Summary of the Ph.D. Thesis

Identification& functional characterization of the Arabidopsis ZINC FINGER PROTEIN 3

PhD thesis

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INTRODUCTION

Plant growth and development are regulated by internal signals like hormones and by external environmental conditions such as light, touch and temperature. One important regulator that coordinates growth and development with responses to the environment is the sesquiterpenoid hormone abscisic acid (ABA). In addition to its role in plant development, ABA plays an important role in the stress response and tolerance of plants to drought, high salinityand to a certain extent cold stress. In the last decades numerous genes controlling ABA biosynthesis and signal transduction were identified by genetic, molecular, biochemical and pharmacological approaches. Many important players of ABA signaling have been identified by genetic screens employing ABA-mediated inhibition of germination or stomatal closure as selection criteria (Koorneef, 1984; Merlot et al., 2002). Screening for ABA insensitive germination led thus to the identification of *ABI1* and *ABI2* genes encoding PP2C type protein phosphatases (Finkelstein and Somerville, 1990), which turned to be components of the ABA receptor complex (Ma et al., 2009; Park et al., 2009; Klingler et al., 2010). Three of the best characterized positive regulators of ABA signaling are transcription factors encoded by the *ABI3*, *ABI4* and *ABI5* genes, which were initially identified by screening for mutants exhibiting ABA-insensitive germination (Koorneef, 1984). ABI3, ABI4 and ABI5 belong to B3, APETALA2 (AP2), and basic leucine zipper (bZIP) domain protein families, respectively, that regulate overlapping subsets of seed-specific and ABA inducible genes, and control seed maturation and germination (Finkelstein and Somerville, 1990; Giraudat et al., 1992; Parcy and Giraudat, 1997; Finkelstein et al., 1998; Finkelstein and Lynch, 2000; Carles et al., 2002; Lopez-Molina et al., 2002; Fujita et al., 2011; Monke et al., 2012). Protein Phosphatase 2CA (AtPP2CA) was identified in a library of cDNA overexpressing lines, which were screened for ability to germinate in the presence of inhibitory concentration of ABA (Kuhn et al., 2006). Similar gain-of-function strategy was employed in the Conditional cDNA Overexpressing System (COS) to identify novel components in ABA signal transduction (Papdi et al., 2008). While ectopic overexpression of regulatory genes may result in dominant dwarfism or reduced fertility (Kasuga et al., 1999; Dinkins et al., 2002), the chemically induced expression in the COS system circumvents such disadvantages and permits the generation of fertile transgenic plants (Rigo et al., 2012). Regulated overexpression of cDNAs was employed in the identification of the small heat shock protein gene*HSP17.6A*, which could confer ABA insensitivity to Arabidopsis, pointing to a novel function of this gene (Papdi et al., 2008).

Here we report on functional characterization of the C2H2-type Zinc Finger Protein 3 (ZFP3), which was identified by screening for ABA-insensitive seed germination using the COS system. *In silico* analysis revealed that there are 176 C2H2-type zinc finger proteins in Arabidopsis, from which 33 are conserved in other eukaryotes, whereas 143 appears to be plant specific (Englbrecht et al., 2004). Members of this gene family have been implicated in the regulation of plant development, including photomorphogenesis, leaf, shoot, flower organogenesis, gametogenesis, seed development and dormancy (Sakai et al., 1995; Chrispeels et al., 2000; Prigge and Wagner, 2001; Dinkins et al., 2002; He and Gan, 2004; Ohno et al., 2004; Takeda et al., 2004). Our data show that ZFP3 and its closest C2H2-type zinc-finger protein homologs (ZFPs) are negative regulators of ABA signaling during germination, influence vegetative development and fertility, and modulate red light signaling in seedling photomorphogenesis.

OBJECTIVES

The COS system was previously created and employed in our laboratory to identify a set of cDNAs conferring dominant stress-tolerance phenotype (Papdi 2008).Our primary interest here has been to use the COS system to identify novel regulators and to understand their role in the complex network of ABA signaling during plant development. For this purpose our goals were as follows:

I. Selection of Arabidopsis plants showing ABA insensitive germination
    a. Transformation of Arabidopsis plants with the inducible COS cDNA library.
    b. Screening for enhanced ABA tolerance and identification of the cDNAs.
    c. Verification of the effect of the identified cDNA by generating independent transgenic lines.

II. Comparing the seedling development and hormone responses in germination and growth assays of loss-of function T-DNA insertion mutants and overexpressing lines for the studied genes.

III. Studying genes closely related to the ones identified and thereby get a better understanding of their function.

IV. Understanding the genetic interaction of the identified genes and other ABA regulatory genes in double mutants and/or double transgenic lines.

V. Identifying the molecular function of the protein of interest using system biology approaches (transcriptome analysis or proteomics).

VI. Propose a model for regulatory pathway(s) controlled by the characterized gene(s).

METHODS

- Transformation and screening of *Arabidopsis thaliana* plants.
- Generation of Arabidopsis transgenic plants by Agrobacterium-mediated transformation.
- Characterization of an Arabidopsis T-DNA insertion mutant.
- Analysis of abiotic stress tolerance in vitro (ABA).
- Protein expression in Arabidopsis protoplasts.

Molecular biology techniques:

- Molecular cloning
- RNA isolation, cDNA synthesis. Gene expression studies by quantitative and semi-quantitative PCR.
- In vivo visualization of reporter constructs (GFP) by confocal microscopy, histochemical staining (GUS)
- Recombinant protein extraction and western blot.

RESULTS

I. We have successfully developed and used the Controlled cDNA OverexpressionSystem, (COS) to identify novel stress regulatory genes.

- Characterization of a cDNA conferring insensitivity to ABA in germination assays has identified the coding region of the small heat-shock protein HSP17.6A suggesting its implication in ABA signal transduction.
- Characterization of the Arabidopsis Zinc Finger Protein 3 (ZFP3), revealed that this nuclear protein acts as a negative regulator of ABA signalling.

II. Further detailed characterization revealed that the Arabidopsis Zinc Finger Protein 3 (ZFP3), together with closely related ZFP factors-ZFP1, ZFP4, ZFP6 and ZFP7, is a negative regulator of ABA signaling.

- We have shown that ZFP3 belongs to the nuclear C2H2 zinc finger protein family and acts as a negative regulator of ABA-suppressed seed germination and early seedling development.

- Over-expression of ZFP3 and the closely related ZFP1, ZFP4, ZFP6 and ZFP7 zinc finger factors confers ABA insensitivity to seed germination while the *zfp3zfp4* double mutant displays enhanced ABA susceptibility.

- Constitutive over-expression of ZFP3ox plants revealed multiple phenotypic alterations in Arabidopsis plants, such as semidwarf growth habit, defects in fertility and enhanced sensitivity of hypocotyl elongation to red but not to far-red or blue light.

- Analysis of genetic interactions with phytochrome and *abi* mutants indicates that ZFP3 enhances red light signaling by photoreceptors other than phyA, and additively increases ABA insensitivity conferred by the *abi2, abi4* and *abi5* mutations.

- We have also shown through genetic studies that ABI5 seem to be epistatic to ZFP3 in control of red light-dependent repression of hypocotyl elongation.

- From the transcriptomic studies we show that subset of genes which are inversely regulated by ABA and ZFP3 are also controlled by light throught a phytochrome light receptor and one or more PIF transcription factors.

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3) Papdi C, **Joseph MP**, Pérez Salamó I, Sabina Vidal, Szabados L (2009) Genetic technologies for the identification of plant genes controlling environmental stress responses. **Funct. Plant Biol**, 36, 696-720. IF:2.471

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Patent:

Szabados L, Koncz C, Ábrahám E, Papdi C, **Joseph MP** (2009) CONTROLLED cDNA OVEREXPRESSION SYSTEM IN ARABIDOPSIS. European Patent: No. PCT/EP2009054946: 24.04.09