cDNAs (Arath;CycB1;3 and Arath;CycB1;4) show the highest sequence similarity with mitotic cyclins. Arath;CycB1;3 is most homologous to the plant CycB1 group cyclins and contains a conserved motif that is typical of this group. Arath;CycB1;4, while homologous to Arath;CycB1;2, has some features that make it different from other known mitotic-like cyclins. These data suggest the presence of several distinct cyclins of CycB1 group in Arabidopsis. Analysis of expression of three members of CycB1 group (Arath;CycB1;2, Arath;CycB1;3 and Arath;CycB1;4) in different tissues by reverse transcription-polymerase chain reaction using primers corresponding to unique regions of their cDNAs shows that they are differentially expressed in different tissues.

Introduction

Cell division in eukaryotes is tightly regulated at key points in the cell cycle. An interaction between two types of proteins, cyclins and cyclin-dependent kinases (Cdks), has been shown to be involved in the regulation of these transitions. Both these proteins are represented in the genome as gene families. In animals, a large family of Cdks has been identified [33, 35, 36, 52]. Similarly a family of cyclin genes has been identified in both yeast [1, 6, 17, 26] and animals [22, 33, 35]. Specific cyclins interact with specific Cdks to regulate the different transition points [31, 33, 52]. Proteins are classified as members of the cyclin family on the basis of sequence similarity limited to a central region (the cyclin-box) of about 200 amino acids that are flanked by divergent N- and C-terminal regions [34]. Cyclins have been generally divided into two groups: mitotic cyclins which are involved in the G2/M transition and G1 cyclins involved at Start and the G1/S transition. Mitotic cyclins have a motif called a destruction box that has been implicated in their destruction through the ubiquitin pathway [16, 26].

Cyclins were first isolated in plants from carrots and soybean in 1991 [18]. Since then, 61 cyclins have been identified in 14 species of plants representing seven angiosperm families [42]. All plant cyclins in this report are referred to by the names suggested by Renaudin et al. [42]. Recently a cyclin has been isolated from a fern, Adiantum capillus-veneris L. [51]. Many of the cyclins were isolated using probes generated by PCR amplification of cyclin box sequences [4, 5, 10, 21, 37, 41, 46, 50] oligonucleotide probes based on the cyclin box [18, 19], or cyclin clones [13, 45]. Because the cyclin box sequence in G1-type cyclins shows very limited homology compared to the homology between the mitotic-type cyclins, all of the cyclins isolated using these methods were of the mitotic-type. However, they were not easily classified as A or B type because their cyclin-box sequences contained conserved residues from both types. Phylogen-
etic analyses by several groups [27, 41, 46] grouped some of the known plant cyclins into A-like and B-like types. The most recent classification, including the 61 reported cyclins, groups the mitotic cyclins into two major groups, CycA and CycB, with CycA having three subgroups and CycB having two subgroups [42]. In Arabidopsis, six different mitotic-like cyclins have been reported [5, 10, 19]. Two are classified in group CycA2, two in CycB1 and two in CycB2.

A different approach based on complementation of a yeast cyclin minus mutant was used by two groups to identify cyclins in Arabidopsis [49] and alfalfa [3]. Three Arabidopsis clones were able to complement this mutant. Analysis of these genes indicates that they are similar to the D-type cyclins [49]. Other D-like cyclins have been isolated from other plant species [42]. The classification scheme proposed by Renaudin et al. [42] includes a CycD group with 3 subgroups. The three Arabidopsis clones are each in a different subgroup (Arath;CycD2;1 being the only member of group 2) [42].

Injection of a p34cdc2/cyclin B-like complex into the stamen hair cells of Tradescantia caused rapid disassembly of the preprophase band of microtubules but not of interphase-cortical, spindle or phragmoplast microtubules. It also accelerated chromatin condensation and nuclear envelope breakdown showing that cyclin/Cdk complexes can initiate nuclear division in plants [23]. Ectopic expression of Arabidopsis cyclin Arath;CycB1;1 was shown to accelerate growth without altering the pattern of lateral root development [7]. In contrast a study using an Arabidopsis p34cdc2 homologue showed no effect of ectopically expressed cdc2 [20]. This suggests that cyclin is the limiting factor for growth [7]. Cdk5 have been isolated in plants [2, 12, 29, 32] but no cyclin-Cdk interactions have been shown.

The mitotic-like cyclins in plants appear to constitute a large family of genes. In maize, four different mitotic-type cyclins were isolated [41]. In Brassica napa two different full-length genes for mitotic-type cyclins plus six different cyclin box sequences were isolated [50]. Seven mitotic-like cyclins have been reported in tobacco [40, 46]. We report here the isolation of two additional mitotic-like cyclins in Arabidopsis. The specific function of any of the isolated mitotic-like cyclins in plants has not been established. Expression patterns of different cyclins suggest there may be cell cycle stage- and tissue-specific functions for the different cyclins although redundancy of function may also be involved [13, 48].

Materials and methods

Materials

Arabidopsis thaliana ecotype Columbia plants were grown under continuous light at 22 °C. Plant parts were collected between 4–6 weeks after germination. Suspension cultures were grown in Murashige and Skoog media (BRL) as described [39]. Samples were taken at 5–7 days following transfer to new medium. Callus was grown on the same medium with 0.7% agar in constant light at 22 °C.

Restriction enzymes, T4 ligase, T4 DNA polymerase and Taq polymerase were purchased from Gibco-BRL or Boehringer Mannheim Biochemicals (BMB). Sequencing was done using Sequenase Version 2.0 DNA Sequencing Kit (USB). Labeling and detection were done using the Genius System (BMB).

Screening and sequence analysis

About 400,000 plaques from each of thee Arabidopsis thaliana libraries (a cDNA library from above-ground tissues in λ-YES vector [8] and a cDNA library from flower buds in λ-ZAPII vector and a whole plant cDNA library in λ-PRL vector obtained from ABRC) were screened. Plaques were transferred onto Hybond N (Amersham) filters [43] and DNA was fixed to the filters using UV light. Duplicate filters were probed with either a 32P-dCTP-labeled probe generated with the Megaprinse DNA Labeling Kit (Amer sham) or dig-dUTP-labeled probe (Boehringer Mannheim) generated by PCR. Second and third screenings were done with dig-dUTP-labeled probe. Hybridization was done at 55 °C. Filters probed with 32P-dCTP-labeled probe were exposed to X-ray film. Detection of dig-dUTP-labeled probe was done colorimetrically. The pBluescript SK plasmid from positive clones was excised from the Zap II vector using the Exassist/SOLR System (Stratagene) and the plasmids contained in the λ-YES and λPRL vectors were excised as in Elledge et al. [8] and Ziplox Kit (Gibco-BRL), respectively. Clones were sequenced by the dideoxynucleotide chain termination method [44] using double-stranded plasmid DNA as a template. Subclones and internal primers were used to determine the complete sequence of each cyclin. Sequences were analyzed using Eugene, MacVector and BLAST programs.