The Effect of Stress Factors on Gene Expression in Higher Plants

Summary of PhD Thesis

of

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Szeged

2006

Introduction

Owing to their sessile lifestyle, plants have to respond to local environmental conditions such as light, temperature, water, nutrients, gravity, pathogen attack, wounding by changing their physiology and redirecting their growth. The perceived stimuli are communicated across the plant body through chemical messengers, hormones, which affects diverse aspects of plant growth and development. Since the rigid cell wall does not allow mobilizing of specialized cells for stress response, plants developed the capacity of making cells competent for the activation of defense responses. The mechanisms for increased tolerance to environmental stress involve physiological changes, or expression of genes that results in modification of molecular and cellular processes.

The information about the perceived stress in the cells is transmitted through a signal transduction pathway. The signalling molecules can regulate the gene expression. The transcription factors recognize multiple regulatory sequences within the promoter regions of the genes ensuring nuanced response to different signals. Studying the gene expression regulation at transcription level may improve the understanding about the regulatory networks acting during stress adaptation.

In the present study, I aimed the examination of the effect of stress factors on gene expression in higher plants. In the first part of our work, I investigated the expression of genes up-regulated in

response to drought/osmotic stress. My approach was based on gene substraction; a set of genes was isolated from drought sensitive and was substracted from the gene set of drought tolerant wheat (*Triticum aestivum* L.) cultivar. This project was done in collaboration with the Department of Plant Physiology of the University of Szeged with the purpose to collect information about the molecular mechanism of drought tolerance of wheat. These data can serve as molecular markers for fast identification of drought tolerant species, as well as for the establishment of drought tolerant transgenic lines at a later stage.

In the second part of our work, we studied how a G2/M regulated gene is involved in the wound stress response. We focused on analyzing the regulation of B-type cyclin dependent kinase (B-type CDK, or CDKB) in alfalfa (*Medicago sativa* L.). The CDKBs are key regulators of the G2/M checkpoint of the cell division cycle progression in plants. They are characteristically regulated at the transcriptional level and promoter studies are a useful approach to define different signal pathways that can affect the expression of these cell cycle kinases. There are two CDKB classes – CDKB1 and CDKB2 (the expression of CDKB1 precedes the expression of CDKB2). In an earlier work, Magyar et al. (1997) demonstrated that two CDK genes, assigned as *cdc2MsD* and *cdc2MsF* (according to the recent nomenclature *Medsa;CDKB1;1* and *Medsa;CDKB2;1*, respectively) accumulate at the G2/M cell cycle-phase transition. In

the present work, we wanted to clone and characterize the upstream region of the Medsa;CDKB2;1 kinase. Up to now, no detailed investigations have been performed on promoters of B2-type CDKs, which highlights the requirement and the interest to improve our understanding about their regulation.

Results

I. Differential gene expression in response to drought/osmotic stress in wheat

In order to study the differentially expressed genes under drought stress in wheat, we constructed **subtractive libraries** using both non-treated and PEG-treated seedlings **from** two **wheat cultivars**, the mild drought tolerant cv. Öthalom and the drought tolerant cv. Kobomugi. Clones from the resulting cDNA populations were sequenced and subjected to database screening. According to the characteristics of the proteins coded by the homologous genes, classification into several categories was performed.

Many of the genes are highly homologous to known **drought-responsive genes** and give ideas about the drought/osmotic stress defense system of the tolerant wheat cv. Kobomugi. The survival strategy seems to involve ABA signal pathways for induction of drought related genes, decreased transpiration to limit water loss, solutes (especially carbohydrates) as osmoprotectants, stabilization of the cell wall and cell membranes, and transport of ions for keeping

the homeostasis. The genes coding for proline-rich protein, Rubisco small subunit, Rubisco activase and low temperature and salt responsive protein (early drought induced protein) were highlighted to have putative role in the drought tolerance of cv. Kobomugi. However, these are only *in silico* results and their importance must be further evaluated by investigations on plants. Our expression studies on selected differentially expressed genes confirmed several genes e.g. Rubisco small subunit, low temperature and salt responsive protein (early drought induced protein) , and an unknown protein, are indeed under drought/osmotic stress regulation.

II. Activation of alfalfa B2-type CDK (Medsa;CDKB2;1) by wounding, and ethylene, in a non-cell division-dependent manner

We focused on the isolation and characterization of the upstream region of alfalfa *CDKB2;1* gene. A 360 bp DNA fragment was cloned and its *in silico* analysis revealed the presence of putative motifs related to the cell cycle regulation, light responsiveness and wounding. Comparison with the upstream regions of Arabidopsis B-type CDKs revealed a characteristic promoter structure and we hypothesized that the order of regulatory elements might be necessary for the correct function of these genes.

In order to unravel the propensity of the alfalfa *CDKB2;1* promoter, it was linked to reporter genes (GUS, and luciferase) and characterized in transgenic plants. It was demonstrated that the

reporter activity was restricted to the actively proliferating tissues and regions of intact plants. More detailed studies using synchronized alfalfa cell cultures **confirmed G2/M cell cycle phase-specificity of the promoter**. The expression data of the reporters were in good correlation with that of the endogenous alfalfa *CDKB2;1*. In addition, we compared the promoter activity in heterologous systems such as stably transformed *Arabidopsis*. In Arabidopsis, the cloned promoter worked in a very similar manner as in alfalfa with reporter activity characteristic for proliferating regions.

Based on the *in silico* results, the **wound inducibility** of alfalfa CDKB2;1 was further **evaluated**. The CDKB2;1 wound response was demonstrated in leaves of young seedlings and in a simplified system of detached leaves. The wound induction occured in a non-cell division-dependent manner. Treatment with ethylene precursor, ethephon, could turn on the promoter in a similar manner as mechanical injury without cell division activation. Ethylene is known as one of the wound response mediators, and on the other hand, it can cause a G2/M arrest and stimulate the endoreduplication. We suggest that ethylene might have role at the switch between mitosis and endoreduplication. The accumulation of alfalfa **CDKB2;1 kinase** due to G2/M progression, or by treatment with ethylene, considered as a G2/M-phase inhibitor, emphasizes the **multifuncional role** of this kinase.

Acknowledgement

I am very grateful for the opportunity to obtain a very rich social and scientific experience in the Biological Research Center in Szeged, Hungary.

First of all, I would like to thank to Professor Dr. Denes Dudits who invited me in the Institute of Plant Biology in the frame of Center of Excellence PhD Exchange proram. This chance allowed me to meet a great person and a perfect supervisor, Dr. Laszlo Szilak, who helped me to learn a lot and to improve myself very much. I am thankful for working also with Dr. Aladar Pettko-Szandtner who was another good example for me to follow, and who supported me during my study.

As well, I would like to thank to all the members of Gabor Horvath's and Attila Feher's groups, and the lab of Sandor Bottka, for their help during my work.

BRC is a big scientific center that allows collaboration with members of groups from different institutes. I am very glad and thankful for finding support in the Institute of Plant Biology in the groups of - Ferenc Nagy, Laszlo Szabados, Gyozo Garab, Imre Vass, Zoltan Gombos; in the Institute of Genetics – in the group of Gyorgy Kiss and specially from Ariana Perhald, as well as from members of other groups - Andras Blastyak, Monika Bodogai, Florentina Rus, Robert Markus; in the Institute of Biophysics in the group of Arpad Pardutz; in the Institute of Biochemistry – from Eva Korpos and Ferenc Deak.

Also, I thank to Eva Stelkovics and Laszlo Krenacs from BayBioMed, Szeged, for their collaboration.

I am confident that I would not be able to complete efficiently my PhD work without the support of my dear friends: Laszlo Szilak, Aladar Pettko-Szandtner, Zsuzsanna Varkonyi, Zoltan Gombos, Sashka Krumova, Ozge Gunduz, Rui Branca, Erika Bereczki, Eva Korpos, Pilaiwanwadee Hutamekalin, Ariana Perhald and the habitants and ex-habitants of the ITC house.

Of course, I am thankful to my dear family who keeps on encouraging me and always supports me.

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Zhiponova MK, Pettkó-Szandtner A, Stelkovics É, Neer Z, Bottka S, Dudits D, Fehér A, Szilák L (2006) Mitosis-specific promoter of the alfalfa cyclin-dependent kinase gene (*Medsa;CDKB2;1*) is activated by wounding, and ethylene, in a non-cell division-dependent manner. (*in press*)

Posters and presentations

A.Barbulova, A. Iantcheva, **M. Zhiponova**, M. Vlahova, A. Atanassov: *Establishment of Embryogenic Potential of Bulgarian Alfalfa Cultivars and Creation of Herbicide Resistant Lines* (poster), From Gametes to Embryos, Xth International Conference on Plant Embryology, Nitra, Slovak Republic, 5-8 September, 2001

D. Dénes, J. Pauk, F. Ertugrul, **M. Zhiponova**, A. Mai, L. Szilák, K. Török, J. Gyögyey: *Transgenic Approaches in Cereal Research*, Chinese – Hungarian Workshop on "Molecular Genetics and Breeding in Wheat", Martonvásár, Szeged, Hungary, 21-26 May, 2002

M. Zhiponova, L. Szilák, L. Erdei, J. Györgyey, D. Dudits: *Comparative Approach for the Isolation of Genes Involved in the Osmotolerance of Wheat* (poster), VII Magyar Növényélettani Kongresszus, Szeged, Hungary, 24-27 June, 2002

M. Zhiponova, L. Szilák, L. Erdei, J. Györgyey, D. Dudits: *Comparative Approach for the Isolation of Genes Involved in the Osmotolerance of Wheat* (lecture), Bulgarian-Hungarian Bilateral Seminar, Szeged, Hungary, 23-24 September, 2002

M. Zhiponova, L. Szilák, J. Györgyey, D. Dudits: *Isolation and Characterization of Genes Involved in the Drought Response of Wheat* (poster), ESF-JSPS Frontier Science Meeting for Young Researchers " Functional Genomics - from the bench to bioinformatics", San Feliu de Guixols, Spain, 25-31 October, 2003