Abstract

An Analysis of *Antirrhinum majus* Cyclin A20 and Cyclin D1 Function in *Arabidopsis thaliana*

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Arabidopsis thaliana is the model system of choice for genetic and molecular studies of plants due to its small completely sequenced genome and well-characterized development. These features also make *A. thaliana* a good model to investigate cell cycle regulation in plants. As in all eukaryotes the cell cycle is controlled at the cellular level by cyclins (Cycs), which interact with, and modulate, the activity of cyclin-dependent kinases (CDKs). Different cyclins are active during different parts of the cell cycle, as a broad example, A- and B-type cyclins are active during mitosis, and D-type cyclins during DNA replication. However, many cyclins present in plant genomes do not have assigned roles. This work aimed to discover possible roles for two cyclins of unknown function, cyclin A20 and cyclin D1, both originating from *Antirrhinum majus* (*Am*). The *AmcycA20* and *AmcycD1* genes were expressed using the inducible *alc* regulon of *Aspergillus nidulans,* allowing the selective expression of transgenes in a regulated manner. The *alc* switch consists of two components, the *alcR* gene, which encodes a transcriptional activator AlcR, and the AlcR recognition sequences from the *alcA* promoter. The AlcR transcription factor is constitutively expressed and allows transgene expression downstream of an *alcA* promoter upon induction with ethanol. A hemagglutinin (HA) tag was included to monitor expression of both transgenes.

The *AmcycA20* and *AmcycD1* genes were cloned into pGPTV-M and pGreen0029-M binary vectors, in gene cassettes containing the *alcA* promoter. Both genes were also cloned with an HA tag at the 5' terminus. The plasmids were then transformed into *A. thaliana* wild type (Columbia) as controls and into *alcR* containing plant strains either with (AGS) or without (SRN) a *GUS* reporter gene.

Transgenic lines of *A. thaliana* were produced that expressed cyclin A20 and cyclin D1 with and without an HA tag. The lines were characterised for the segregation of selectable markers and the progeny were analysed by PCR to confirm the integrity of the transgenes. RT-PCR was used to confirm ethanol regulated transcription and Western blot analysis to monitor expression in the case of the HA-tagged cyclins. Detailed phenotypic analysis of these lines has revealed that expression of the cyclin genes resulted in significant alterations to plant growth. The phenotypic characteristics observed in *A. thaliana* demonstrated that *A. majus* cyclin A20 caused an increase in cell size, and a corresponding increase in plant size and weight. This pattern was repeated in the cyclin D1 plants, but to a lesser extent, and the expression of the *AmcycD1* led only to a very subtle phenotype. Expression of *AmcycA20* and *AmcycD1* genes did not affect overall developmental timing of the plant. This study indicates that the function of cyclin A20 may be involved in regulating cell expansion.