Functional and Developmental Analysis of Cell Cycle Control Genes in Alfalfa (*Medicago sativa*) and Snapdragon (*Antirrhinum majus*)

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INTRODUCTION

Cell division is a complex process that is fundamental to the survival and propagation of all organisms, including higher plants. It has been shown that the basic mechanism of cell cycle control is conserved among eukaryotes. Compared to most animals, plants have a somewhat unusual life style, to which many aspects of their morphogenesis and cell division are adapted. Presence of a rigid cell wall prevents the cell migration and consequently organogenesis is dependent on cell division and cell expansion at the site of formation of new organs. Most cell proliferation occurs in specialized regions called meristems. As the cells are displaced from the meristem, cells differentiate and expand. Expansion is sometimes associated with endoreduplication, a process by which the DNA in re-replicated without intervening mitoses. There is many debates as to whether cell proliferation is a driving force in plant morphogenesis or merely a response to growth and sub-divides space. The cells that go to form organ primordia behave differently from those that remain in the meristem. Those that remain in the meristem are "stem" cells, that is they divide relatively slowly but indefinitely and do not differentiate. Their progeny, which are displaced from the meristem, can have one of two fates. One fate involves the formation of stem tissue and cells following this path tend to expand extensively and rarely divide. The other fate involves the formation of leaves. Cells following this path initially divide very rapidly and then differentiate. The meristem identity itself can change from a vegetative to a reproductive phase, resulting in the formation of flowers. A key problem in plant biology is understanding why these different populations of cells behave differently. The plant development is mainly post-embryonic. During embryogenesis, the main developmental event is the establishment of the root-shoot axis. Most plant growth occurs after germination, by iterative development at the meristems. Structurally, the plant cell cycle differs from that of animals, particularly in the early stages of mitosis; before spindle formation, a microtubule array called the pre-prophase band defines the future division plane of the cell. At the end of mitosis, another microtubule array, the phragmoplast, is involved in

cytokinesis. Mutants defective in cytokinesis have dramatic effects on plant development (Jurgens, 1997).

Aim of this study

In this thesis, several approaches have been used to characterize the cell cycle control genes. Mainly it has been tried to understand how the cell cycle is altered when some of the cell cycle control genes were over expressed (transient over-expression experiments). Hormone experiments were done as they play a central role in cell cycle induction, suppression and somatic embryogenesis with unclear mechanisms. A mutant was partially characterized to understand the better mechanism that led to dorsoventality in the lateral organs of plants and the role of a cell cycle control gene in this phenomena (cycD3A Antma;*CycD3*;1 gene and *phantastica* mutant of *Antirrhinum majus*). New techniques are optimized and used to over come the limited sample source and complications of already existing methods (expressional analysis of cell cycle related genes with RT-PCR in leaf protoplast and use of CyQuant cell proliferation assay). All this study was done to get a better picture of plant cell cycle control and development.

EXPERIMENTAL PROCEDURES

Cell Cultures and plants: Two alfalfa cell suspension cultures were used prepared from two different lines of alfalfa ; A2 and KS10. A2 was maintained in Muroshige-Skoog medium with 1 mg/l 2,4-D and 0.2 mg/l kinetin.. KS10 was maintained in SHF medium with 10 μM NAA and 1 μM kinetin. *In vitro* plants were maintained on hormone free UM medium. For mRNA *in situ* hybridization experiments *Antirrhinum majus* wild type plants and *Phantastica* mutant were used. They were grown in growth chambers. For transformation experiments; protoplasts were isolated from *Nicotiana tabaccum* in vitro plants; alfalfa A2 line cell suspension and maize HE/89 cell suspension were used.

Molecular Materials: The cDNA library was prepared from auxin treated alfalfa microcallus suspension and screened as described in Sambrook *et al.* The plasmid

constructs were maide by using basic cloning techniques. The mRNA was isolated by using Quiagen columns as described in the procedure and northerns were performed as described in Sambrook *et al.* For RT-PCR the mRNA was reverse transcribed by Superscript II reverse transcriptase (Gibco BRL). Primers were designed for each gene separately and synthesized in the conventional way.

Other procedures: In all experiments PEG mediated direct DNA uptake method was used. mRNA *in situ* hybridization was done as described in the thesis in detail and performed on shoot apex of inflorescence *A. majus* plants.

RESULTS and DISCUSSION

In this thesis several approaches were used to analyse the cell cycle related genes functionally and developmentally.

- Analysis of the transcriptional regulation of cell cycle genes in alfalfa:

Effects of phytohormones are analysed by applying different concentrations of phytohormones for different durations (2,4-D, NAA, kinetin, 24-epibrassinolide, ABA for 8 and 24 hours)

Analysis of mRNA transcription of cell division cycle genes were perfomed during auxin-induced somatic embryogenesis.

- Analysis of a *MYB* transcription factor mutant '*Phantastica*' was performed. The results gave information about the relation between D-type cyclins and MYB transcription factors and their effect on cell cycle.

- For the functional analysis of alfalfa cyclin-dependent kinases and cyclin genes, the genes of interest are overexpressed by cloning them under a constitutive promoter of 35S CaMV. One homologous and two heterologous (*N. tabaccum* and *Z.* mays) systems were tested. To monitor the changes in the S-phase the GUS gene under histone H3 and H4 promoter that are proved to be S-phase specific were used as S-phase markers. In heterologous system overexpression of cdc2*Ms*A was found to be

give the highest GUS activity, in homologous system cyclin over expression resulted in was slightly stronger GUS activity than cdc2*Ms*A.

- Reinitiation of cell division cycle by leaf mesophyll protoplasts and analysis of genes of interest by RT-PCR led the use of a new application to overcome the material limitation as well as introducing a different way of synchronization of cells.

CONCLUSION

Several techniques were used and developed to analyse the cell cycle related genes functionally and developmentally. All of the experiments gave information for the response of cell cycle genes in different conditions. Use of mRNA *in situ* hybridization and the analysis of Phantastica mutant enabled us to monitor the expression of D-type cyclins at different developmental stages.

Further studies should be carried out both with hormone response and analysis of cmutants thoroughly in order to draw the complete picture of cell cycle and the involvement of cdks and cyclins in the control mechanism.

This thesis is based on following publications

1) Dudits, D., Bögre, L., Bako, L., **Dedeoglu, D.**, Magyar, Z., Kapros, T., Felföldi, F., Györgyey, J., (1993) "Key components of cell cycle control during auxin-induced cell division" In :J.C. Ormond, D. Francis (editors), *Molecular and cell biology of the plant cell cycle*, Dordrecht, Kluwer Academic Publishers, pp 111-132.

2) Magyar, Z., Bako, L., Bogre, L., **Dedeoglu D.**, Kapros, T. and Dudits, D. (1993) "Active cdc2 genes and cell cycle phase specific cdc2 related kinase complexes in hormone-stimulated alfalfa cells" *Plant Journal*, 4(1), 151-161.

3) Bilgin, M., **Dedeoglu, D.**, Peres, A., Engler, G., Inze, D., Dudits, D., and Feher, A. (1999) "Meristem and cell division associated expression of wheat histone H4 promoter is modified by 2-4,D and ABA in transgenic maize plants" (*Plant Science*, in press).

4) Doonan, J.H., **Dedeoglu, D.**, Fobert, P., Gaudin, V. and Lunness, P. (1997) "Spatial and temporal regulation of cell division during plant growth" *Cell Biol. Int.*, 21:861-863.

5) **Dedeoglu, D.**, Bilgin, M., Setenci, F., Kapros, T., Feher A., and Dudits D., (1999) "Transient overexpression of cyclin or cyclin dependent kinase genes activates S-phase in protoplast-derived plant cells" (submitted to *Plant Cell Reports*).

Unrelated Publication

A. PERES , K. NIKOVICS, J. DE ALMEIDA-ENGLER, **D. DEDEOGLU**, G. ENGLER, D. INZÉ , D. DUDITS , A. FEHÉR

Functional analysis of the *Arabidopsis thaliana* cycB1;1*At* promoter region in transgenic maize (submitted to *Cereal Research Communications*)

Selected Presentations and Meeting Abstracts

Dedeoglu D, Bogre, L., Bako, L., Magyar, Z., Dudits, D. (1991) "Molecular events in the activation of cell cycle" 8th Protoplast Symposium, Uppsala, Sweden, 16-22 June 1991

Dedeoglu D., Gyorgyey J., Magyar Z., and Dudits D. (1993) "Complex Cyclin gene family in alfalfa: Differential Expression in plant organs and during somatic embryo development" 10th European Cell Cycle Conference, La Rochelle, France, September 20-24, 1993.

Gyorgyey J., Magyar Z., **Dedeoglu D**., Kapros T., and Dudits D. (1993) "The expression of cdc2 and cyclin genes in differentiating and synchronized cell cultures of alfalfa" Canterbury Meeting, 29 March-2 April 1993, 14th Annual Conference of the European Society for Comperative Physiology and Biochemistry

Bilgin M., **Dedeoglu D**., Setenci F., Ayaydin F., Mavituna M., and Dudits D. (1995) "Utilization of replication-dependent Histone H3/H4 promoters linked to GUS marker gene as an indicator of S-phase activation in plant protoplast".Control of Cell Cycle Division in Higher Plants, EMBO Workshop October 5-7 1995, Szeged.

Peres A., **Dedeoglu D**. Ferreira P.C.G., Inze D., Dudits D. and Bilgin M. (1995) "Transformation of maize with a chimeric gene construct based on Arabidopsis cyclin (cyc1At) promoter fused to GUS reporter gene" Control of Cell Cycle Division in Higher Plants, EMBO Workshop October 5-7 1995, Szeged"

Meszaros T., Miskolczi P., **Dedeoglu D**., Setenci F., Bako L., Koncz Cs., Deak M., Magyar Z., Dudits D. (1995) "Hormone treatment of alfalfa cultured cells alters the *in vitro* histone kinase activity of cdk complexes", Control of Cell Cycle Division in Higher Plants, EMBO Workshop October 5-7 1995, Szeged

Dudits D., Magyar Z., Meszaros T., Miskolczi P., **Dedeoglu D.**, Horvath G.V., Bilgin M, Ayaydin F., Kelemen Z., Gyorgyey J , Feher A. (1998) "The pivotal role of plant division kinases (PDKs) in hormonal and stress control of cell cycle in plants", IX. International Congress of Plant Tissue and Cell Culture, Jerusalem, Israel, June 14-19, 1998.

D. Dedeoglu, M. Bilgin, Zs. Ponya, B. Barnabas, D.Dudits and A. Fehér "Transient overexpression of cell cycle genes in isolated somatic and egg cells", Körber Workshop on "From Single Cell to Plant"; 18-20. 05. 1998. Hamburg, Germany (oral presentation)

Társszerzoi lemondó nyilatkozat

Alulírott nyilatkozom, hogy a Jelölt téziseit ismerem, a tézisekben foglalt tudományos eredményeket tudományos fokozat megszerzéséhez nem használtam fel, s tudomásul veszem, hogy azokat ilyen célból a jövoben sem használhatom fel.

Dátum.....

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