UPPSALA
UNIVERSITET

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 13

HDZip I Transcription Factors in *Arabidopsis thaliana*

Expression and Function in Relation to Environmental Stress Conditions

ANNA OLSSON

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2005

ISSN 1651-6214
ISBN 91-554-6150-6
urn:nbn:se:uu:diva-4787

Dissertation presented at Uppsala University to be publicly examined in Lecture Hall, room 3041, Department of Physiological Botany, Uppsala, Friday, March 18, 2005 at 13:00 for the degree of Doctor of Philosophy. The examination will be conducted in English.

Abstract
Olsson, A S B. 2005. HDZip I Transcription Factors in *Arabidopsis thaliana*. Expression and Function in Relation to Environmental Stress Conditions. Acta Universitatis Upsaliensis. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 13. 52 pp. Uppsala. ISBN 91-554-6150-6

The homeodomain leucine zipper (HDZip) proteins constitute a plant-specific family of transcription factors, that based on sequence criteria have been grouped into four classes, HDZip I-IV. This thesis describes the phylogeny, function, expression patterns and regulation of the HDZip class I genes in the model species *Arabidopsis thaliana*.

The phylogenetic analyses, traced duplication history and exon/intron organisation of the 17 class I genes in Arabidopsis show that the genes form six monophyletic groups, clades, with an origin in early plant evolution. All genes are expressed in broad tissue distribution patterns and the majority are responsive to water availability and/or light conditions. The expression of the genes show different patterns and dependence on environmental stress conditions, indicating evolutionary changes within and between clades. Ectopic expression of the genes suggest that they regulate genes in part by conserved mechanisms. Therefore, different functional roles seem to have evolved by a divergence mainly in the regulatory properties of the genes.

Detailed expression analyses of the paralogous HDZip I genes *ATHB7* and *ATHB12* show that they have essentially overlapping patterns of activity in response to abscisic acid, ABA, or water deficit in leaves, stems and roots. The water deficit response of *ATHB7* and *-12* is mediated by ABA and depends on the protein phosphateses ABI1 and ABI2. Transgenic plants with ectopic expression of *ATHB7* and/or *-12*, and *athb7* and *athb12* mutants, reveal that the genes in roots mediate the growth inhibitory effects of ABA. In this aspect of their function they do not overlap. In leaves and stems, the genes might act as growth regulators redundantly with other factors.

Taken together these data suggest that *ATHB7* and *-12* regulate growth in response water deficit and that other HDZip I genes have related functions in response to environmental stress conditions.

Keywords: homeodomain leucine zipper, ABA, transcription factor, water deficit, Arabidopsis thaliana

Anna S. B. Olsson, Department of Evolution, Genomics and Systematics, Department of Physiological Botany, Villav. 6, Uppsala University, SE-75236 Uppsala, Sweden

ISSN 1651-6214
ISBN 91-554-6150-6
urn:nbn:se:uu:diva-4787 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-4787)

This thesis is based on the following papers, which will be referred to in the text by their roman numerals:

I Eva Henriksson, Anna S. B. Olsson, Henrik Johannesson, Henrik Johansson, Johannes Hanson, Peter Engström and Eva Söderman. Class I HDZip genes in *Arabidopsis*: expression patterns and phylogeny. (In manuscript)

II Mattias Hjellström, Anna S. B. Olsson, Peter Engström and Eva Söderman. 2003. Constitutive expression of the water deficit-inducible homeobox gene *ATHB7* in transgenic *Arabidopsis* causes a suppression of stem elongation growth. Plant, Cell and Environment 26, 1127-1136.

III Anna S. B. Olsson, Peter Engström and Eva Söderman. 2004. The homeobox genes *ATHB12* and *ATHB7* encode potential regulators of growth in response to water deficit in *Arabidopsis*. Plant Molecular Biology 55, 663-677.

IV Anna S. B. Olsson, Elin M. J. Övernäs, Peter Engström and Eva Söderman. Involvement of the HDZip genes *ATHB7* and *ATHB12* in ABA related responses in *Arabidopsis*. (In manuscript)

Eva Henriksson and Anna S. B. Olsson contributed equally to paper I.

TABLE OF CONTENTS

INTRODUCTION 7
Arabidopsis thaliana 7
The Arabidopsis genome 7
Transcription factors 9
Arabidopsis as a model organism 11
Environmental stress signalling 12
ABA 12
ABA responses 13
Stress signalling – cross-talk and signal convergence 14
Homeobox genes 18
HDZip genes 18
Functions of HDZip I-IV genes in Arabidopsis 20
Phylogeny in relation to function 22
RESULTS AND DISCUSSION 23
HDZip class I genes 23
Phylogeny of HDZip I genes 23
Divergence in function and regulatory patterns of HDZip I genes 27
The β and γ proteins activate transcription 29
ATHB7 and *ATHB12* 31
ATHB7 and *ATHB12* show wide and overlapping promoter activity patterns 31
ATHB7 and *ATHB12* are regulated by ABA, ABI1 and ABI2 32
ATHB7 and *ATHB12* control growth in response to water deficit conditions 34
SUMMARY 40
Genväg till torktolerans (Swedish summary) 41
ACKNOWLEDGEMENT 43
REFERENCES 45

ABBREVIATIONS

ABA	abscisic acid
ABRE	ABA responsive element
DNA	deoxyribonucleic acid
G-protein	guanine nucleotide-binding protein
HDZip	homeodomain leucine zipper
mRNA	messenger RNA
mya	million years ago
PP2C	protein phosphatase type 2C
RNA	ribonucleic acid
T-DNA	transfer-DNA

The following nomenclature is used in this thesis:
Protein names are written in capital letters, e.g. ATHB12.
Gene names are written in capital italic letters, e.g. *ATHB12*.
Mutant names are written in italic letters, e.g. *athb12-*

INTRODUCTION

Arabidopsis thaliana

The dicot plant *Arabidopsis thaliana,* Arabidopsis (Figure 1), belongs to the crucifer family (Brassicaceae) and is closely related to broccoli, cabbage, cauliflower, radish and rapeseed. In contrast to these, Arabidopsis is not an agricultural crop but grows naturally throughout the temperate parts of the world. Due to the plant's small size and modest growth requirement it thrives *in vitro* and on soil in laboratory conditions. Further, Arabidopsis is self-fertilising, but can easily be cross-pollinated, has a short generation time (6-8 weeks) and produces a large number of seeds compared to other flowering plants.

The Arabidopsis genome

Among the flowering plants Arabidopsis has one of the smallest known genomes. The small genome size was an advantage when sequencing the whole Arabidopsis genome, a project that was finished in the year 2000 when it was published as the first complete plant genome (Arabidopsis Genome Initiative, 2000). The annotation of the genome sequence that was first reported has since been refined (Wortman *et al.,* 2003). A recent estimate of the genome size is 119 Mb and the predicted gene number is 26207 (Wortman *et al.,* 2003; Rensink and Buell, 2004). The sequence is publically available (at http://www.tigr.org/tdb/e2k1/ath1/ath1.shtml) and provides a good genetic basis for detailed functional characterisation of all predicted genes.

No other plant genome has so far been completely sequenced but a draft sequence from the rice genome is published (Goff *et al.,* 2002; Yu *et al.,* 2002). The Arabidopsis genome is significantly smaller than the rice genome that is estimated to be 358 Mb. As a further reference, *Physcomitrella patens*, a model moss, has an estimated genome of 460 Mb (Schaefer and Zrÿd, 2001). The estimated number of genes in rice, 45 000, is around twice that of Arabidopsis (Rensink and Buell, 2004). It has been indicated that rice has the same but larger gene families as compared to Arabidopsis (Goff *et al.*, 2002). One-third of the genes in Arabidopsis and

Figure 1. The model plant *Arabidopsis thaliana*, ecotype Columbia, in different stages of the life cycle. The scale is given for the plants indicated by the horizontal lines.

rice seem to be plant-specific, since they are found in both the plants but not in other organisms with completely sequenced genomes, such as *Drosophila melanogaster*, *Caenorhabditis elegans*, yeast or bacteria (Goff *et al.,* 2002).

The plant and animal kingdoms evolved independently from unicellular eukaryotes into contrasting life forms. Among the present land plants, the mosses derive from the oldest branch in the evolutionary tree of plants. This branch separated from the vascular plants 430 million years ago. Thereafter, the ferns diverged from the seed plant lineage. After the split between monocots and dicots, at least two major duplication events can be traced in the Arabidopsis genome. The resulting paralogous genes are found in segmental duplications, tandem arrays or are scattered all over the chromosomes. One of these duplication events occurred 100 mya or earlier, before the cotton and the Arabidopsis lineages diverged from each other, and the most recent dates to 20 to 60 mya before the divergence of *Brassica rapa* from the Arabidopsis lineage. After this last event major gene loss occurred. This is known since species closely related to Arabidopsis, e.g. *Arabidopsis hallari,* have eight chromosomes whereas Arabidopsis has only five (Blanc *et al.,* 2003 and references therein). These polyploidisation events are the reason why Arabidopsis has a larger proportion of duplicated genes, than Drosophila or C. elegans (Arabidopsis Genome Initiative, 2000).

Plants do not use alternative promoters and splicing as much as animals (Arabidopsis Genome Initiative, 2000). However, new gene functions might have evolved from gene duplication events that either has resulted in the development of a new, altered and/or total loss of original gene function. Blanc and Wolfe (2004) have suggested that gene duplicates involved in signal transduction and transcription preferentially are retained in the genome, and further that those involved in DNA repair have preferentially been lost. This might imply that polyploidisation could be a mechanism for increasing the complexity of regulatory networks and adaptability of plants, resulting in selective advantages to the plant. As plants are sessile organisms, in contrast to animals, these specific gene functions are likely associated with increased plasticity to survive changes in environmental conditions. This thesis describes one gene class; the HDZip I genes, with respect to their function and regulation in response to environmental conditions.

Transcription factors

In all living organisms the process referred to as transcription transforms the inherited genetic information, DNA, into RNA. In eukaryotes the

enzyme RNA polymerase II is responsible for transcription of protein-coding genes into messenger RNAs, mRNAs. The mRNA is then translated to the corresponding protein sequence by the ribosomes and the sum of all proteins will result in the corresponding characteristics, the phenotype, of the individual. mRNAs are short-lived, being degraded soon after synthesis. This means that the composition of mRNAs in a cell quickly can be restructured by altered transcription rates from specific genes. Even though post-transcriptional regulation also influences where and when genes are expressed, the transcription initiation is the stage where the most critical regulation occurs, being the level of regulation that determines which genes to express (Latchman, 1998).

RNA polymerase II associates with general transcriptional regulators, which include the TATA-box binding and associated proteins. The TATA-box is a short AT-rich sequence about 30 base pairs upstream of the transcription start that can be found in most genes. This DNA element ensures accurate positioning of the transcription start site and provides a core promoter, which can produce basal levels of transcription. Transcription by RNA polymerase II is further controlled by regulatory transcription factors, in this thesis referred to as transcription factors. These transcription factors bind to specific short sequences, *cis*-elements, in the promoter of each gene. These elements differ depending on where and in response to what signal the particular gene is expressed. The transcription factors can interact with other factors and with general transcriptional regulators, which affect the activity, binding and stability of the polymerase complex in order to activate and/or repress transcription (Figure 2; Latchman, 1998).

According to the Arabidopsis Genome Initiative (2000), the Arabidopsis genome consists of at least 1533 transcription factors, corresponding to 5.9% of the genes. The number of transcription factors is two or three times higher for Arabidopsis than for Drosophila, C. elegans or yeast (Riechmann *et al.*, 2000). Only 8 to 23% of Arabidopsis transcription factors have related genes in other eukaryote genomes, reflecting an extensive and independent evolution of plant-specific transcription factors. In Arabidopsis 29 classes of transcription factors have been identified and 16 of these appear to be plant-specific. Some of these have DNA binding domains unique to plants and some have plant-specific combinations of domains, which by themselves are found in other organisms (Arabidopsis Genome Initiative, 2000). The large and numerous transcription factor families with a high degree of plant linage specificity indicate that the transcriptional control have been significant in the evolution of the plant kingdom.

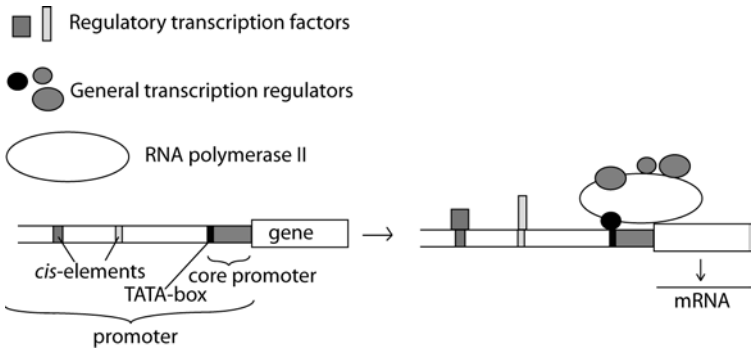

Figure 2. Components involved in the initiation of transcription in eukaryotes. The transcriptional complex consists of RNA polymerase II associated with general transcriptional regulators, including TATA-box binding proteins, and produces basal levels of mRNA. This complex is regulated by regulatory transcription factors that bind to specific control sequences, *cis*-elements, in the promoters, resulting in increased or repressed transcriptional activity.

Arabidopsis as a model organism

The scientific interest in Arabidopsis has led to its adoption as a model organism that is today used by thousands of plant biologists all over the world. During the past 20 years this has resulted in the development of a wide range of techniques to create mutants and transgenic plants, which is of great importance in order to investigate the function of specific genes, as the phenotype of these plants reflects the function of the manipulated gene (reviewed by Somerville and Koornneef, 2002). Furthermore, knowledge gained from Arabidopsis is more or less directly applicable to other plants.

Amazed by plants' plasticity in adapting to changes in the environmental conditions I chose Arabidopsis as a model system to study a class of genes encoding plant-specific transcription factors with a functional genetic approach. Results from this study are described in this thesis.

Environmental stress signalling

Most plants are bound to the place where the seed germinated, well anchored through the root system. The survival of a plant depends on the availability of light, nutrients, temperature and water, which are highly variable factors. In suboptimal growth conditions the plants are stressed. Stress is a naturally and often occurring phenomenon in plants. Plants can respond to stress by altered development to acquire increased tolerance to harsh environmental conditions.

Plants sense signals from the environment by different mechanisms that start molecular processes, which pass on knowledge of the new growth conditions throughout the plant. In response to these signal cascades, adaptive responses are activated. Some response signals are more or less local, whereas others have to be communicated across the whole plant body. Long-distance signalling is often achieved by use of hormones. Plant hormones involved in stress signalling include abscisic acid (ABA), ethylene, brassinosteroids, salisylic acid and jasmonate, of which the most well studied is ABA.

ABA

The stress hormone ABA is a 15-carbon compound (Figure 3) found ubiquitously in vascular plants. ABA is synthesised from carotenoids in cells that contain chloroplasts or amyloplasts (reviewed by: Taiz and Zeiger, 2002; Schwartz *et al.*, 2003), which includes almost all plant cells. The hormone can be stored in the chloroplast, from which it can be released to the apoplast, the cell wall continuum of a plant or organ.

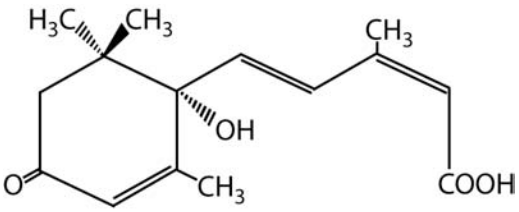

Figure 3. The chemical structure of the naturally occurring *cis*-isomer of abscisic acid, ABA.

ABA acts as an antagonist to the generally non-stress-associated hormones gibberelic acid, cytokinin and auxin, which in contrast to ABA often are referred to as growth activators (Taiz and Zeiger, 2002). The physiological and developmental outcome of hormone signalling is a combinatorial effect that depends on the ratios and levels of concentrations of different hormones more than the absolute concentration of a specific hormone (reviewed by Fedoroff, 2002).

ABA responses

ABA is required in normal growth conditions to regulate the synthesis of seed storage proteins and lipids, to promote seed desiccation tolerance and dormancy, to inhibit the phase transition from embryonic to germinative growth and from germinative to vegetative growth, to regulate growth during the whole lifecycle and is also needed for fertilisation (reviewed by: Leung and Giraudat, 1998; Rock, 2000; Himmelbach *et al.,* 1998; Cheng *et al.,* 2002). In plants exposed to conditions with water deficit or high osmotic potential, ABA accumulates and acts as an endogenous stress signal. Not all stress responses are mediated through ABA and therefore the signalling pathways are sometimes referred to as ABA dependent or ABA independent.

One fast ABA-mediated response to water deficit stress is the closure of stomatal pores to reduce further water loss by transpiration. These pores are situated between two specialised cells, guard cells, in the epidermal cell layer on aerial parts of plants (Figure 4). ABA synthesis increases in leaves only when the turgor falls below zero. This implies that in conditions with only mild water deficit the ABA transport from the roots to the shoot through the xylem is essential for stress responses in the leaves, such as stomatal closure. ABA can also be loaded from the leaves to the phloem and be transported to the roots where it can be deposited or recirculated to the xylem vessels (reviewed by Sauter *et al.,* 2001).

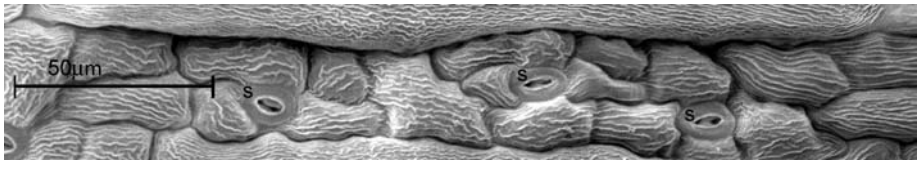

Figure 4. Scanning electron micrograph of a sepal from an Arabidopsis flower bud that shows three stomatal pores (s).

Long-term adaptations to water deficit conditions include stomatal differentiation, synthesis of cuticular waxes on the aerial part of the plants, formation of thorns and hairs, growth inhibition and differential gene regulation for metabolic and developmental adjustments. Many of these processes can also be induced by external application of ABA (Trewavas and Jones, 1991). ABA inhibits shoot and root growth when exogenously applied to well-watered plants, however in water deficit conditions the ABA sensitivity and response changes (Sharp, 2002). At low water potentials, ABA mediates the maintenance of root elongation regulated by the increased ABA level and the change in environmental conditions that alters the growth response. At low water potentials in the shoot, ABA is suggested to both promote and inhibit growth, depending on the duration of water deficit (Sharp, 2002). Not much is known of the differences between shoot and root ABA signalling but one difference is that the ABA signal in roots depends on a functional ethylene response, which the ABA signal in the shoots does not require (Cheng *et al.,* 2002).

Mutant plants that are unable to synthesise ABA have been identified. These *aba*-mutants are similar to wild-type plants when grown under optimal growth conditions. In water deficit conditions *aba*-mutants wilt earlier than the wild-type plants, which as a result of the elevated ABA levels respond with physiological adaptations. ABA induces tolerance to these conditions as well as to ionic, hypoxic, cold, wound or pathogenic stress conditions (Leung and Giraudat, 1998; Rock, 2000; Shinozaki and Yamaguchi-Shinozaki, 2000).

Mutants that have a reduced response to ABA have also been isolated. Two of the most studied are the ABA insensitive, *abi1-1* and *abi2-1* (Bertauche *et al.,* 1996; Leung *et al.,* 1997). Compared to wild-type, the mutant plants both exhibit similar phenotypic alterations, including reduced seed dormancy, reduced ABA sensitivity during germination, root elongation and stomatal closure (Koornneef *et al.,* 1984; Finkelstein and Sommerville, 1990; Leung *et al.,* 1997). In addition to these mutants several ABA insensitive as well as hypersensitive mutants have been isolated (reviewed by Finkelstein, 2002) indicating a complex ABA signalling pathway.

Stress signalling – cross-talk and signal convergence

The first step in environmental stress signalling is the recognition of the stress. Plants have potential receptor-like kinases suggested to act as osmosensors in plants (Urao *et al.,* 1999; Tamura *et al.,* 2003). These are believed to transmit the messages of stress to ABA receptors, not yet identified, or to the ABA independent pathway (reviewed by: Zhu, 2002;

Finkelstein *et al.,* 2002; Yamazaki *et al.,* 2003). Osmotic stress signals are further downstream mediated by components such as G-proteins, phospholipases, Ca^{2+} and other secondary messengers, protein kinases, protein phosphatases, pH, redox status, a wealth of transcriptional regulators and factors involved in RNA and protein processing, that enable the plant to respond to the prevalent environmental condition (reviewed by: Fedoroff, 2002; Zhu *et al.,* 2002; Himmelbach *et al.,* 2003; Chinnusamy *et al.*, 2004).

Early events in ABA signalling involve G-proteins, phopholipases, protein kinases and phosphatases that are not specific to ABA or osmotic stress signalling. Examples of this are that both auxin and ABA signalling are dependent on the single G_{α}-subunit, GPA1, of G-proteins (Ullah *et al.,* 2001; Wang *et al.*, 2001). The mitogen activated protein kinases, MAPKs, are also assumed to converge diverse signals (reviewed by Chinnusamy *et al.,* 2004). The fast and slow ABA response pathways are another example of pathways that share components. When plants respond to ABA by closing their stomata, this occurs within minutes and involves changes in activity of various signalling molecules and ion channels. The long-term adaptive processes, e.g. inhibition of growth and germination, are much slower and involve regulation of gene expression. However, based on mutants such as *abi1-1* and *abi2-1,* which have defects in both the fast and the slow responses, it is evident that the two sets of responses share signal components (Leung *et al.,* 1994, 1997; Meyer *et al.,* 1994). The concept of ABA-dependent and ABA-independent pathways seemed for a while to represent two straightforward roads of signal transduction. However, some stress-responsive genes are regulated by both pathways and some are dependent on only one of the two (Shinozaki and Yamaguchi-Shinozaki, 2000).

Ca^{2+} is an example of a stress signalling components that have been shown to confer specificity. The Ca^{2+} levels oscillate in specific patterns that depend on the kind of stress condition. Down stream it is suggested that the specificity of induced Ca^{2+} oscillation is preserved based on the notion that the Ca^{2+} sensory SOS3-SOS2 pathway is specific for ion-homeostasis and the SCaBP5-PKS3 is specific for the ABA-induced Ca^{2+} signalling (Guo *et al.,* 2002; reviewed by Chinnusamy).

Finally, since plants have so many transcription factors they have the potential to fine-tune the response to the prevalent environmental condition, by use of transcriptional regulation. Independently of the type and former path of the signal a cell perceives, the signal is transduced to the nucleus where transcription factors are the final components in the pathways that determine which genes to activate or repress and thereby determine their activities (Figure 5). Accumulating evidence (e.g. Seki 2002

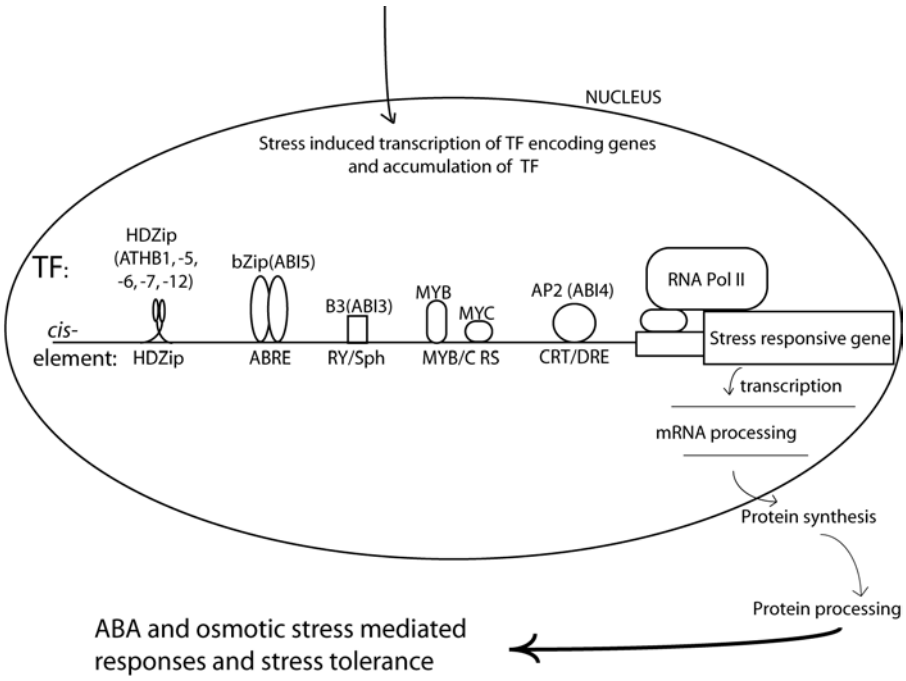

Figure 5. A schematic representation of ABA and osmotic stress induced gene expression. ABA or osmotic stress signals are transmitted to the nucleus (indicated with an arrow into the nucleus), where examples of different classes of transcription factors (TFs) accumulating in response to these signals are shown. Transcription factors mentioned in the text are given as examples of the different classes, shown within parentheses. These factors bind to recognition sequences, *cis*-elements, found in the promoters of stress-inducible genes and direct the RNA polymerase II to transcribe these genes. Known *cis*-elements are the HDZip binding site (Homeodomain leucine zipper site; Johannesson *et al.,* 2001; Paper I), ABRE (ABA responsive element), RY/Sph element, MYBRS and MYCRS (MYB and MYC recognition sequences respectively) and DRE/CRT (drought/cold responsive element; reviewed by: Schinozaki and Yamaguchi-Shinozaki, 1997; Finkelstein *et al.,* 2001; Zhu, 2002). mRNA processing, protein synthesis, protein modifications and degradation is also regulated by ABA and stress signals (Lois *et al.,* 2003; reviewed by; Kuhn and Schroeder, 2003; Fedoroff, 2002) and influence the expression of genes, resulting in responses in the cell that increase the stress tolerance (adopted from Himmelbach *et al.,* 2003).

a, b) shows that many types of transcription factors are regulated by and regulate stress responses (reviewed by Shinozaki, 2003). Among the genes with ABA or osmotic stress inducible transcript levels, members of the following gene families are found: AP2, Zinc-finger, WRKY, MYB, bHLH, NAC, HD, bZip and others (Seki *et al.,* 2002a, b). Examples of transcription factor with well-documented roles in ABA signal transduction are ABI3, ABI4 and ABI5. The *abi3, abi4* and *abi5* mutants show reduced ABA responses that mainly are restricted to seed development and all are suggested to act in the same signalling pathway (Finkelstein, 1994; Nakamura *et al*., 2001; Lopez-Molina *et al*., 2002).

Some physiological stress symptoms, like reduced growth, are common to different stress signals. Water deficit stress can be imposed by limited watering, salt and low temperature, all resulting in osmotic and associated oxidative stress conditions. Other symptoms are more specific. This is illustrated by that plants exposed to soil-water deficit respond by reducing stomatal and cuticular water loss and maximising water uptake by the roots; salt stressed plants respond primarily by osmotic adjustments; and during low temperature the prevention of ice-nucleus formation, through water export from intra- to extracellular space, plays a major role in maintaining osmotic homeostasis.

It is evident that the stress signalling pathways constitute a complex network where cross-talk and convergence occur at different levels and where transcription factors act to regulate the specificity of the signal to mediate an optimal response and stress tolerance in plants.

Homeobox genes

Homeosis is the phenomenon where one part of a body, or an organ, attains features typical for another part or organ of the body. Mutations causing such abnormalities are referred to as homeotic mutations and have been found in a range of organisms, including plants. One well-studied example of this phenomenon is the Drosophila mutant *antennapedia* that has legs on the head in the position normally occupied by antennae. The corresponding gene *Antp* was the first homeotic gene to be identified by cloning (McGinnis *et al.,* 1984; Scott and Weiner, 1984). It was shown that this and other genes with related functions have a 180 base pair sequence in common that became known as the homeobox. This DNA motif encodes a 60 amino acid domain, the homeodomain, HD, which can bind to DNA in a sequence specific manner (Gehringer, 1990; Kissinger *et al.,* 1990). Homeodomain proteins have been shown to act as transcription factors.

Homeobox genes are present in all eukaryotes. Homeobox genes in animals are evolutionarily conserved and act as essential molecular switches to control the establishment of the body plan, the identities of different body segments and specific organs. The homeobox genes in plants are diverse; the gene family in Arabidopsis including 89 different genes, mostly of unknown functions.

HDZip genes

Among the 89 homeobox genes in Arabidopsis (Reichmann *et al.*, 2000) 47 encode proteins in which the homeodomain at the C-terminal end is flanked by a leucine zipper (Ruberti *et al.,* 1991; Mattsson *et al.,* 1992; Schena and Davis, 1992; Schrick *et al.,* 2004; Paper I). These are plant-specific proteins and constitute the HDZip family. The leucine zipper domain forms a α-helix with leucine or methionine residues (Landschulz *et al.,* 1988) at every seventh position on the same face of the helix, allowing dimerisation through hydrophobic interactions between two such factors (Figure 6; Landschulz *et al.,* 1988, Sessa *et al.,* 1993; Meijer *et al.,* 1997; 2000; Frank *et al.*, 1998; Johannesson, 2001). HDZip protein dimers interact with defined pseudopalindromic DNA sites (Sessa *et al.,* 1993, 1998) either as homo or hetero dimers (Sessa *et al.,* 1993; Meijer *et al.,* 1997, 2000; Frank 1998; Johannesson *et al.,* 2001). Based on sequence similarity and shared

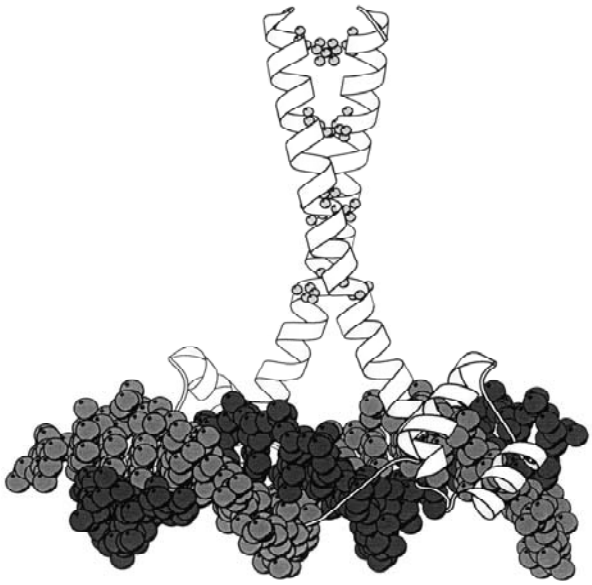

Figure 6. A model of two HDZip protein domains that bind DNA, based on the three-dimensional structure of the *Drosophila melanogaster* engrailed homeodomain (Kissinger *et al.,* 1990) and the yeast GCN4 leucine zipper (Ellenberger *et al.,* 1992; Figure by K. Johansson, H. Johannesson and E. Söderman, unpublished). The homeodomain consist of three α-helices and an extended N-terminal arm. The N-terminal arm fits into the minor groove and the most conserved helix, helix three, fits directly into the adjacent major groove of DNA (Kissinger *et al.,* 1990)

intron/exon patterns, four classes of HDZip genes, I-IV, are recognised (Sessa *et al.,* 1994). The class I HDZip member ATHB5 has been shown to form homodimers *in vitro* and can heterodimerise with the class I members ATHB6, -7, -12 and -16 but not with ATHB1, which is also a class I protein (Johannesson *et al.*, 2001). Further, *in vivo* experiments in yeast show that ATHB16 can form heterodimers with ATHB6 (Wang, 2001). In a yeast two hybrid screen for interacting partners to the class II HDZip protein CPHB-1 from *Craterostigma plantagineum*, only interaction with the class II protein CPHB-2 could be confirmed (Frank *et al.,* 1998). This suggests that HDZip heterodimer formation is selective and differs between and within class I and II. The leucine zippers of HDZip III and IV proteins differ from the ones of classes I and II. The leucine zipper domain of HDZip III proteins is most similar to the classic leucine zippers found in all eukaryotic organisms and the class IV leucine zippers have an internal loop (Schrick *et al.,* 2004), which possibly excludes heterodimerisation between members of different classes. In addition to the HDZip domain, class II, III and IV but not class I

proteins have other characteristic domains. HDZip II proteins contain a set of conserved cysteine residues in the dimerisation domain, and this motif is also found within the variable helix of HDZip IV proteins. The cysteine residues are suggested to mediate redox regulation of DNA binding activity (Tron *et al.,* 2002). HDZip III and IV members differ from the class I and II proteins by having a lipid/sterol-binding START domain (Ponting and Aravind, 1999; Schrick *et al.,* 2004).

Functions of HDZip I-IV genes in Arabidopsis

In contrast to the founding members of the homeobox genes in Drosophila, the loss-of-function, by mutation, of HDZip genes has not been associated with severe phenotypic effects. The data on functions of these gene products have mostly been obtained by the use of gain-of-function transgenic plants harbouring constructs with a CaMV 35S promoter fused to the cDNA of individual HDZip genes.

HDZip I

In Arabidopsis the HDZip class I includes 17 genes (Paper I). Detailed data on the functional roles of ATHB7 and -12 as negative regulators of growth in water deficit conditions are discussed in a separate chapter of this thesis (Papers II, III and IV). *ATHB3, -13, -20* and *-23*, are referred to as the POC genes based on the pointed cotyledons caused by high-level expression of any of those genes in transgenic Arabidopsis (Hanson, 2000). This phenotypic deviation is caused by reduced lateral expansion of the cotyledon cells. The POC phenotype caused by *ATHB13* depends on sucrose sensing, and ATHB13 also affects sucrose responses on gene expression (Hanson *et al.,* 2001). Adult plants that constitutively express the POC genes have an increased degree of serration of the leaf edges, are severely dwarfed and have delayed reproductive development. Beside the POC-phenotype the plants that constitutively express *ATHB3* show inhibited primary root development and premature initiation of adventive roots. It is further suggested that the POC genes regulate the transcription of each other (Hanson, 2000). Transgenic plants with high-level expression of *ATHB5* have short hypocotyls, roots and petioles of the cotyledons. In addition these plants show increased sensitivity to ABA inhibition of seed germination and seedling growth and also increased ABA responsiveness in the ABA-induced accumulation of *RAB18* gene expression (Johannesson *et al.,* 2003). Transgenic plants with constitutive expression of *ATHB6* or *-16* suggest that those genes reduce the response to photoperiod in the transition from vegetative to reproductive development. These plants also show an

increased degree of serration of the leaf edges, like the plants with elevated levels of the POC gene transcripts (Hanson, 2000; Wang *et al.,* 2003; Henriksson, 2004). Ectopic expression of *ATHB6* or *-16* also reduces cell expansion in leaves and stem, and *ATHB16* also in flowers and siliques (Wang *et al.,* 2003; Henriksson, 2004). Further, *ATHB16* as well as *ATHB5* and *-6,* acts as a positive regulator of blue light inhibition of hypocotyls' growth (Wang *et al.*, 2003; Henriksson, 2004). ATHB6 also acts as a negative regulator of the ABA signal pathway during seed germination and stomatal closure (Himmelbach *et al.,* 2002). Constitutive expression of *ATHB1* in transgenic plants causes changes in leaf development including deetiolated phenotypes in the dark (Aoyama *et al.,* 1995). The effect on plants of altered levels of transcript of the other seven HDZip I genes has not been determined.

Given these data it appears as if a common function of the HDZip class I members is to regulate cell expansion in relation to environmental stimuli.

HDZip II

The Arabidopsis genome contains nine HDZip II/HAT genes: *ATHB2/HAT4, ATHB4* and *-17, HAT1, -2, -3, -9, -14* and *-22*. The RNA accumulation of *ATHB2* and *-4* are increased by far-red-rich light (FR light, associated with dusk, dawn or canopy shade; Carabelli *et al.,* 1993, 1996). The distinct and coordinated effects on cell expansion and cell proliferation in the hypocotyls and cotyledons of plants in environments with low R:FR ratios are conducted by ATHB2 (Steindler *et al.,* 1999). ATHB2 induced hypocotyl elongation seems to be dependent on the auxin transport system. HAT2 has been found to regulate auxin-mediated morphogenesis. Both HAT2 and ATHB2 negatively regulate their own expression (Ohgishi *et al.,* 2001; Sawa *et al.,* 2002) and *35S::HAT2* plants have also been found to have decreased mRNA levels of other HDZip II genes including *ATHB2*, and *-4, HAT1, -3, -9* and *-22* (Sawa *et al.,* 2002).

HDZip III

Five class III genes exist in Arabidopsis. *PHB* and *PHV* are paralogous HDZip III genes and form a clade with *REV/IFL1*. All three contribute to the establishment of a functional apical meristem and to adaxial tissues in lateral organs, where REV mostly is involved in vascular development (Emery *et al.,* 2003). The other two HDZip III genes, *ATHB8* and *ATHB15*, are likewise involved in vascular development. *ATHB8* is positively regulated by auxin and acts in wounding recovery (Baima *et al.,* 1995, 2001).

HDZip IV

There are 16 genes that belong to class IV in Arabidopsis. *GL2/ATHB10* was the first HDZip gene that was found to be linked to a mutant phenotype (Reire *et al.*, 1994). The mutant phenotype shows that GL2 promotes trichome differentiation in the shoot epidermis (Reire *et al.,* 1994) and suppresses root-hair formation in the root epidermis (Di Christina *et al.,* 1996). Other genes of the class IV have also been implicated in regulating gene expression specific to the outermost cell layer (Abe *et al.,* 2003 and references therein).

Phylogeny in relation to function

The functions and structures of HDZip III and IV genes are different from HDZip I and II genes. This correlates with HDZip I and II sharing a common origin, distantly related to the HDZip III and IV genes (Chan *et al.,* 1998). Both class I and II genes are implicated in the plant's growth response to environmental signals, but the genes differ in their heterodimer preferences, which can be linked to their evolutionary divergence. The connection between function and phylogeny within subclass I has not been examined before but is addressed in this thesis (Paper I).

RESULTS AND DISCUSSION

HDZip class I genes

The Arabidopsis genome has been shaped by ancient polyploidisation events followed by chromosomal rearrangements (Arabidopsis Genome Initiative, 2000; Blanc *et al.*, 2003). These events have created gene families, of which the HDZip genes constitute one example. In addition to ten previously reported HDZip class I genes (*ATHB1, -3, -5, -6, -7, -12, -13, -16, -20* and *-23*), searches of Arabidopsis databases resulted in the identification of seven additional genes with sequence similarities specific to the HDZip class I genes (Paper I). These were named *ATHB21, -22, -40, -51, -52, -53* and *-54* (Paper I).

Phylogeny of HDZip I genes

To reconstruct the phylogeny of the Arabidopsis HDZip I genes, the amino acid sequences of the HDZip domains of the class I genes and the class II gene *HAT1* were subjected to a parsimony analysis, which resolved six clades with bootstrap support over 50% (Figure 7A; Paper I). We called these clades α (containing *ATHB3, -20, -13* and *-23*), β (containing *ATHB1, -5, -6* and *-16*), γ (containing *ATHB7* and *-12*), δ (containing *ATHB21, -40* and *-53*), ε (containing *ATHB22* and *-51*) and Φ (containing *ATHB52* and *-54*). To further verify the evolutionary relationships, the nucleotide sequences, which correspond to the HDZip matrix described above, were used to reconstruct the phylogeny by use of Bayesian interference algorithms (Figure 7B; Paper I). The analyses gave similar result except that *ATHB1*, in relation to clade β differed. In both analyses, the statistical significance for the association of *ATHB52* and *-54*, in clade ϕ, was weak.

Within the HDZip motifs, introns are found in four different positions, all different from the locations of the two introns in the HDZip class II gene *HAT1* (Figure 7C). The α, β and γ genes have one intron just downstream of the fifth leucine in the leucine zipper. *ATHB1* has an additional intron in helix 1 of the homeobox, a unique intron position among the HDZip I genes. The δ and ε genes share another intron position, localised between the fourth and fifth leucine, whereas the Φ genes lack introns in this sequence. This supports the phylogenetic association between clade α, β and γ as well as between clade δ and ε.

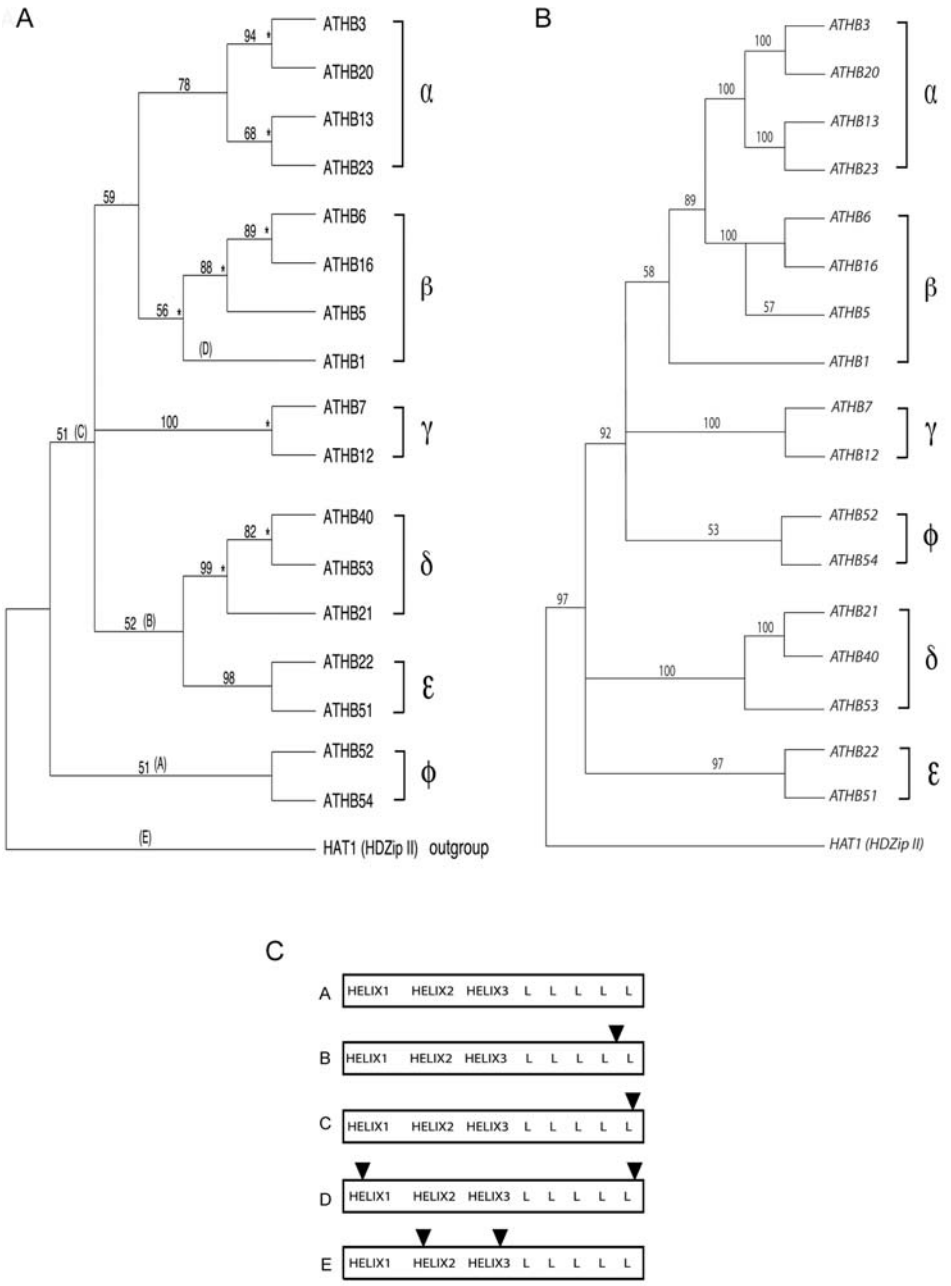
A
ATHB3
ATHB20
ATHB13
ATHB23
ATHB6
ATHB16
ATHB5
ATHB1
ATHB7
ATHB12
ATHB40
ATHB53
ATHB21
ATHB22
ATHB51
ATHB52
ATHB54
HAT1 (HDZip II) outgroup
α
β
γ
δ
ε
ϕ
B
ATHB3
ATHB20
ATHB13
ATHB23
ATHB6
ATHB16
ATHB5
ATHB1
ATHB7
ATHB12
ATHB52
ATHB54
ATHB21
ATHB40
ATHB53
ATHB22
ATHB51
HAT1 (HDZip II)
C
A
B
C
D
E
HELIX1
HELIX2
HELIX3
L

Figure 7. The phylogenetic relationship between the HDZip class I genes reconstructed with (A) parsimony analyses or (B) with Bayesian interference algorithms. (A) Bootstrap support is given in % on the branches and the letters, A to E, indicate the intron/exon patterns within the HDZip encoding motifs (illustrated in C). The asterisk (*) indicates phylogenetic relationships supported by gene duplication data (Paper I). Monophyletic clades with more than 50% in bootstrap support from parsimony analyses are indicated by brackets and named α, β, γ, δ, ε and ϕ. (B) The numbers given on the branches are the estimated posterior probability distribution, in %, of the characters of the tree, given a priori likelihood distribution of different parameters including the sequence matrix and a model for nucleotide substitution rates. The tree depicted is the resulting majority rule tree.

The Arabidopsis genome contains numerous large duplicated chromosome segments that share similar genes in the same order. A map of these traceable duplication blocks has been constructed by Blanc *et al.* (2003). By use of this map we have reconstructed the duplication history of the HDZip class I genes (Paper I). An overview of the duplication events is depicted in Figure 8. A complete duplication of the genome occurred 20-60 mya (Blanc and Wolfe, 2004), resulting in the duplicated gene pairs *ATHB3/20*, *ATHB13/23, ATHB6/16*, *ATHB7/12* and *ATHB21/40*. Older duplicates, *ATHB5/6* and *ATHB40/53*, originate from polyploidisation events in early angiosperm evolution, around 100 mya. By use of a pseudogenome (Blanc *et al.,* 2003), in which all the recently duplicated blocks have been merged, even older polyploidisation events can be traced. This map indicates that *ATHB1/5* and *ATHB21/53* potentially are duplicates with ancient origins, and supports the association of *ATHB1* to the β clade as indicated by the parsimony analyses (Figure 7A). The genome localisations of *ATHB22, -51, -52* and *-54* are covered by duplicated blocks, but no traceable duplicates to these genes are found (Paper I), possibly due to gene loss or major gene alterations. Thus the duplication history supports the monophyletic origins of the β, δ and γ clades and *ATHB3/20* and *ATHB13/23* within the α clade.

No duplications were traced between the α, β, γ, δ, ε and ϕ genes, probably reflecting an ancient establishment of the different clades. In rice HDZip I genes have been found that possess highest sequence similarity to Arabidopsis HDZip I clade β and γ genes (Meijer *et al.,* 2000), suggesting that the clades were established before the split between the mono- and the dicot lineages (Figure 8). Analyses of moss and fern genes show that HDZip genes are present in these plant but that the α-ϕ clades were established after the divergence of the ferns from the lineage leading to the angiosperms (Aso *et al.*, 1999; Sakakibara *et al.,* 2001). Furthermore, genes with high similarity to class I, II and III genes have been found in the moss *Physcomitrella patens*. Thus, the genomic events that gave rise to these classes' founding members may have taken place in early plant evolution,

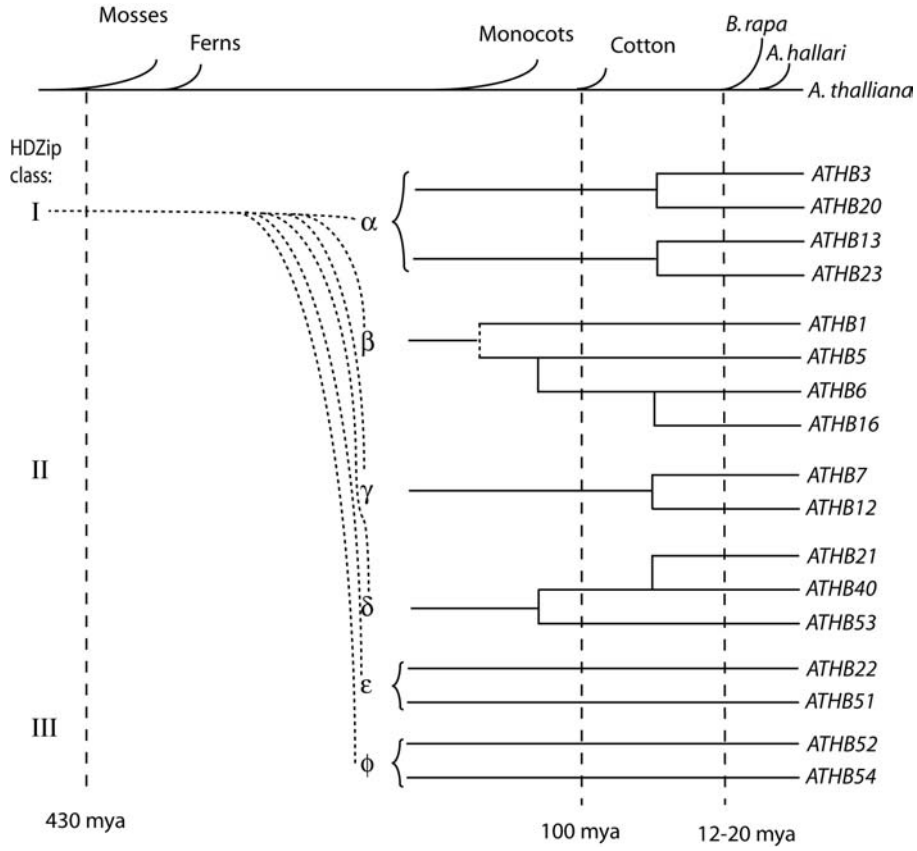

Figure 8. Model of evolutionary events that have formed the HDZip class I in *Arabidopsis thaliana*. The x-axis indicates major events in the evolution of plants. Dashed vertical lines indicate an approximate time scale. Lines separating from the x-axis upwards indicate divergences of the *A. thaliana* lineage from *Physcomitrella patens* (a moss), *Ceratopteris richardii* (a fern), rice (a monocot), cotton, *Brassica rapa* and *Arabidopsis hallari.* The first appearance of class I, II and III genes and the assumed timing of the genes ancestral to HDZip class I α, β, γ, δ, ε and Φ clades are indicated by their position. The traceable duplication events and the approximate time when these occurred are indicated by the positions of branch points that join the resulting gene pairs. The duplication event resulting in *ATHB1* and *ATHB5,* is ancient and the approximation of timing uncertain indicated with a dashed branch line. The model is based on references given in the text.

before vascular plants and the mosses separated, 430 mya (Sakakibara *et al.,* 2001). The most ancient evolutionary event that have shaped the HDZip genes is traced based on the notion that homeodomains and leucine zippers are found in separate protein classes in eukaryotic organisms outside the plant kingdom. The juxtaposition of the domains in HDZip proteins is thought to have occurred after the divergence of plants and animals, as a result of an exon capture event (Schena and Davis, 1994).

Divergence in function and regulatory patterns of HDZip I genes

Genes originating from a duplication event and, thus, from one ancestral gene might be expected to show similarities in expression pattern and function. We found that most HDZip I genes have broad expression patterns (Figure 9A). Organs with no transcription of a certain HDZip I gene had mRNA of at least one other gene of the same clade, indicating that the regulatory sequences that determine the location of α, δ, ε and ϕ gene expression have been subjected to evolutionary change among the genes in the clades. All 17 members are transcribed, and hence no pseudogenes exist in the HDZip class I in Arabidopsis. This and the many duplicates present within HDZip class I, suggest that the HDZip genes have been preferentially retained at high frequency in the genome during evolution rather than being lost by mutation (Paper I).

Since water deficit, ABA or light regulate the transcript levels of *ATHB5, -6, -7, -12* and *-16* (Söderman *et al.,* 1996, 1999; Lee and Chun, 1998; Johannesson *et al.,* 2001; Henriksson, 2004) we investigated if these conditions also affected the expression of other HDZip class I genes (Figure 9; Paper I). The majority of the HDZip class I genes' transcript levels were responsive to ABA or water deficit treatments and were expressed at different levels in dark, white or blue light grown seedlings (Figure 9B; Paper I). Regulatory properties have been conserved between members of clades γ and δ, respectively, since all these genes showed increased transcript levels in response to ABA and water deficit stress, and to a lesser extent between genes within α and β (Figure 9B; Paper I). This is illustrated by the down- regulation of the transcript level of three genes (*ATHB1, -5* and *-16*) within β in response to water deficit treatments and the up-regulation of one *(ATHB6*). It is known that *ATHB5* transcription is impaired in *abi3-1* but not in *abi1-1* or *abi2-1* mutant backgrounds (Johannesson *et al.,* 2003), whereas *ATHB6* transcript levels are reduced in

A

		5d	12d	R	L	St	F	Si
α	*ATHB3*	+	+	+	-	+	+	+
	ATHB20	+	+	+	+	+	+	+
	ATHB13	+	+	-	+	-	+	-
	ATHB23	+	+	+	+	+	-	+
β	*ATHB1*	+	+	+	+	+	+	+
	ATHB5	+	+	+	+	+	+	+
	ATHB6	+	+	+	+	+	+	+
	ATHB16	+	+	+	+	+	+	+
γ	*ATHB7*	+	+	+	+	+	+	+
	ATHB12	+	+	+	+	+	+	+
δ	*ATHB21*	+	+	+	+	+	+	+
	ATHB40	+	+	+	-	-	+	+
	ATHB53	-	-	+	-	-	+	-
ε	*ATHB22*	+	+	-	-	-	-	+
	ATHB51	+	+	+	+	+	+	+
ϕ	*ATHB52*	+	+	+	-	-	+	-
	ATHB54	-	-	-	+	+	+	+

B

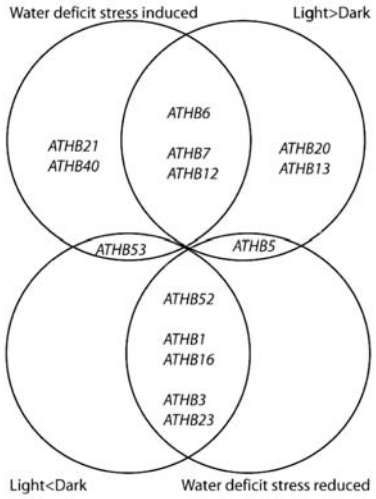

Figure 9. Expression patterns of the HDZip class I genes. (A) The presence (+) or absence (-) of transcript of the HDZip I genes in 5-day-old seedlings (5d), 12-day-old seedlings (12d), roots (R), rosette leaves (L), stems (St), flowers (F) and siliques (Si) detected with reverse transcriptase-mediated PCR analysis (Paper I). The relations between the genes as interpreted from parsimony analyses are indicated to the left. (B) Based on RNA gel blot analyses (Paper I) genes are grouped in genes with transcript levels that increase in response to water deficit stress imposed by salt treatment (upper left circle), genes with higher transcript levels in light compared to darkness (upper right circle), genes with higher transcript levels in darkness as compared to light (lower left circle) and genes negatively regulated by water deficit stress (lower right circle).

abi1-1 and *abi2-1* but not in *abi3-1* (Söderman *et al.,* 1996), demonstrating that the upstream components in the ABA signalling pathway, which regulate HDZip gene expression, differ within the β clade. Furthermore, the light regulation within the β clade has diverged, as *ATHB1* and *-16* transcript levels were higher in darkness than in white light and the *ATHB5* and *-6* levels were slightly down-regulated. In clade α one gene from each of the two duplicates was higher in light than in darkness (*ATHB13* and *-20*), whereas the other two were higher in the dark conditions (*ATHB3* and *-23*; Figure 9B; Paper I). Divergence in regulatory responses within HDZip I subclass δ have also been shown by Son *et al.* (2005), who recently reported that the transcript levels of *ATHB40* and *-53,* but not *ATHB21,* increase in response to auxin treatments and that cytokinin inhibits the auxin effect on *ATHB53* but not *-40*. Although the duplicated genes once had the same function and expression profiles, their responsiveness to different

environmental stimuli as well as the distribution of transcripts in different organs have diverged.

Ectopic expression of the α, β and γ genes show that they are all involved in different aspects of growth-regulation but that the gene function has diverged, especially between but also within, subclasses as evident from the β genes (Aoyama *et al.,* 1995; Hanson *et al.,* 2001; Himmelbach *et al.*, 2002; Johannesson *et al.*, 2003; Wang *et al.,* 2003; Henriksson *et al.*, 2004; Paper II, III). Ectopic expression of either *ATHB6* or *-16*, indicate a conservation of function between the two duplicated gene products (Wang *et al.,* 2003; Henriksson, 2004), whereas elevated levels of *ATHB1* and *-5* cause different effects indicating that the β gene products differ from each other (Aoyama *et al.,* 1995; Johannesson *et al.,* 2003). In contrast, the effect of reduced transcript levels of these genes on the blue light growth response of hypocotyls show that their functions partly overlap (Henriksson, 2004). In contrast to the other class I genes the transcript level of *ATHB1* in blue light was reduced both in comparison to white light and darkness. Supported by previous functional data, this implicates *ATHB1* in light-regulated processes (Aoyama *et al.,* 1995; Paper I). Further, the four α genes (*ATHB3, -20, -13* and *-23*) act similarly as negative regulators of lateral cell expansion in leaves when ectopically expressed (Hanson, 2000; Hanson *et al.,* 2001; Johannesson *et al.,* 2001). The function of *ATHB13* differs from *ATHB3, -20* and *-23* in being dependent on sucrose (Hanson *et al.,* 2001). The α genes are an example of a subclass where the function of the gene products seems to be largely conserved but where the regulation of gene expression has diverged more extensively. The γ genes *ATHB7* and *-12* differ from the other duplicated genes in class I by showing conservation in both function of the gene product and expression patterns (Figure 9; Paper I, II, III, IV). Analyses of mutant or transgenic plants with reduced transcript levels of *ATHB5, -6, -7, -12* or *-13,* caused by transfer-DNA, T-DNA, insertion mutation or ectopic expression of the cDNA in antisense orientation, have not demonstrated any drastic effects on plant phenotype (Hanson *et al.,* 2001; Johannesson *et al.*, 2003; Henriksson, 2004; Paper II, III, IV). This might indicate a functional overlap between the genes and common target genes to the HDZip I members. Data on the φ, δ or ε genes' phenotypical effects are as yet missing. Interestingly the φ gene *ATHB52* and the δ gene *ATHB53* transcript levels were up-regulated in darkness suggesting darkness-related functions.

The β and γ proteins activate transcription

A subset of HDZip I and II members have been shown to activate and repress transcription in plants, respectively (Aoyama *et al.*, 1995; Meijer

1997, 2000; Sessa *et al*., 1998; Steindler, 1999; Sawa *et al*., 2002). These proteins interact with the pseudopalindromic DNA site CAATNATTG with different preferences for the base in the central position (Sessa *et al.,* 1993; Gonzalez *et al.,* 1997; Meijer *et al.,* 1997; Frank *et al*., 1998; Johannesson *et al.,* 2001). Within class I, in *in vitro* experiments, homodimers of ATHB3, -13, -5, -6 and -16 bind to this sites, whereas ATHB7 and -12 do not, indicating that the targets of these two factors differ from the other HDZip I members (Sessa *et al.,* 1993; Johannesson *et al.,* 2001).

To examine if these β and γ factors function similarly as transcriptional regulators and bind to the CAATNATTG site *in vivo*, transient transactivation assays in Arabidopsis rosette leaves were performed. ATHB1 has previously been found to activate transcription when bound to these sites (Aoyama *et al*., 1995; Sessa *et al*., 1998) and was thus used as a positive control. Similarly, ATHB5, -6, -7, -12 and -16 activated transcription interacting with the same DNA site (Paper I). This supports the notion that these class I genes interact with the same target sites and regulate genes by similar mechanisms.

On the one hand, the different phenotypic effects resulting from ectopic expression of α, β and γ genes and of the different binding specificities *in vitro* between the proteins, indicate that their target genes are distinct. On the other hand, ectopic expression of either of *ATHB1, -5, -6, -7, -12* or *-16*, show that all gene products have the ability to reduce plant cell expansion and, further, all activate transcription in Arabidopsis leaves suggesting common target genes. This might indicate that the selective heterodimer formation between the class I proteins and their different regulatory patterns could contribute to the functional difference between the HDZip I members. Together the class I HDZip genes might act to integrate different environmental input stimuli to regulate the expression of common target genes. Possibly, such an integrating network could be an evolutionary advantage that could explain why the HDZip I class members have been retained in the Arabidopsis genome.

ATHB7 and *ATHB12*

The Arabidopsis HDZip class I paralogous genes *ATHB7* and *-12* share over 80% identity in the deduced amino acid sequence of their HDZip domains. The transcript levels of both genes are similarly regulated by ABA or water deficit conditions (Söderman *et al.,* 1996; Lee and Chun, 1998). From these facts we formulated the hypothesis that *ATHB7* and *-12* are regulated by the same mechanisms and might have related functions. During this project I have examined this hypothesis and characterised the function of these two factors.

ATHB7 and *ATHB12* show wide and overlapping promoter activity patterns

Analogous promoter fragments of *ATHB7* and *-12* were cloned as translational fusions to the *uidA/GUS* reporter gene (Paper II and III) and Arabidopsis plants were transformed with these constructs. The promoters were noticeably similar in two regions containing ABRE sites in close proximity to coupling-element-like sites, a complex shown to confer ABA-induced transcription (Izawa *et al.,* 1993; Shen and Ho, 1995; Shen *et al.,* 1996).

In plants grown in optimal growth conditions the GUS staining patterns caused by the *ATHB7* or *-12* promoter activities were observed in axillary shoot primoria, flower buds, anther filaments and developing seeds. ATHB12 activity was also observed in lateral root primordia, young leaves, inflorescence stems and in a zone below stigmatic papillae after pollination and later in nectaries (Paper III). Further, weak *ATHB7* activity was shown in two zones on opposite sides of the differentiation/elongation zone of root tips (Paper III). The *ATHB7* promoter seems to be active mainly in the same cells as the *ATHB12* promoter, whereas the *ATHB12* promoter is active in a broader pattern in normal growth conditions.

When exposed to ABA or water deficit conditions, the *ATHB7* and *-12* promoter activity patterns expanded and more or less totally overlapped with distinct activities in the elongation/differentiation zone of the root tips, lateral root primordia, whole roots but especially the vasculature, whole cauline and rosette leaves, cotyledons, inflorescence stems and in flower organs (Paper II, III). Based on the similarities in putative ABA regulatory sequences and the GUS staining patterns the regulatory properties that are responsive to water deficit stress in *ATHB7* and *-12* seem to have been conserved. These regulatory data suggest that *ATHB7* and *-12* are active in a broad and overlapping pattern in plants and that they function in response

to water deficit conditions (Paper II, III). As the *uidA* constructs are translational fusions it is likely that the sub cellular pattern of both *ATHB7* and *-12* promoter activity, which can be seen in Paper III, reflect nuclear localisations of the hybrid proteins, consistent with the role of HDZip proteins as transcriptional regulators (Paper III).

ATHB7 and *ATHB12* are regulated by ABA, ABI1 and ABI2

As regards the time course of expression in response to water deficit, *ATHB7* and *-12* were nearly identical (Figure 10A). Treatment of plants with ABA also increased the mRNA accumulation of *ATHB7* and *-12* following parallel time courses (Figure 10B; Paper III). ABA deficient *aba* mutants did not respond to water deficit conditions with increased *ATHB7* and *-12* transcript levels as the wild-type did, showing that these transcriptional responses are dependent on ABA (Söderman *et al.,* 1996; Paper III).

ATHB7 and *-12* transcript levels were neither responsive to the stress hormones ethylene or salisylic acid, nor to stress conditions caused by chilling, flooding, or wounding, indicating that the genes are responsive to water deficit specific signals (Paper III). However according to Jiao *et al.,* (2003) the shift from darkness to blue light strongly up-regulates *ATHB7* and *-12* transcript levels, a response that they share with many other transcription factor genes involved in water deficit signalling. It is suggested that this might be a mechanism by which etiolated seedlings

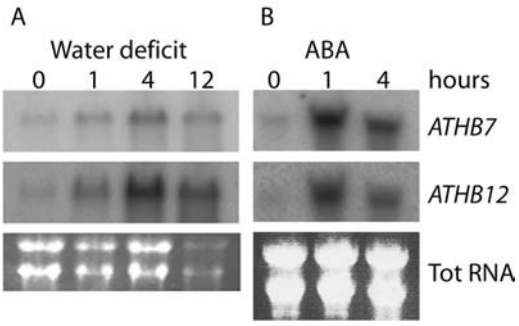

Figure 10. Time course of *ATHB7* and *ATHB12* transcript accumulation in response to water deficit or ABA treatments. Transcript levels of *ATHB7* and *ATHB12* in two-week-old Arabidopsis seedlings grown in liquid culture and (A) transferred to filter paper to dehydrate for 0, 1, 4 and 12 hours or (B) treated with 10 μM ABA for 0, 1 and 4 hours. The bottom panels show the ethidium-bromide-stained RNA gels before blotting.

prepare for a photomorphogenic development, which requires more water for respiration and photosynthesis (Jiao *et al.,* 2003). Compared to wild-type, the blue light receptor *cry1*/*cry2* double mutant shows stronger induction of both genes in blue light (Jiao *et al.,* 2003; our unpublished results), suggesting a common signalling mechanism in the light regulation of *ATHB7* and *-12*. Both *ATHB7* and *-12* are further reported as regulated by plant pathogen response signalling but by different mechanisms (Chen *et al.,* 2002; our preliminary results), showing that the genes can respond to stress conditions other than water deficit.

In the dominant negative *abi1-1* and *abi2-1* and in the putative *ABI1* and *ABI2* loss-of-function mutants *abi1-1R1*, *abi1-1R5*, *abi2-1R1*, *abi1-1R4*/*abi2-1R1* and *abi1-1R5*/*abi2-1R1*, the ABA-induced *ATHB7* and *-12* transcript levels were reduced compared to the wild-type levels (Paper III). However, in assays of ABA responses involved in seed germination and growth the *abi* and the *abiR* plants show opposite effects in response to ABA, being insensitive and hypersensitive, respectively (Koornneef *et al.,* 1984; Finkelstein and Sommerville, 1990; Leung *et al.,* 1997; Gosti *et al.,* 1999; Merlot *et al.,* 2001). The wild-type proteins ABI1 and ABI2, are Serine/Threonine protein phosphatases type 2C, PP2C, which phosphatase activities increase in response to ABA treatment. Both the original *abi* and the *abiR* mutants do have reduced phosphatase activities (Bertauche *et al.*, 1996; Leung *et al.,* 1997; Gosti *et al.,* 1999; Merlot *et al.,* 2001), indicating that the reduced ABA induction of both *ATHB7* and *-12* correlates with the low phosphatase activities in these plants.

ABA-induced *ATHB7* and *-12* transcript levels were lower in the double mutants *abi1-1R4*/*abi2-1R1* and *abi1-1R5*/*abi2-1R1* compared to the single mutants *abi1-1R4*, *abi1-1R5* and *abi2-1R1*, indicating that ABI1 and ABI2 are partially redundant with respect to their regulation of *ATHB7* and *-12* (Paper III). In the double *abiR* mutants *ATHB7* and *-12* mRNA levels were not as severely altered as in the *abi1-1* or *abi2-1* single mutants. This indicates that there might be additional redundant PP2Cs that compensate for the loss of the ABI-phosphatase activities in the *abiR* mutants. Three other phylogenetically close PP2C proteins, AtPP2CA, HAB1 and HAB2, are involved in ABA signalling and therefore likely to act with related function to ABI1 and ABI2 (Sheen, 1998; Rodriguez *et al.,* 1998; Tähtiharju and Palva, 2001; Saez *et al.*, 2004).

Based on that ATHB7 and -12 interact with the HDZip I consensus site in Arabidopsis leaves but not in *in vitro* experiments, like the other HDZip I factors tested, it is likely these two factors require cell-mediated modifications to bind DNA. Since ATHB6 has been shown to interact with ABI1 in yeast (Himmelbach *et al.,* 2002) it is tempting to suggest that the PP2C proteins also at the protein levels could affect ATHB7 and -12. A critical serine residue within the homeobox of ATHB6 contributes to the ABI1 interaction and ATHB7 and -12 also have this serine as well as additional putative phosphorylation sites that are absent from the other

HDZip I homeoboxes. However, ATHB7 and ABI1 cannot interact in yeast, suggesting that other modifying enzymes might be involved.

ATHB7 and *ATHB12* control growth in response to water deficit conditions

To address the question of what function *ATHB7* and *-12* have in plants, we studied the phenotypic effects of ectopic expression of *ATHB7* and/or *-12* driven by CaMV 35S promoter in transgenic Arabidopsis plants. Since ectopic effects can cause phenotypic defects, that do not directly reflect the endogenous gene function, we also screened available mutant collections for T-DNA insertions in *ATHB7* or *-12*. We identified mutants with inserts either in the promoter, which reduced the transcript level of the mutated gene, or in the C-terminal end, which probably generate a truncated protein without the domain suggested to be required for transcriptional activation (Lee *et al.,* 2001). The T-DNA insertions therefore likely cause loss-of-function mutations in *ATHB7* and *-12*, respectively (Tables 1 and 2; Paper III, IV).

Table 1. *Summary of transgenic plants with ectopic expression of ATHB7 and/or ATHB12.*

Plant line	Inserted construct	Effect on transcript levels	Ecotype	Reference
M4	*35S::ATHB7*	elevated *ATHB7*	Ws	II, III
S5	*35S::ATHB7*	highly elevated *ATHB7*	Ws	II, III
12s	*35S::ATHB12*	elevated *ATHB12*	Ws	III
*12s7s**	*35S::ATHB7/-12*	elevated *ATHB7 and -12*	Ws	III

* Constructed by transformation of the M4 line with the *35S::ATHB12* construct.

Table 2. *Summary of identified mutant alleles.*

Allele	Position of T-DNA	mRNA of mutated gene	Collection	Eco-type	Ref.
*athb7-1**	*ATHB7* UTL	data missing	Feldman	Ws	*
athb7-2	*ATHB7* 2nd exon	truncated	Wisconsin-basta	Col	IV
athb7-3	*ATHB7* UTR	no ABA response	GABI-Kat	Col	IV
athb12-1	*ATHB12* 2nd exon	truncated	T. Jack	Col	III,IV
athb12-2	*ATHB12* UTR	non-detectable	Wisconsin-basta	Ws	III
athb12-3	*ATHB12* UTR	reduced level	Wisconsin-alfa	Ws	III
athb12-4	*ATHB12* UTR	reduced level	Wisconsin-basta	Ws	III
*athb12-5**	*ATHB12* UTR	reduced level	Wisconsin-alfa	Ws	*
athb12-6	*ATHB12* UTL	non-detectable	GABI-Kat	Col	IV

* Mutant allele identified but not further characterised. Abbreviations: Ref=reference, UTL=untranslated leader, UTR=untranscribed region of the promoter.

As ATHB7 and -12 have similar sequences, promoter activities and expression patterns, it is likely that their functions are similar. The effect of the loss of one of the genes could be covered by the function of the other and we therefore crossed the *athb7* mutants with *athb12* mutants. The resulting double *athb7/12* mutants were studied to examine if the genes act functionally redundant to each other.

Since the transcript levels of the *ATHB7* and *-12* are responsive to water deficit conditions and depend on ABA, ABI1 and ABI2, we analysed the response to ABA of the transgenic and mutant plants. In one such assay, transgenic plants with high-level expression of *ATHB7* and/or *-12* all showed enhanced response to ABA inhibition of root elongation as compared to wild-type (Figure 11; Paper III). Further, both *athb7* and *athb12* single mutants were less sensitive to ABA inhibition of root elongation than wild-type (Figure 11; Paper III, IV). This indicates that ATHB7 and -12 have unique functional roles in the roots that do not overlap completely with any other gene (Paper III, IV). Double *athb7/-12* mutants also showed reduced sensitivity to ABA to degrees comparable to that of the single *athb7* and *athb12* mutants', indicating that the functions of ATHB7 and -12 do not overlap, but likely affect the same or similar targets as components of the same protein complex, possibly an ATHB7/-12 heterodimer. The promoter activity patterns of the genes show that the genes are active in the same cells in the expanding part of the roots, in response to ABA exposure, which would support the possibility of such an interaction and the role of the gene products as regulator of growth. Ectopic expression of *ATHB7* and/or *-12* did not cause increased sensitivity to ABA in roots to the same severe degree as shown for *abi1-1R5/abi2-1R1* and *athb7/12* mutants did not show as severe insensitivity as *abi1-1* and *abi2-1*. Based on these notions it is likely that ATHB7 and -12 act parallel to other factors with similar functions, in ABA signal transduction in the roots.

Neither the transgenic plants with ectopic expression of *ATHB7* and/or *-12* nor the *athb7* or *athb12* mutants showed any difference from wild-type in assays that analyse seed dormancy, seed germination in response to ABA or water loss through stomata. This suggests that these processes are independent of *ATHB7* and *-12* or that additional, functionally overlapping, genes are present in the plants (Paper II, III, IV).

Wild-type plants exposed to water deficit conditions reduce their shoot growth (Figure 12A) as well as increase transcript levels and promoter activities of *ATHB7* and *-12* in stems and leaves (Figure 12B; Paper III). Like plants exposed to water deficit, 12s, 12s7s, M4 and S5 plants have high levels of *ATHB7* and/or *-12* transcripts and showed reduced growth of these organs (Figure 12C). These notions suggest that ATHB7 and -12 are involved in the water deficit response in above ground organs and might act as growth regulators. The time of flower initiation of these plants did not differ but the onset of stem elongation was delayed

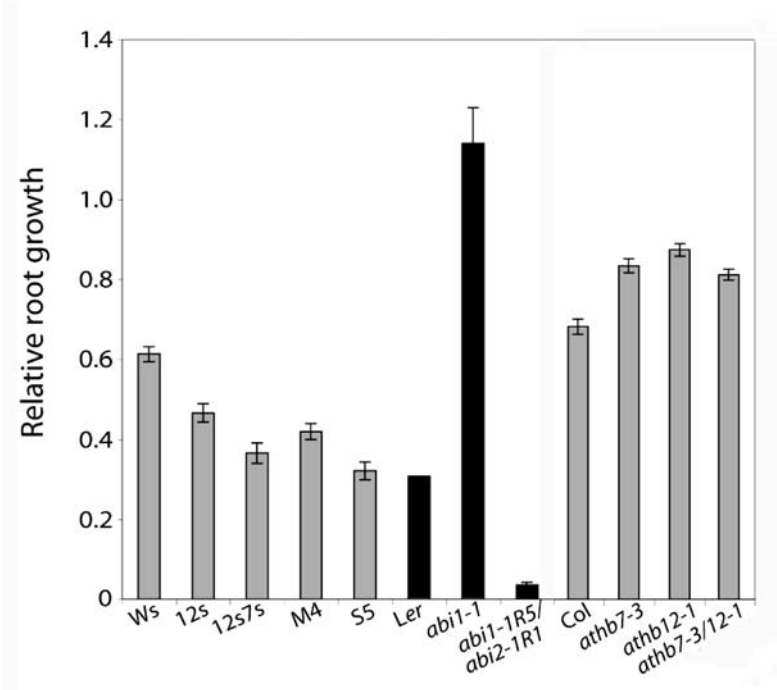

Figure 11. Inhibition of root growth in response to ABA. Four days' growth of roots on media containing 10 μM ABA expressed as a fraction of seedlings' growth on control media. Transgenic plants with elevated levels of *ATHB7* (M4 and S5), *ATHB12* (12s) or *ATHB7* and *-12* (12s7s) and representative lines for *athb7* and *athb12* mutants. L*er*, *abi1-1* and *abi1-1R1*/*abi2-1R1* were included as ABA-insensitive and ABA-hypersensitive controls; the corresponding data is coloured in black.

(Paper II, III) and based on results from *35S::ATHB7* plants this reduction in the stem growth was due to effects on expansion rather than on cell division (Paper II). The ratio between stem length in normal growth conditions and stem length in water deficit conditions was similar when comparing wild-type plants with the *35S::ATHB7* plants (Paper II). This indicates that ATHB7 acts in concert with other components mediating the water stress inhibition of stem elongation. Further, the minor effect on stem elongation caused by *athb7*/*athb12* mutant indicates functional overlap between ATHB7, -12 and other factors.

High-level expression of *ATHB7* and/or *-12* in transgenic plants also resulted in increased number of branches. This phenotype and the reduced elongation growth of stems, leaves as well as roots in response to ABA was enhanced in the 12s7s line as compared to 12s or M4 plants. The S5 plants express *ATHB7* at a higher level than the 12s7s, but these two

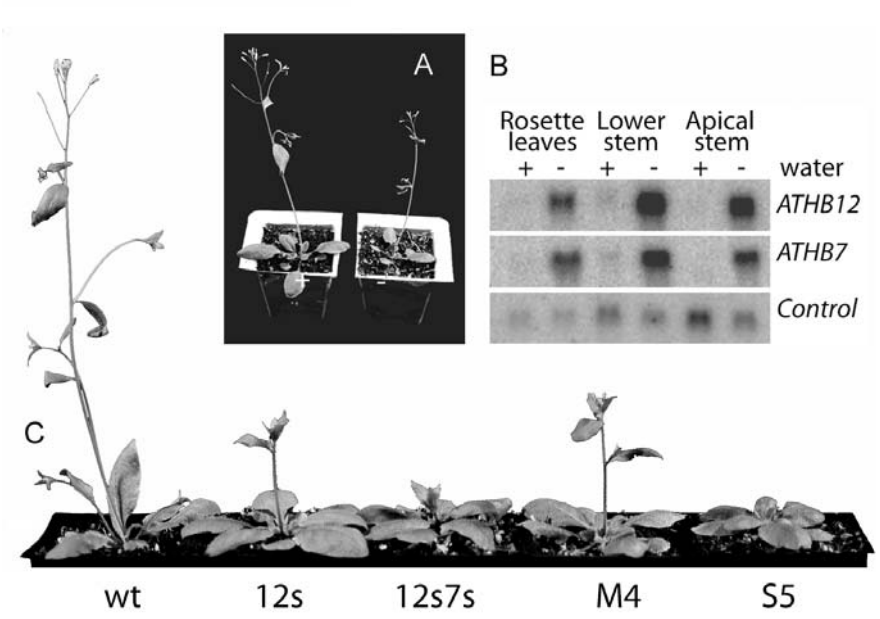

Figure 12. ATHB7 and ATHB12 mediate the regulation of growth in water deficit conditions. (A) Arabidopsis wild-type plants grown in normal (+) or water deficit conditions (-). (B) mRNA levels of *ATHB7* and *ATHB12* in rosette leaves, basal and apical parts of the inflorescent stem of 28-day-old wild-type plants grown under normal (+) or water deficit (-) conditions. The control panel shows the same membranes with RNA rehybridised with a ribosomal gene fragment (Baima *et al.,* 1995; Paper III). (C) Transgenic plants with high-level mRNA accumulation of *ATHB12* (12s), *ATHB12* and *ATHB7* (12s7s) or *ATHB7* (M4 and S5) 28 days after germination.

lines show phenotypes with similar deviations from wild-type (Paper III), supporting the suggestion that ATHB7 and -12 can have the same target genes or their target genes have similar effects. Our transient expression assays in Arabidopsis leaves also showed that both the factors bind to the same DNA site, supporting that the factors have common target genes (Paper I).

Interestingly, the ATHB7 and -12 inhibitory functions on stem growth were independent of ABA or water conditions, while their involvement in root growth was dependent on ABA (Paper II, III). This indicates that ATHB7 and -12 have similar functions above and below ground and that it must be other components that define the shoot/root differences of growth responses in relation to ABA.

The rosette leaves of plants with ectopic expression of *ATHB7* and/or *-12* had shorter petioles and the leaf blades were more rounded in

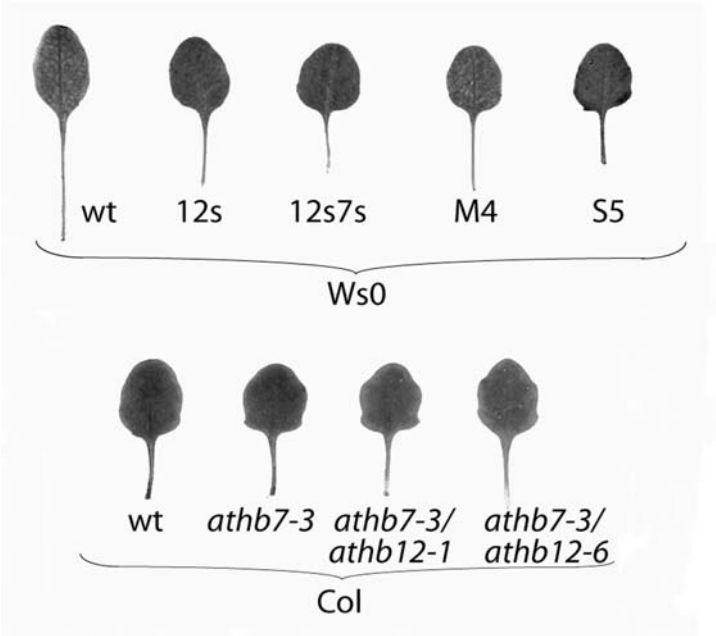

Figure 13. The fourth rosette leaf from representative individuals of 21-day-old Ws0 wild-type (wt) or transgenic plants harbouring the construct *35S::ATHB12* (12s), *35S::ATHB7* and *35S::ATHB12* (12s7s) or *35S::ATHB7* (M4 and S5) and 22-day-old Colombia wild-type (wt) plants and with the mutated *athb7-3, athb7-3/athb12-1* or *athb7-3/athb12-6* alleles.

shape than the wild-type (Figure 14; Paper II, III). The area of the *35S::ATHB7* plants' rosette leaves showed no difference as compared to wild-type, therefore the reduction in growth is probably not caused by reduced photo assimilation (Paper II). The only indication from mutant data of a functional role for either of the genes in leaves was displayed by one of the *athb7* mutants, *athb7-3*, which had an increased degree of serration of the leaf edges (Figure 13; Paper IV). ATHB7 might therefore have a unique endogenous function in leaf development. Based on that *ATHB7* transcript in *athb7-3* are found at levels similar to wild type in normal conditions but do not increase in response to ABA it is likely that this ATHB7 function is dependent on ABA. Further, since *athb7-2* produce a truncated *ATHB7* transcript and do not display the leaf serration phenotype it is likely that this function do not dependent on the ability of ATHB7 to activate gene expression.

Taken together, transgenic plants with elevated transcript levels and mutant plants with loss-of-function of *ATHB7* and/or *-12* suggest functional roles of the factors in the ABA-mediated growth response in roots. The deviations in stem and leaf phenotypes of the transgenic plants resemble the

growth pattern of wild-type plants subjected to water deficit conditions (Figures 12 and 13; Paper II, III). The results indicate that ATHB7 and -12 are involved in the regulation of growth in water deficit conditions. This model is in agreement with the notion that the transcript levels as well as promoter activities of *ATHB7* and *-12* in stems, leaves and roots are clearly up-regulated in response to ABA or water deficit conditions (Figure 12B; Paper II, III). The subtle phenotypic deviation displayed by the single and double *athb7*/*athb12* mutants indicate that the functions of *ATHB7* and *-12* overlap with the function of other genes. We have found other class I HDZip genes, *ATHB6, -21, -40* and *-53*, that show similar ABA and osmotic stress-regulated expression and therefore might compensate for the loss of *ATHB7* and *-12* functions (Paper I). The potential functional redundancy emphasises the importance of analysing mutants with loss-of-function alleles of more than one HDZip I gene. Examining these mutants in a broad range of growth conditions might reveal the plants' requirements for the genes. Interestingly, the potential functional redundancy, the many retained duplicates within HDZip I, the responsiveness of the HDZip I genes to environmental signals, and their wide and divergent transcript distribution might reflect their function to integrate different environmental signals to regulate plant growth. Such a network would give plants an ability to adapt to changes in the environment, and therefore could explain why HDZip I members have been retained in the Arabidopsis genome.

SUMMARY

There are 17 HDZip class I genes in *Arabidopsis thaliana,* derived by genome duplications from six clades established early in plant evolution. No psudogenes are found within HDZip I indicating a selective advantage for retention of the HDZip I factors in the Arabidopsis genome. The genes are expressed in broad tissue distribution patterns and the transcript levels of many HDZip I genes are responsive to water and light conditions. The regulatory properties of the genes show extensive divergence between anciently as well as recently duplicated genes. All tested HDZip I members can regulate growth and act as transcriptional activators that bind to the CAAT(N)ATTG site *in planta,* indicating more conserved functional roles of the gene products.

ATHB7 and -12 are potential regulators of growth in response to water deficit conditions. This conclusion is based on the fact that transgenic plants with high-level expression of *ATHB7* and/or *-12* show reduced growth of stems and leaves and the ABA or water deficit dependent expression patterns of the genes support endogenous functions in these organs. The transcript levels of both *ATHB7* and *-12* are regulated by water deficit conditions in similar manners, dependent on ABA and the phosphatases ABI1 and ABI2. Based on the fact that the *athb7* and *athb12* mutants and the transgenic plants expressing *ATHB7* and/or *-12* at high level were insensitive and hypersensitive, respectively, to ABA-mediated inhibition of root elongation growth, *ATHB7* and *-12* are shown to be involved in the post-germinative ABA growth response. Double *athb7*/*12* mutants showed ABA responses in root similar to those of the single *athb7* and *athb12* mutants, indicating that ATHB7 and -12 interact and that both have unique functional roles in root elongation growth in response to water deficit conditions. Since the phenotypic deviations in stem and leaf in *athb7* and *athb12* mutants were subtle, it is likely that ATHB7 and -12 act functionally redundant to other factors in stems and leaves.

Genväg till torktolerans (Swedish summary)

Växter har ett sensoriskt system för att kunna läsa av signaler i omgivningen och anpassa sig till rådande miljöbetingelser. Tillgång på vatten och ljus är två viktiga faktorer som styr växtens tillväxt och utveckling, t.ex. är det avgörande för om ett frö ska gro eller inte, hur mycket växten kan växa och för när blommor ska bildas. Om tillgången på vatten är låg, utsätts växten för stress och måste hushålla med det vatten den har. Genom att minska sin tillväxt av skottet klarar växter sig bättre under torka, växtens torktolerans ökar således. Som svar på förändringar i omgivningen startar signalkedjor i växten som aktiverar olika responser. Vid vattenbrist ökar produktionen av stresshormonet abskissinsyra, ABA, och många av växtens transkriptionsfaktorer aktiveras. Transkriptionsfaktorer är proteiner som styr när och var andra gener ska uttryckas. Detta sker genom transkription, som är den process genom vilken den ärvda genetiska informationen, DNA, omvandlas till RNA. RNA omvandlas i sin tur till protein och alla proteiner tillsammans avgör hur organismen är utformad. Andelen gener som kodar för transkriptionsfaktorer är större i växter än i andra organismer. Denna mångfald kan vara en orsak till att växter har kunnat anpassas till ett liv fast förankrade i marken.

I min forskning har jag studerat transkriptionsfaktorer, som karakteriseras av en homeodomän, som kan binda till DNA, och en leucinzipper som kan binda två liknande proteiner till varandra. Framförallt har jag studerat en klass av dessa, HDZip klass I, med avseende på deras inbördes släktskap, reglering och funktion. För att kunna göra detta har jag använt modellväxten *Arabidopsis thaliana* (Backtrav). Arabidopsis kräver inte stora utrymmen och är lätt att odla, har kort generationstid (ca sex veckor), och producerar mycket frön. Forskare över hela världen använder därför Arabidopsis för att studera hur växter fungerar på molekylär nivå.

Inom min forskningsgrupp har vi visat att 17 gener i Arabidopsis tillhör HDZip klass I. Våra evolutionära studier visar att dessa gener härstammar från sex ursprungliga gener som fanns redan innan monokotyledoner och dikotyledoner utvecklades. Generna har under årmiljonernas lopp dubblerats och muterats till de sex subklasser av HDZip klass I-gener som finns idag. Våra studier visar att de flesta finns uttryckta i groddplantor, rötter, rosettblad, blommor och fröskidor, men några av generna har större organspecificitet. Torka eller olika ljusbetingelser visade sig styra hur många av generna var uttryckta. Resultaten från uttrycksanalyserna tyder på att det fortfarande finns vissa likheter i HDZip I-genernas regulatoriska egenskaper men främst tycks denna aspekt av genernas funktion ha utvecklats åt skilda håll under evolutionen.

Två av generna, *ATHB7* och *-12*, utgör en egen subklass inom HDZip klass I och deras reglering och funktion har jag undersökt noggrant.

ATHB7 och *-12* är de gener inom HDZip I som höjer sitt uttryck mest när växter utsätts för torka eller behandlas med ABA. Båda generna torkrespons följer samma tidsmönster och styrs av ABA och två proteinfosfataser, ABI1 och ABI2.

I Arabidopsis är det relativt lätt att introducera en ny DNA-sekvens i växtens arvsmassa. Inom mitt forskningsprojekt har vi producerat sådana transgena växter där genernas reglerande sekvens, promotorn, styr en reportergen. När reporterproteinet uttrycks resulterar det i att en blå fällning kan observeras i växtens celler. *ATHB7* och *-12* visade sig vara aktiverade i ett mönster som överlappar på cellulärnivå i hela växten under torka. Det är troligt att gener som aktiveras under torka också har en funktionell effekt under dessa förhållande.

För att kunna studera funktionen av en specifik gen kan vi förändra växten genetiskt så att uttrycksnivån av genen höjs eller sänks. Transgena Arabidopsisplantor som i likhet med torkstressade plantor uttrycker *ATHB7* och/eller *-12* på höga nivåer visar att ATHB7 och -12 minskar tillväxten av stammar och blad och gör att rötter svarar starkare på ABA. Den reducerade tillväxten gör att de transgena växterna liknar en växt utsatt för torka och indikerar att generna kan styra växtens torktolerans.

Efter att ha studerat effekten av ett förhöjt uttryck av generna studerade jag effekten av ett minskat uttryck. Växter isolerades i vilka generna *ATHB7* och *-12* är muterade, vilket gör att deras funktion är reducerad. Mutanternas rötter kan inte svara på ABA lika bra som omuterade växter vilket tyder på att generna behövs för att växten ska kunna anpassa sig optimalt till torkbetingelser. En av *athb7*-mutanterna har sågtandade bladkanter vilket kan tyda på att ATHB7 har en unik roll i bladutvecklingen. I övrigt ser mutanterna ut som normala Arabidopsisplantor. Likheter mellan generna *ATHB7* och *-12* kan tyda på att de är så lika i sin funktion att om den ena tas bort ur växten kan den andra överta den förlorade genens funktion. För att undersöka denna möjlighet studerade jag dubbelmutanter med reducerat uttryck av båda generna. Dubbelmutanter visade samma ABA-beroende avvikelse i rötter som de enkla mutanterna. Slutsatsen av detta är att de två genprodukterna har separata roller i rotens svar på ABA, som inte är funktionellt likvärda, men att de troligen är komponenter i samma proteinkomplex.

Sammanfattningsvis tyder våra resultat på att ATHB7 och -12 i respons på torka, kan minska tillväxt och att det finns andra gener i Arabidopsis med liknande funktion. Fyra andra HDZip I-gener höjer sitt uttryck likt *ATHB7* och *-12* i respons på ABA och torka och skulle därför kunna reglera tillväxten på liknande sätt.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to colleagues, friends and family who have helped me in various ways to complete this thesis:

To my supervisor Peter Engström for accepting me as a PhD student, for his efforts in teaching me scientific writing and for commenting on my manuscripts.

To my other supervisor Eva Söderman for all time and continuous support she has provided concerning practical things in and outside the lab as well as reading and commenting on my manuscripts.

To Johannes Hanson, Eva Henriksson, Mattias Hjellström, Henrik Johannesson, Henrik Johansson, Yan Wang and Elin Övernäs for small and big HDZip talks.

To Birgitta Ebendal and Anita Wallin for help with administrative matters.

To all of you who have solved computer problems; Jen Sundström, Johannes Hanson, Joel Sohlberg, Pia Lindberg, Fernando Lopes Pinto, Håkan Svensson and Elin Övernäs.

To Anette Axén, Gunn-Britt Berglund, Eva Büren, Marie Englund, Britt-Marie Fogelholm, Gunilla Hjort, Marie Lindersson, Agneta Ottosson, Staffan Tjus och Cecilia Wärdig for their contributions with technical assistance.

To Stefan Gunnarsson and Gary Wife for advice, encouragement and help with microscopes and picture handling.

To Annelie Carlsbecker for sharing her phylogenetic skills.

To Johannes Hansson, Eva Henriksson, Henrik Johansson and Lars Nilsson for critical reading drafts of this thesis along the way.

To Joel and Eva H for being great office-mates, friends and company on conferences and courses during this whole project.

To everyone at Fysbot who in one way or the other contributes in creating a special and positive atmosphere.

To my friends, who have kept me in touch with the world outside the growth-room. With the help of clay, pyssel, food and excursions you have kept my creative side awake.

To my parents for always believing in me and supporting me in every possible way. To my little big sister for always having encouraging answers to everything concerning science as well as life in general. To all my other family members – you are the best!

To Tommy for bringing balance to this project with love, joy, owls and endless support.

To the foundations of Liljewalchs, Hierta-Retzius and Olof Ahlöf for covering travel expenses to conferences.

TACK!

REFERENCES

Abe, M., Katsumata, H., Komeda, Y. and Takahashi, T. (2003). Regulation of shoot epidermal cell differentiation by a pair of homeodomain proteins in *Arabidopsis*. Development 130, 635-643.

Arabidopsis Genome Initiative. (2000). Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 14, 796-815.

Aoyama, T., Dong, C., Wu, Y., Carabelli, M., Sessa, G., Ruberti, I., Morelli, G. and Chua, N. (1995). Ectopic expression of the *Arabidopsis* transcriptional activator *Athb-1* alters leaf cell fate in tobacco. Plant Cell 7, 1773-1785.

Aso, K., Kato, M., Banks, J. A. and Hasebe, M. (1999). Characterization of homeodomain-leucine zipper genes in the fern *Ceratopteris richardii* and the evolution of the homeodomain-leucine zipper gene family in vascular plants. Mol. Biol. Evol. 16, 544-552.

Baima, S., Nobili, F., Sessa, G., Lucchetti, S., Ruberti, I. and Morelli, G. (1995). The expression of the *Athb-8* homeobox gene is restricted to provascular cells in *Arabidopsis thaliana*. Development 121, 4171-82.

Baima, S., Possenti, M., Matteucci, A., Wisman, E., Altamura, M. M., Ruberti, I. and Morelli, G. (2001). The *Arabidopsis* ATHB-8 HD-Zip protein acts as a differentiation-promoting transcription factor of the vascular meristems. Plant Phys. 126, 643-655.

Bertauche, N., Leung, J. and Giraudat, J. (1996). Protein phosphatase activity of abscisic acid insensitive 1 (ABI1) protein from *Arabidopsis thaliana*. Eur. J. Biochem. 241, 193-200.

Blanc, G., Hokamp, K. and Wolfe, K. H. (2003). A recent polyploidy superimposed on older large-scale duplications in the *Arabidopsis* genome. Genome Res. 13, 137-144.

Blanc, G. and Wolfe, K. H. (2004). Functional divergence of duplicated genes formed by polyploidy during *Arabidopsis* evolution. Plant Cell 16, 1679-1691.

Carabelli, M., Morelli, G., Whitelam, G. and Ruberti, I. (1996). Twilight-zone and canopy shade induction of the *ATHB-2* homeobox gene in green plants. Proc. Natl. Acad. Sci. USA 93, 3530-3535.

Carabelli, M., Sessa, G., Baima, S., Morelli, G. and Ruberti, I. (1993). The *Arabidopsis Athb-2* and *-4* genes are strongly induced by far-red-rich light. Plant J. 4, 469-479.

Chan, R. L., Gago, G. M., Palena, C. M. and Gonzalez, D. H. (1998). Homeoboxes in plant development. Biochimica et Biophysica Acta 1442, 1-19.

Chen, W., Provart, N. J., Glazebrook, J., Katagiri, F., Chang, H. S., Eulgem, T., Mauch, F., Luan, S., Zou G., Whitham, S. A., Budworth, P. R., Tao, Y., Xie, Z., Chen, X., Lam, S., Kreps, J. A., Harper, J. F., Si-Ammour, A., Mauch-Mani, B., Heinlein, M., Kobayashi, K., Hohn, T., Dangl, J. L., Wang, X. and Zhu, T. (2002). Expression profile matrix of *Arabidopsis* transcription factor genes suggests their putative functions in response to environmental stresses. Plant Cell 3, 559-574.

Cheng, W. H., Endo, A., Zhou, L., Penney, J., Chen, H.C., Arroyo, A., Leon, P., Nambara, E., Asami, T., Seo, M., Koshiba, T. and Sheen, J. (2002). A

unique short-chain dehydrogenase/reductase in *Arabidopsis* glucose signaling and abscisic acid biosynthesis and functions. Plant Cell 14, 2723-2743.

Chinnusamy, V., Schumaker, K. and Zhu J.-K. (2004). Molecular genetic perspectives on cross-talk and specificity in abiotic stress signalling in plants. J. Exp. Bot. 55, 225-236.

Di Cristina, M., Sessa, G., Dolan, L., Linstead, P., Baima, S., Ruberti, I. and Morelli, G. (1996). The *Arabidopsis* Athb-10 (GLABRA2) is an HD-Zip protein required for regulation of root hair development. Plant J. 10, 393-402.

Ellenberger, T. E., Brandl, C. J., Struhl, K. and Harrison, S. C. (1992). The GCN4 basic region leucine zipper binds DNA as a dimer of uninterrupted alpha helices: crystal structure of the protein-DNA complex. Cell 24, 1223-1237.

Emery, J. F., Floyd, S. K., Alvarez, J., Eshed, Y., Hawker, N. P., Izhaki, A., Baum, S. F. and Bowman, J. L. (2003). Radial patterning of *Arabidopsis* shoots by class III HD-ZIP and KANADI Genes. Current Biol. 13, 1768-1774.

Fedoroff, N.V. (2002). Cross-talk in abscisic acid signaling. Science's STKE 140, RE10.

Finkelstein, R. R. (1994). Mutations at two new Arabidopsis ABA response loci are similar to the *abi3* mutations. Plant J. 5, 765-771.

Finkelstein, R. R. and Sommerville, C. R. (1990). Three classes of Abscisic acid (ABA) – insensitive mutations of *Arabidopsis* define genes that control overlapping subsets of ABA responses. Plant Physiol. 94, 1172-1179.

Finkelstein, R. R., Gampala, S. S. L. and Rock, C. D. (2002). Abscisic acid signalling in seeds and seedlings. Plant Cell 14, Suppl:S15-45.

Finkelstein, R. R., Wang, M. L., Lynch, T. J., Rao, S. and Goodman, H. M. (1998). The Arabidopsis abscisic acid response locus *ABI4* encodes an APETALA 2 domain protein. Plant Cell 10, 1043-1054.

Frank, W., Phillips, J., Salamini, F. and Bartels, D. (1998). Two dehydration-inducible transcripts from the resurrection plant *Craterostigma plantagineum* encode interacting homeodomain-leucine zipper proteins. Plant J. 15, 413-421.

Gehring, W. J., Muller, M., Affolter, M., Percival-Smith, A., Billeter, M., Qian, Y. Q., Otting, G. and Wuthrich, K. (1990). The structure of the homeodomain and its functional implications. Trends Genet. 6, 323-329.

Goff, S. A, Ricke, D., Lan, T. H., Presting, G., Wang, R., Dunn, M., Glazebrook, J., Sessions, A., Oeller, P., Varma, H., Hadley, D., Hutchison, D., Martin, C., Katagiri, F., Lange, B. M., Moughamer, T., Xia, Y., Budworth, P., Zhong, J., Miguel, T., Paszkowski, U., Zhang, S., Colbert, M., Sun, W. L., Chen, L., Cooper, B., Park, S., Wood, T. C., Mao L., Quail, P., Wing, R., Dean, R., Yu, Y., Zharkikh, A., Shen, R., Sahasrabudhe, S., Thomas, A., Cannings, R., Gutin, A., Pruss, D., Reid, J., Tavtigian, S., Mitchell, J., Eldredge, G., Scholl, T., Miller, R. M., Bhatnagar, S., Adey, N., Rubano, T., Tusneem, N., Robinson, R., Feldhaus, J., Macalma, T., Oliphant A. and Briggs, S. (2002). A draft sequence of the rice genome (*Oryza sativa L. ssp. japonica*). Science 296, 92-100.

Gonzalez, D. H., Valle, E. M. and Chan, G. G. (1997). Interaction between proteins containing homeodomains associated to leucine zippers from sunflower. Biochimica et Biophysica Acta 20, 137-149.

Gosti, F., Beaudoin, N., Serizet, C., Webb, A. A., Vartanian, N. and Giraudat, J. (1999). ABI1 protein phosphatase 2C is a negative regulator of abscisic acid signaling. Plant Cell 11, 1897-1910.

Guo, Y., Xiong, L., Song, C., Gong, D., Halfter, U. and Zhu, J. (2002). A calcium sensor and its interacting protein kinase are global regulators of abscisic acid signaling in *Arabidopsis*. Dev. Cell 3, 233-244.

Hanson, J. (2000). Functional characterization of the pointed cotyledon subclass of HDZip genes in *Arabidopsis thaliana*. ISBN 91-554-4846-1, Uppsala: Acta Universitatis Upsaliensis.

Hanson, J., Johannesson, H. and Engström, P. (2001). Sugar-dependent alterations in cotyledon and leaf development in transgenic plants expressing the HDZip gene *ATHB13*. Plant Mol. Biol. 45, 247-262

Henriksson, E. (2004). The HDZip class I transcription factors in *Arabidopsis thaliana* Characterisation of HDZip genes involved in the mediation of environmental signals. ISBN 91-554-6018-6, Uppsala: Acta Universitatis Upsaliensis.

Himmelbach, A., Hoffmann, T., Leube, M., Höhener, B. and Grill, E. (2002). Homeodomain protein ATHB6 is a target of the protein phosphatase ABI1 and regulates hormone responses in *Arabidopsis*. EMBO J. 21, 3029-3038.

Himmelbach, A., Iten, M. and Grill, E. (1998). Signalling of abscisic acid to regulate plant growth. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 353, 1439-1444.

Himmelbach, A., Yang, Y. and Grill, E. (2003). Relay and control of abscisic acid signaling. Curr. Opin. Plant Biol. 6, 470-479.

Izawa, T., Foster, R. and Chua, N. H. (1993). Plant bZIP protein DNA binding specificity. J. Mol. Biol. 230, 1131-1144.

Jiao, Y., Yang, H., Ma, L., Sun, N., Yu, H., Liu, T., Gao, Y., Gu, H., Chen, Z., Wada, M., Gerstein, M., Zhao, H., Qu, L. J. and Deng, X. W. (2003). A genome-wide analysis of blue-light regulation of *Arabidopsis* transcription factor gene expression during seedling development. Plant Physiol. 133, 1480-1493.

Johannesson, H., Wang, Y. and Engström, P. (2001). DNA-binding and dimerization preferences of *Arabidopsis* homeodomain-leucine zipper transcription factors in vitro. Plant Mol. Biol. 45, 63-73.

Johannesson, H., Wang, Y., Hanson, J. and Engström, P. (2003). The *Arabidopsis thaliana* homeobox gene *ATHB5* is a potential regulator of abscisic acid responsiveness in developing seedlings. Plant Mol. Biol. 51, 719-729.

Kissinger, C. R., Liu, B. S., Martin-Blanco, E., Kornberg, T. B. and Pabo, C. O. (1990). Crystal structure of an engrailed homeodomain-DNA complex at 2.8 A resolution: a framework for understanding homeodomain-DNA interactions. Cell 63, 579-90.

Koornneef, M., Reuling, G. and Karssen, C. M. (1984). The isolation and characterization of abscisic acid-insensitive mutants of *Arabidopsis thaliana*. Physiol. Plant. 61, 377-383.

Kuhn, J. M. and Schroeder, J. I. (2003). Impacts of altered RNA metabolism on abscisic acid signaling. Curr. Opin. Plant Biol. 6, 463-469.

Landschulz, W. H., Johnson, P. F. and McKnight, S. L. (1988) The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. Science 240, 1759-64.

Latchman, D. S. (1998). Eukaryotic transcription factors. 3rd ed. Academic press. London.

Lee, Y. H. and Chun, J. Y. (1998). A new homeodomain-leucine zipper gene from *Arabidopsis thaliana* induced by water stress and abscisic acid treatment. Plant Mol. Biol. 37, 377-84.

Lee, Y. H., Oh, H. S., Cheon, C. I., Hwang, I. T., Kim, Y. J. and Chun, J. Y. (2001). Structure and expression of the *Arabidopsis thaliana* homeobox gene *Athb-12*. Biochem Biophys Res Commun. 284, 133-41.

Leung, J., Bouvier-Durand, M., Morris, P. C., Guerrier, D., Chefdor, F. and Giraudat, J. (1994). *Arabidopsis* ABA response gene *ABI1*: features of a calcium-modulated protein phosphatase. Science 264, 1448-52.

Leung, J. and Giraudat, J. (1998). Abscisic acid signal transduction. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49, 199-222.

Leung, J., Merlot, S. and Giraudat, J. (1997). The Arabidopsis *ABSCISIC ACID-INSENSITIVE2 (ABI2)* and *ABI1* genes encode homologous protein phosphatases 2C involved in abscisic acid signal transduction. Plant Cell 9, 759-771.

Lois, L. M., Lima, C. D. and Chua, N. H. (2003). Small ubiquitin-like modifier modulates abscisic acid signaling in *Arabidopsis*. Plant Cell 15, 1347-1359.

Lopez-Molina, L., Mongrand, S., McLachlin, D. T., Chait, B. T. and Chua, N. H. (2002). ABI5 acts downstream of ABI3 to execute an ABA-dependent growth arrest during germination. Plant J. 32, 317-328.

Mattsson, J., Söderman, E., Svenson, M., Borkird, C. and Engström, P. (1992). A new homeobox-leucine zipper gene from *Arabidopsis thaliana*. Plant Mol. Biol. 18, 1019-1022.

McGinnis, W., Levine, M. S, Hafen, E, Kuroiwa, A. and Gehring, W. J. (1984). A conserved DNA sequence in homoeotic genes of the *Drosophila* Antennapedia and bithorax complexes. Nature 308, 428-433.

Meijer, A. H., de Kam, R. J., d'Erfurth, I., Shen, W. and Hoge, J. H. C. (2000). HD-Zip proteins of families I and II from rice: interactions and functional properties. Mol. Gen. Genet. 263, 12-21

Meijer, A. H., Scarpella, E., van Dijk, E. L., Qin, L., Taal, A. J., Rueb, S., Harrington, S. E., McCouch, S. R., Schilperoort, R. A. and Hoge, J. H. (1997). Transcriptional repression by Oshox1, a novel homeodomain leucine zipper protein from rice. Plant J. 11, 263-276.

Merlot, S., Gosti, F., Guerrier, D., Vavasseur, A. and Giraudat, J. (2001). The ABI1 and ABI2 protein phosphatases 2C act in a negative feedback regulatory loop of the abscisic acid signalling pathway. Plant J. 25, 295-303.

Meyer, K., Leube, M. P. and Grill, E. (1994). A protein phosphatase 2C involved in ABA signal transduction in *Arabidopsis thaliana*. Science 264, 1452-1455.

Nakamura, S., Lynch, T. J. and Finkelstein, R. R. (2001). Physical interactions between ABA response loci of *Arabidopsis*. Plant J. 26, 627-635.

Ohgishi, M., Oka, A., Morelli, G., Ruberti, I. and Aoyama, T. (2001). Negative autoregulation of the *Arabidopsis* homeobox gene *ATHB-2*. Plant J. 25, 389-398.

Ponting, C. P. and Aravind, L. (1999). START: a lipid-binding domain in StAR, HD-ZIP and signalling proteins. Trends Biochem. Sci. 24, 130-132.

Rensink, W. A, and Buell C. R. (2004). Arabidopsis to rice. Applying knowledge from a weed to enhance our understanding of a crop species. Plant Physiol. 135, 622-629.

Rerie, W. G., Feldmann, K. A. and Marks, M. D. (1994). The *GLABRA2* gene encodes a homeo domain protein required for normal trichome development in *Arabidopsis*. Genes Dev. 15, 1388-1399.

Riechmann, J. L., Heard, J., Martin, G., Reuber, L., Jiang, C., Keddie, J., Adam, L., Pineda, O., Ratcliffe, O. J., Samaha, R. R., Creelman, R., Pilgrim, M., Broun, P., Zhang, J. Z., Ghandehari, D., Sherman, B. K. and Yu, G. (2000). *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. Science 290, 2105-2110.

Rock, C. (2000). Pathways to abscisic acid-regulated gene expression. New Phytol. 148, 357-396.

Rodriguez, P. L., Leube, M. P. and Grill, E. (1998). Molecular cloning in *Arabidopsis thaliana* of a new protein phosphatase 2C (PP2C) with homology to ABI1 and ABI2. Plant Mol. Biol. 38, 879-883.

Ruberti, I., Sessa, G., Lucchetti, S. and Morelli, G. (1991). A novel class of plant proteins containing a homeodomain with a closely linked leucine zipper motif. EMBO J. 10, 1787-1791.

Saez, A., Apostolova, N., Gonzalez-Guzman, M., Gonzalez-Garcia, M. P., Nicolas, C., Lorenzo, O. and Rodriguez, P. L. (2004). Gain-of-function and loss-of-function phenotypes of the protein phosphatase 2C HAB1 reveal its role as a negative regulator of abscisic acid signalling. Plant J. 37, 354-369.

Sakakibara, K., Nishiyama, T., Kato, M. and Hasebe, M. (2001). Isolation of homeodomain-leucine zipper genes from the moss *Physcomitrella patens* and the evolution of homeodomain-leucine zipper genes in land plants. Mol. Biol. Evol. 18, 491-502.

Sauter, A., Davies, W. J. and Wolfram, H. (2001). The long-distance abscisic acid signal in the droughted plant: the fate of the hormone on its way from root to shoot. J. Exp. Bot. 52, 1991-1997.

Sawa, S., Ohgishi, M., Goda, H., Higuchi, K., Shimada, Y., Yoshida, S. and Koshiba, T. (2002). The *HAT2* gene, a member of the HD-Zip gene family, isolated as an auxin inducible gene by DNA microarray screening, affects auxin response in *Arabidopsis*. Plant J. 32, 1011-1022.

Schaefer, D. G. and Zrÿd, J.-P. (2001). The *Physcomitrella patens*, now and then. Plant Physiol. 127, 1430-1438.

Schena, M. and Davis, R. W. (1992). HD-Zip proteins: members of an *Arabidopsis* homeodomain protein superfamily. Proc. Natl. Acad. Sci. USA 1, 3894-3898.

Schena, M. and Davis, R. W. (1994). Structure of homeobox-leucine zipper genes suggests a model for the evolution of gene families. Proc. Natl. Acad. Sci. USA 91, 8393-8397

Schrick, K., Nguyen,, D., Karlowski, W. M. and Mayer, K. F. (2004). START lipid/sterol-binding domains are amplified in plants and are predominantly associated with homeodomain transcription factors. Genome Biol. 5:R41.

Schwartz, S. H., Qin, X. and Zeevaart, J. A. (2003). Elucidation of the indirect pathway of abscisic acid biosynthesis by mutants, genes, and enzymes. Plant Physiol. 131, 1591-1601.

Scott, M. P. and Weiner, A. J. (1984). Structural relationships among genes that control development: sequence homology between the *Antennapedia, Ultrabithorax,* and *fushi tarazu* loci of *Drosophila*. Proc. Natl. Acad. Sci. USA. 81, 4115-4119.

Seki, M., Ishida, J., Narusaka, M., Fujita, M., Nanjo, T., Umezawa, T., Kamiya, A., Nakajima, M., Enju, A., Sakurai, T., Satou, M., Akiyama, K., Yamaguchi-Shinozaki, K., Carninci, P., Kawai, J., Hayashizaki, Y. and Shinozaki, K. (2002a). Monitoring the expression pattern of around 7,000 *Arabidopsis* genes under ABA treatments using a full-length cDNA microarray. Funct. Integr. Genomics 2, 282-291.

Seki, M., Narusaka, M., Ishida, J., Nanjo, T., Fujita, M., Oono, Y., Kamiya, A., Nakajima, M., Enju, A., Sakurai, T., Satou, M., Akiyama, K., Taji, T., Yamaguchi-Shinozaki, K., Carninci, P., Kawai, J., Hayashizaki, Y. and Shinozaki, K. (2002b). Monitoring the expression profiles of 7000 *Arabidopsis* genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant J. 31, 279-292.

Sessa, G., Borello, U., Morelli, G. and Ruberti, I. (1998). A transient assay for rapid functional analysis of transcription factors in *Arabidopsis*. Plant Mol. Biol. Rep. 16, 191-197.

Sessa, G., Carabelli, M., Ruberti, I., Lucchetti, S., Baima, S. and Morelli, G. (1994). Identification of distinct families of HD-Zip proteins in *Arabidopsis thaliana* In: Plant Molecular Biology, ed. G. a. P. Coruzzi, P.: Springer Verlag, Berlin, Germany, 411-426.

Sessa, G., Morelli, G. and Ruberti, I. (1993). The ATHB-1 and -2 HD-Zip domains homodimerize formaing complexes of different DNA binding specificities. EMBO J. 12, 3507-3517.

Sharp, R. E. (2002). Interaction with ethylene: changing views on the role of abscisic acid in root and shoot growth responses to water stress. Plant Cell Environ. 25, 211-222.

Sheen, J. (1998). Mutational analysis of protein phosphatase 2C involved in abscisic acid signal transduction in higher plants. Proc. Natl. Acad. Sci. USA 95, 975-980.

Shen, Q. and Ho, T. H. (1995). Functional dissection of an abscisic acid (ABA)-inducible gene reveals two independent ABA-responsive complexes each containing a G-box and a novel cis-acting element. Plant Cell 7, 295-307.

Shen, Q., Zhang, P. and Ho, T. H. (1996). Modular nature of abscisic acid (ABA) response complexes: composite promoter units that are necessary and sufficient for ABA induction of gene expression in barley. Plant Cell 8, 1107-1119.

Shinozaki, K. and Yamaguchi-Shinozaki, K. (2000). Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. Curr. Opin. Plant Biol. 3, 217-223.

Shinozaki, K., Yamaguchi-Shinozaki, K. and Seki, M. (2003). Regulatory network of gene expression in the drought and cold stress responses. Curr. Opin. Plant Biol. 6, 410-417.

Somerville, C. and Koornneef, M. (2002). A fortunate choice: the history of *Arabidopsis* as a model plant. Nat. Rev. Genet. 3, 883-889.

Son, O., Cho, H. Y., Kim, M. R., Lee, H., Lee, M. S., Song, E., Park, J. H., Nam, K. H., Chun, J. Y., Kim, H. J., Hong, S. K., Chung, Y. Y., Hur, C. G., Cho, H.

T. and Cheon, C. I. (2005). Induction of a homeodomain-leucine zipper gene by auxin is inhibited by cytokinin in *Arabidopsis* roots. Biochem. Biophys. Res. Commun. 326, 203-209.

Steindler, C., Matteucci, A., Sessa, G., Weimar, T., Ohgishi, M., Aoyama, T., Morelli, G. and Ruberti, I. (1999), Shade avoidance responses are mediated by the ATHB-2 HD-zip protein, a negative regulator of gene expression. Development 126, 4235-4245.

Tamura, T., Hara, K., Yamaguchi, Y., Koizumi, N. and Sano, H. (2003). Osmotic stress tolerance of transgenic tobacco expressing a gene encoding a membrane-located receptor-like protein from tobacco plants. Plant Physiol. 131, 454-462.

Taiz. L and Zeiger E. (2002). Plant Physiology, 3rd ed. Sinauer Associates, Inc. Sunderland, MA, USA.

Trewavas, A. J. and Jones, H. G. (1991). An assessment of the role of ABA in plant development. Abscisic acid and physiology and biochemistry Oxford, UK, Bios. Scientific Publishers.

Tron A. E., Bertoncini C. W., Chan R. L. and Gonzalez D. H. (2002). Redox regulation of plant homeodomain transcription factors. J. Biol. Chem. 277, 34800-34807.

Tähtiharju, S. and Palva, T. (2001). Antisense inhibition of protein phosphatase 2C accelerates cold acclimation in *Arabidopsis thaliana*. Plant J. 461-470.

Ullah, H., Chen, J. G., Young, J. C., Im, K. H., Sussman, M. R. and Jones, A. M. (2001). Modulation of cell proliferation by heterotrimeric G protein in *Arabidopsis*. Science 15, 2066-2069.

Urao, T., Yakubova, B., Satoha, R., Yamaguchi-Shinozakia, K., Sekib, M., Hirayamab, T. and Shinozakib, K. (1999). A Transmembrane Hybrid-Type Histidine Kinase in *Arabidopsis* Functions as an Osmosensor. Plant Cell 11, 1743-1754.

Wang, X. Q., Ullah, H., Jones, A. M. and Assmann, S. M. (2001). G protein regulation of ion channels and abscisic acid signaling in *Arabidopsis* guard cells. Science 15, 2070-2072.

Wang, Y. (2001). The role of the homeobox gene *ATHB16* in development regulation in *Arabidopsis thaliana*. ISBN 91-554-4983-2 Uppsala: Acta Universitatis Upsaliensis.

Wang, Y., Henriksson, E., Söderman, E., Henriksson, K. N., Sundberg, E. and Engström, P. (2003). The Arabidopsis homeobox gene, *ATHB16*, regulates leaf development and the sensitivity to photoperiod in *Arabidopsis*. Dev. Biol. 1, 228-239.

Wortman, J. R, Haas, B. L., Hannick, L. I., Smith, R. K. Jr, Maiti, R., Ronning, C. M., Chan, A. P., Yu, C., Ayele, M., Whitelaw, C. A., White, O. R. and Town C. D. (2003). Annotation of the *Arabidopsis* genome. Plant Physiol. 132, 461-468.

Yamazaki, D., Yoshida, S., Asami, T. and Kuchitsu, K. (2003). Visualization of abscisic acid-perception sites on the plasma membrane of stomatal guard cells. Plant J. 35, 129-139.

Yu, J., Hu,.S., Wang, J., Wong, G. K., Li, S., Liu, B., Deng, Y., Dai, L., Zhou, Y., Zhang, X., Cao, M., Liu, J., Sun, J., Tang, J., Chen, Y., Huang, X., Lin, W., Ye, C., Tong, W., Cong, L., Geng, J., Han, Y., Li, L., Li, W., Hu, G., Huang, X., Li, W., Li, J., Liu, Z., Li, L., Liu, J., Qi Q., Liu, J., Li, L., Li,

T., Wang, X., Lu, H., Wu, T., Zhu, M., Ni, P., Han, H., Dong, W., Ren, X., Feng, X., Cui, P., Li, X., Wang, H., Xu, X., Zhai, W., Xu, Z., Zhang, J., He, S., Zhang, J., Xu, J., Zhang, K., Zheng, X., Dong, J., Zeng, W., Tao, L., Ye, J., Tan, J., Ren, X., Chen, X., He, J., Liu, D., Tian, W., Tian, C., Xia, H., Bao, Q., Li, G., Gao, H., Cao, T., Wang, J., Zhao, W., Li, P., Chen, W., Wang, X., Zhang, Y., Hu, J., Wang, J., Liu, S., Yang, J., Zhang, G., Xiong, Y., Li, Z., Mao, L., Zhou, C., Zhu, Z., Chen, R., Hao, B., Zheng, W., Chen, S., Guo, W., Li, G., Liu, S., Tao, M., Wang, J., Zhu, L., Yuan, L. and Yang, H. (2002). A draft sequence of the rice genome (*Oryza sativa L. ssp. indica*). Science 296, 79-92.

Zhu, J.-K. (2002). Salt and drought stress signal transduction in plants. Annu. Rev. Plant Biol. 53, 247-273.

Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 13

Editor: The Dean of the Faculty of Science and Technology

A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology. (Prior to January, 2005, the series was published under the title "Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology".)

Distribution: publications.uu.se
urn:nbn:se:uu:diva-4787

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2005