This is to certify that the
dissertation entitled

TRANSCRIPTIONAL NETWORKS INVOLVED IN
RESPONSE TO LOW TEMPERATURE STRESS IN
ARABIDOPSIS THALIANA

presented by

Colleen J. Doherty

has been accepted towards fulfillment
of the requirements for the

Ph.D. degree in Biochemistry and Molecular Biology

Major Professor's Signature

7/31/08

Date

TRANSCRIPTIONAL NETWORKS INVOLVED IN RESPONSE TO LOW TEMPERATURE STRESS IN *ARABIDOPSIS THALIANA*

By

Colleen J. Doherty

A Dissertation

Submitted to
Michigan State University
In partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Biochemistry and Molecular Biology

2008

ABSTRACT

Transcriptional Networks Involved in Response to Low Temperature Stress in *Arabidopsis thaliana*

By

Colleen J. Doherty

Desirable agricultural land for crop growth is limited by extremes in temperature. Low temperatures place limits both geographically and temporally on the amount of crop land available for use. To survive in climates of low and freezing temperatures, some plants have the ability to cold acclimate, a process where plants can detect low temperature and adjust to survive freezing. Understanding how plants are capable of detecting low temperature and the signaling process involved in refocusing the transcriptome, proteome, and metabolome to levels that allow for survival of freezing temperatures will allow identification of targets for traits important to increase freezing tolerance in plants, potentially extending the growing season for some species. A complete understanding of plant cold acclimation would require creating a network of the cold response in plants as they cold acclimate. To elucidate this network two key questions need to be answered. The first, how do plants sense low temperature and secondly are there multiple mechanisms for cold acclimation and to what extent do known pathways in cold acclimation contribute?

To address the first question our goal is to identify upstream components of a known pathway of cold response. The C-Repeat Binding Factor (CBF) cold response pathway has an important role in cold acclimation, the process whereby plants increase in freezing tolerance in response to low nonfreezing temperatures. In Arabidopsis, a cold acclimating plant, three AP2 domain-containing transcription factors, CBF 1, 2, and 3 (DREB 1B, C, and A, respectively), are induced rapidly in response to low temperatures. Induction of CBF transcription factors is followed by expression of the CBF target genes, resulting in increased freezing tolerance. A key objective is to determine how plants sense low temperature and activate expression of the CBF genes. A cis-acting region of the CBF2 promoter that is sufficient for cold-induction of a reporter gene was identified. Here, CAMTA3, a calmodulin binding transcriptional activator, is identified as a trans-acting factor involved in the regulation of CBF2 in response to low temperature through this element, providing a potential link between calcium signaling and the CBF cold response pathway.

Secondly, to identify the requirement for the CBF pathway in response to low temperature, plants expressing a dominant negative version of CBF, CBF2ΔC, were analyzed for their ability to cold acclimate and for changes in the cold-regulated transcriptome. This analysis showed that the expression of 40% of the cold-induced genes was affected in the CBF2ΔC plants. However, although not to the same extent as WT plants, CBF2ΔC plants were still able perceive and responds to low temperature through the process of cold acclimation. This result indicates that multiple pathways for cold acclimation exist.

ACKNOWLEDGEMENTS

First of all I would like to thank Dr. Michael Thomashow for the opportunity to work in the lab, for the support he has given me to try following my interests into the world of bioinformatic analysis, for his patience when many of the attempts did not work, and for reminding me to focus on the question.

Thanks to my committee members, Dr. Arnosti, Dr. LaPres, Dr. Keegstra, and Dr. Kuo for their enthusiasm and support of my projects, their sound advice which I wish I had followed more closely, and their editorial skills to make this a readable document.

I would like to thank my parents for their support. I am grateful that they were always proud of me, no matter how small the accomplishment and that they encouraged my love of "projects". I would especially like to thank them for the interest they have shown in my work- "We found an error in your proposal, you say this is 'interesting'."

Thanks are due to the past and present members of the Thomashow lab, especially: Heather Van Buskirk and Jonathan Vogel, who taught me molecular biology; their patience still astounds me to this day. Jonathan, thanks for showing me the real value of an autoclave. Susan Myers for her endless work, I'm not sure how many hundreds of RNA samples you tirelessly extracted, but this document would be years away from being complete without your efforts. Chin-Mei Lee, thanks for reminding me that science IS fun. Michael Mikkelson, Ryan Warner, Dona Canella, and Kanchan Pavangadkar, thanks for the science talks. Sarah Gilmour thanks for all of your help on lab projects, listening to talks, correcting grammar, keeping me organized, but mostly for the occasional chats on any subject imaginable. Catherine Le, Kelly McRay, Megan Sargent, Ryan Sartor, and Laura Stewart, thanks for your enthusiasm, hard work, and positive attitude. Thanks also to Marcela Carvallo, Diane Constan, Malia Dong, Bonnie St. John, Ritu Sharma, and Dan Zarka for being great lab mates.

Many thanks to the biochemsitry, plant biology, and PRL students, especially Andrea Braeutigam, Lori Imboden, Joonyul Kim, Mark Linka, Janet Paper, Hiroshi Maeda, Dean Shooltz, and Dorothy Tappenden for the science discussions, encouragement, and advice. I wish I had time to do a tenth of the great ideas that were discussed.

Thanks to the Biochemistry department and PRL for providing a supportive and encouraging environment for students.

Dona, Andrea, and Mark, thanks for giving the journal club a go, next time, let's meet over beer- if no one else wants to join us, we'll still have a good time. Heather, Bonnie, Dorothy, Malia, Chin-Mei, Dean, Jessy, and Vash, without your support for the past year this would not be getting turned in now. Thanks Joe, for the stories and the whiskey. Thanks to Dunstan, Molly, and Allie, for being happy to see me no matter how late the experiments ran.

Special thanks to Vash for your faith in me and for reminding me that pi is mystical, science is exciting, plants are amazing, dorkiness is acceptable, and oxygen is good!

Love & Peace!

PREFACE

In Chapter 2, the experiments were conducted by the author of this thesis and Dr. Heather Van Buskirk. Dr. Van Buskirk was responsible for the promoter analysis and deletion experiments, the EMSA, and the construction of the CAMTA overexpression constructs.

In Chapter 3, the experiments were conducted by the author of this thesis and Dr. Sarah J. Gilmour. Dr. Gilmour was responsible for the identification of the CBF2ΔC mutant, the creation and characterization of the CBF2ΔC/35s::CBF2 crosses. The metabolite analysis was a joint effort with Dr. Gilmour.

In Chapter 4, Dr. Van Buskirk performed analysis of the CM2/CM1-Box:Gus reporter.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Images in this dissertation are presented in color

KEY TO ABBREVIATIONS

ABA: Abscisic Acid

ABRE: Abscisic Acid Response Element

AP2: APETALA2

bHLH: Basic-Helix-Loop-Helix

BP: Base Pair

CAMTA: Calmodulin Binding Transcriptional Activator

CAS: Cold Acclimation Specific genes

CBF: C-repeat binding factor

CaMV 35s: Cauliflower Mosaic Virus 35s promoter

CBF2ΔC: CBF2 with stop codon upstream of activation domain

CG-1 Element: DNA consensus sequence vCGCGb

CRT: C-repeat

CM-Box: Consevered Motif sequences identified in both the CBF2 and ZAT12 promoters

Col-0: Columbia Ecotype of *Arabidopsis thaliana*

COR: COld Responsive genes

DRE: Dehydration Responsive Element

DREB: Dehydration Responsive Element Binding factor

EAR: ERF-Associated amphiphilic Repression domain

ERF: Ethylene Response Factor

EMS: Ethyl MethaneSulfonate

EMSA: Electrophoretic Mobility Shift Assay

GI: GIgantea

GO: Gene Ontology

GOLS: GalactinOL Synthase

GST: Glutathione S-Transferase

GUS: beta-GlUcuronidaSe

HDAC: Histone DeACetylase

HOS: Hypersensitive to Osmotic Stress

ICE: Inducer of CBF Expression

ICEr: Inducer of CBF Expression region

ICICLEs: Independent of CBF Influence Cold Late Expressed

KB: KiloBase pair

MYB: MYeloBlastosis viral oncogene homolog

MYC: MYeloCytomatosis viral oncogene homolog

NAM: Non-Apical Meristem

PCR: Polymerase Chain Reaction

PKCε: Protein Kinase Cε

PKD: Protein Kinase D

qRT-PCR: Quantitative RT-PCR

ROS: Reactive Oxygen Species

RT-PCR: Reverse Transcriptase PCR

SUMO: Small Ubiquitin related MOdifier

T-DNA: Transfer DNA

TBX: Telo-box cis-element

WS: Wassilewskija ecotype of *Arabidopsis thaliana*

WT: Wild-Type

ZAT: Zn transporter of *Arabidopsis Thaliana*

ZT: ZeiTgeber

CHAPTER ONE

LITERATURE REVIEW

Introduction

Plants live in complex environments and are often bombarded with signals from multiple sources at once. While cold stress is a major factor affecting the growth and development of a plant, plants do not experience cold in isolation but rather in the context of signals of nutrient stress, biotic stress, day length, light quality and quantity. These factors have an influence on the way a plant responds to low temperature. It is no surprise therefore that plants have developed a complex integrated system for handling these multiple inputs, allowing some inputs to act in synergy to give a combined response, while other times when signals conflict the plant uses these cues to inhibit its response to some signals. Understanding the response to low temperature in the midst of these other factors is providing new insights into how plants respond and integrate multiple input signals and how plants can survive and adapt in the complex and constantly changing world. Evaluating the cold response in the light of multiple signals can shed insights into the understanding of the mechanisms of cold acclimation.

Historically, analysis of the cold response in plants has focused on isolating the cold response from all other variables. This focus on cold alone has yielded a wealth of information and provided the basis of our understanding of how plants respond to low temperature. The range of response to low temperature varies greatly between plants. Some tropical plants, such as tomato, *Lycopersicon esculentum*, are not able to withstand low temperatures and are considered both chilling sensitive and freezing sensitive (Zhang et al. 2004). Other plants, such as potato and some wild versions of tomato are chilling tolerant but freezing sensitive since they are able to survive low, non-freezing temperatures, but are not able to withstand temperatures that reach below freezing (Ballou et al. 2007). While no known wild-type plants are able to go directly from warm growth conditions to freezing temperatures, some temperate plants, such as wheat and *Arabidopsis* are able to cold acclimate after exposure to low, non-freezing temperatures. During this process of cold acclimation, large changes in transcription, metabolism, membrane composition and accumulation of cryoprotectant molecules allow plants to prepare for and survive freezing temperatures (Thomashow 1999; Viswanathan and Zhu 2002). Even among the freezing tolerant plants there is a range of ability for cold acclimation, with *Arabidopsis* being able to make changes to survive down to -5°C while rye after acclimation can survive temperatures as low as -40°C (Jaglo-Ottosen et al. 1998; Thomashow 1999).

CBF Pathway Contributes to the Cold Acclimation Ability of *Arabidopsis*

One important pathway that contributes to the process of cold acclimation is the CBF cold-responsive pathway. CBFs are a family of AP2 domain containing DNA binding transcription factors defined by their signature sequences flanking the AP2 DNA binding domain. In the model plant, *Arabidopsis*, there are 6 members of the CBF family, three of which are cold-induced (CBF1, 2, and 3). CBFs bind the CRT/DRE (C-Repeat Element/

Drought Response Element) element in the promoter of their target genes and induce transcription of these genes in response to low temperature. CBFs are important components of the cold acclimation process; overexpression of the three cold-inducible CBFs leads to induction of the CBF target genes at warm temperatures and results in the ability of the plants to be freezing tolerant without a requirement for a period of cold acclimation (Thomashow 2001). It is clear that expression of CBFs is sufficient for cold acclimation in *Arabidopsis*, however, two important questions remain: How are CBFs themselves induced in response to low temperature? And, how much do CBFs contribute to the ability of plants to cold acclimate?

Regulation of CBFs in Response to Low Temperature

The accumulation of CBF 1,2, and 3 mRNA in response to low temperature occurs rapidly, within 15 minutes after exposure to 4°C (Zarka et al. 2003). The CBF transcripts peak at around 2h and then are reduced after continued time in the cold to a level slightly higher than their expression in warm grown plants. Upon return to warm temperature the CBF transcripts are rapidly degraded (Zarka, Vogel et al. 2003). ICE1 (Inducer of CBF expression1), a MYC-like bHLH protein that binds to MYC recognition sites, was shown to be involved in the induction of CBFs (Chinnusamy et al. 2003). A point mutation in this bHLH transcription factor has a dramatic reduction in CBF3 expression in response to low temperature. ICE1 is a target for ubiquitination by HOS1, an E3 ligase and negative regulator of cold acclimation (Dong et al. 2006). SIZ1, a

SUMO-E3 ligase, prevents ubiquitination of ICE1, leading to an increase in accumulation of CBFs in response to low temperature (Chinnusamy et al. 2007).

Although the promoters of CBF1 and 2 have MYC recognition sequences, potential binding elements for ICE1, there is little effect on the expression level of CBF1 and 2 in the *ice1* mutant, indicating that there may be independent regulation of the three CBF transcription factors in response to low temperature. In contrast to the *ice1* point mutation, a complete knock out of this transcription factor does not have any significant effect on expression levels of CBF1, 2, or 3, indicating that there may be redundant factors that allow for regulation of CBF3 in the absence of ICE1. Overexpression of ICE1 increases the accumulation of CBF2 and CBF3 mRNA in response to low temperature. However, ICE1 overexpression does not induce any of the three CBFs in the warm, indicating that expression of ICE1 alone is not sufficient for induction of CBFs. Perhaps there are repressive factors that ICE1 can not overcome, there are modifications of ICE1 or other regulators that occur only at low temperature, or there is a change in stability of CBF mRNA that only allows accumulation of CBF mRNA at low temperature. Cycloheximide treatment also induces the accumulation of CBF mRNA, one interpretation of this result is translation of a potential negative regulatory element is important for the warm-repression of CBFs (Zarka, Vogel et al. 2003).

MYB15 and ZAT12 were identified as negative regulators of CBFs. Overexpression of either MYB15 or ZAT12 reduces the cold-induced accumulation of CBFs (Agarwal et al. 2006, Vogel et al. 2005). Loss of MYB15 results in increased accumulation of these potential factors in response to low temperatures (Agarwal et al. 2006). However, the effect seen in the knockout plants of either MYB15 or ZAT12 on the CBFs is slight, perhaps due either to redundancy among the large MYB and Zinc finger families in *Arabidopsis* or to the function of other potential negative regulatory elements on CBF regulation.

Regulation of CBFs in Response to Other Environmental Factors

As we start to look at the overlapping responses of plants to different environmental signals, a complex regulation of CBFs in response to multiple environmental conditions is revealed. Circadian rhythms have an effect on CBF induction. CBF3 mRNA cycles in the warm due to circadian rhythms, peaking early in the morning (Harmer et al. 2000). Importantly, the circadian clock has an effect on the cold-induction of CBF, with the clock gating the induction. When plants are shifted to low temperature at ZT4 (dawn), which coincides with the peak of the CBF3 circadian expression, the induction of CBFs in the cold is higher than when shifted to cold at the trough of CBF expression (Fowler et al. 2005). This indicates that the clock works together with cold signals to ensure induction of CBFs and the cold response at the appropriate times.

In a similar manner, light quality has an effect on cold-induction of CBFs and their target transcripts. A reporter gene composed of four copies of the CRT fused to GUS was shown to require light for cold induction. A 10min pulse of red light was sufficient for this response; however, this response was eliminated when the pulse of red light was followed by a pulse of far-red light (Kim et al. 2002) . This reversal of the red light response when followed by treatment with far red light is indicative of regulation by phytochrome signaling. Kim et al. demonstrated that this is a phytochrome B dependent response. The involvement of light in regulating CRT elements suggests that there is either a regulation of the accumulation of the CBFs themselves in response to light or that light signals are integrated at a downstream checkpoint between CBF mRNA accumulation and CBF target gene regulation. Light quality signals have been shown to regulate the induction of CBF transcripts at 16°C. A low ratio of Red/Far Red light causes an increase in the circadian based induction of CBF at 16°C. Additionally, this light treatment of low red/far red can be used to increase freezing tolerance of seedlings grown at 16°C, but not at 22°C (Franklin and Whitelam 2007). Thus integration of light signals is important for both CBF induction and activity.

Additionally, it has been shown that CBFs are also transcriptionally induced by another environmental factor, mechanical agitation. In response to touch or agitation of *Arabidopsis* plants, CBFs 1, 2, and 3 are rapidly induced to levels equal or greater than that of their cold level of induction (Gilmour et al. 1998). Integrating the information from these multiple methods of CBF regulation may provide clues to the mechanisms of CBF induction in response to low temperature.

Calcium as a Potential Mechanism for the Induction of CBFs

One common thread among all of these environmental regulators of CBF is a spike in cytosolic calcium. A transient increase in calcium levels is observed in response to a shift to low temperature, mechanical agitation, or treatment with red light. In addition, cytosolic calcium has a circadian regulation with a peak around dawn, coincident with circadian induction of CBFs (Knight et al. 1996, Knight et al. 1992, Shacklock et al. 1992, Johnson et al. 1995)

While calcium signals appear to be ubiquitous in most environmental responses, it is interesting to speculate that the calcium signature maybe a common thread in these conditions to which CBF responds. Perhaps calcium may provide a mechanism for the induction of CBF in response to these treatments. One CBF-target transcript has been shown to be up-regulated in response to treatment with calcium ionophores in the warm and inhibited from cold-induction by treatment with calcium chelators and calcium channel inhibitors (Knight et al. 1996). Similarly, cold-regulated transcripts were shown to be inducible at warm temperatures by treatment with calcium ionophores in alfalfa (Monroy and Dhindsa 1995). There are no reports of the regulation of CBFs by calcium. However, in light of the role of circadian rhythms and light quality in control of CBF induction, experiments to determine the effect of calcium on CBF induction will need to take into consideration the time of day and light quality when CBF induction is analyzed.

This involvement of calcium in cold induction is suggested to be through the CRTs. Treatment with okadaic acid inhibited the cold induction of a 4xCRT reporter construct (Kim et al. 2002). Okadaic acid has been shown to inhibit sharp peaks in calcium accumulation while having little effect on gradual calcium accumulation, reducing calcium influx through inhibition of protein phosphatase activity (Hescheler et al. 1988; Kuo et al. 1996). The concentration required for inhibition of the 4xCRT reporter was specific to the inhibition of type 2C protein phosphatases (Kim et al. 2002). Two type 2C protein phosphatases, ABI1 and ABI2 are known components of ABA signaling upstream of calcium influx in Arabidopsis guard cells (Allen et al. 1999). The specific effect of okadaic acid on cold-induced calcium levels is unknown; however, this experiment raises the possibility that the calcium signal activates the CBF target genes through the CRT element.

The role of calcium in cold signaling is not straightforward in that overexpression of a calmodulin results in a reduced cold-induction of a CBF target gene, Kin1 (Townley and Knight 2002). This surprising result suggests perhaps that the response to the calcium signal is tightly regulated to a specific concentration threshold or signature of calcium and its downstream signals. Perhaps adjusting the levels of any portion of the calcium signal away from this critical threshold can result in feedback inhibition of the response pathway.

Reactive oxygen species (ROS) mediated signaling pathways may also have significant involvement in the induction of CBFs. FRO1 is a NADH dehydrogenase subunit of mitochondrial respiratory chain complex I, and the *fro1* mutant shows constitutive accumulation of ROS (Lee et al. 2002). The *fro1* mutant is sensitive to freezing, have reduced induction of cold-responsive transcripts, COR15a, COR47, Kin1, and RD29a. This reduction leads to a decrease in freezing tolerance of *fro1* plants. It is interesting to note that the only change seen in CBF induction in response to low temperatures was seen at 12h, where CBF levels when increased in *fro1* mutants compared with WT levels. Perhaps this indicates that ROS signals are involved in the circadian or light-base trough of CBF expression. It would be interesting to examine the circadian and R/FR regulation of CBFs in the *fro1* mutant background. Extensive crosstalk exists between ROS signaling and calcium signaling. For example, ROS signaling can activate Ca^{2+} permeable ion channels in the plasma membranes while Ca^{2+} regulates ROS scavenging mechanisms(Mori and Schroeder 2004; Yan et al. 2006).

The evidence for involvement of calcium signaling in the response to regulation of transcripts at low temperature is clear. However, it remains unknown if this involvement is upstream of the CBF factors. Involvement of calcium in the induction of CBFs could be a key link in understanding the response of CBF to these multiple environmental signals.

Evidence for CBF-Independent Pathways of Cold–Acclimation

While it is clear that CBFs are important in the cold-regulation and that their induction is complex and involves integration of many environmental cues, it remains unknown if there are other pathways that can independently lead to an increase in freezing tolerance.

Interestingly, many freezing sensitive plants, such as tomato contain cold-inducible and functional CBF proteins. CBF1 in tomato is cold-inducible and is functional in *Arabidopsis*, leading to constitutive freezing tolerance of *Arabidopsis* when overexpressed (Zhang, Fowler et al. 2004). However, even with this functional and cold-inducible CBF protein, tomato is not freezing tolerant. This suggests that either there is a required component downstream of CBF that is missing in tomato or that there are other pathways that are required for freezing tolerance present in *Arabidopsis* that are missing from tomato.

Mutational analysis has revealed mechanisms of affecting freezing tolerance independent of the CBF pathway. The *esk1* mutant is constitutively freezing tolerant, however it does not show a significant increase in CBFs or many of the CBF target genes (Xin and Browse 1998; Xin et al. 2007). *esk1* mutants accumulate high-levels of proline, a cryoprotectant, suggesting a possible mechanism for the increase in freezing tolerance. However, ESK1 encodes a novel protein with a domain of unknown function, so the mechanisms of the increase in proline levels and this increase in freezing tolerance remain unclear. A mutation in a MYB transcription factor, *hos10,* which has an effect on the regulation of ABA biosynthetic gene NCED3, reduces freezing tolerance yet has an increase in expression of CBF regulon genes, COR15a, RD29a, ADH, and KIN1 (Zhu et al. 2005). This reduction in freezing tolerance in the presence of a functional CBF pathway indicates that CBF alone may not be sufficient to provide full wild-type levels of freezing tolerance.

Analyzing how the cold-response pathway is affected by mutations in other environmental and developmental responses may help to reveal the importance of the CBF pathway in contributing to cold acclimation and help to identify potential CBF-independent pathways. Gigantea (GI), a protein shown to be involved in developmental regulation of flowering in response to day-length and circadian clock, may play a role in CBF-independent cold acclimation. GI itself is cold induced and loss of GI in *gi1-3* plants resulted in increased sensitivity to freezing without and effect on CBFs or their target genes, COR15a, KIN1, and Rd29a (Cao et al. 2005). Additionally, the delay in flowering caused by the gi1-3 mutation is enhanced by intermittent cold treatment, indicating a potential link to developmental regulation by cold through GI.

Additional evidence for CBF-independent pathways comes from microarray data which indicates that the transcriptional regulation of a majority of cold-regulated genes is not affected by overexpression of CBFs in warm grown plants, even though constitutive expression of CBF is sufficient for freezing tolerance (Fowler and Thomashow 2002; Vogel et al. 2005). These transcripts may be entirely CBF independent or they may require CBF and an additional factor present only at low temperatures. Additionally, CBF2 overexpressing plants still have a significant and dramatic increase in freezing tolerance after a period of cold acclimation over their warm-grown levels. This additional increase in freezing tolerance may be due to CBF independent pathways that contribute to freezing tolerance or a quantitative effect due to the increase in the total CBF mRNA levels in the cold due to the additional accumulation of the endogenous CBF mRNA in response to their induction at low temperature.

Cold Acclimation in the Absence of CBFs

An ideal way to address the contribution of CBF independent pathways to the process of cold acclimation would be to knock out CBF function and assess the remaining ability of these mutants to cold acclimate. However, the three cold-induced CBF genes lie in tandem, making recombination of single T-DNA insertion lines impractical. No triple, CBF1, 2, and 3 knock-out plants have been generated to examine the ability of Arabidopsis to acclimate in the absence of CBFs.

Knock-out and miRNA studies of individual CBFs revealed that they have distinct roles in regulation of the target genes, not evident from overexpression (Novillo et al. 2004; Novillo et al. 2007). These distinct roles are in agreement with their tissue specific expression. Promoter GUS fusion reporters demonstrated that during development warm-grown tissue expresses CBF2 highly in the shoots and not in the roots, while CBFs 1 and 3 accumulate in the roots, but not the shoots. It is unknown if these genes are preferentially expressed in different tissue during low temperature induction. Mature plants showed little staining for the reporter genes in the warm, and the cold-induction of the three CBFs was similar in response to low temperatures in shoot tissue. However, root tissue of mature plants was not examined for differences in cold-induced expression of CBFs1, 2, and 3. Double miRNA constructs targeting CBF1 and CBF3 show a dramatic reduction in freezing tolerance of acclimated plants (Novillo, Medina et al. 2007). However, it is not clear if this remaining freezing tolerance is due to CBF2 activity or CBF-independent pathways. It is still unknown if Arabidopsis plants lacking all three cold-inducible CBFs are still able to cold acclimate.

ABA as a Potential Component of a CBF-Independent Cold-Acclimation Pathway

Cross-talk between cold and other stresses may provide clues to potential CBF-independent mechanisms of achieving freezing tolerance. Levels of ABA, a plant growth hormone, important in signaling in response to drought stress, also increase in response to low temperature in *Arabidopsis* and pre-treatment with ABA increases freezing tolerance (Lang et al. 1994). Many cold regulated transcripts are also regulated in response to drought conditions, in an ABA dependent manner. It is not clear if the effects of ABA on cold-responsive transcripts and freezing tolerance are due to direct involvement of ABA in the cold-response or are secondary to the overlap in response between drought and cold. Support for the latter argument comes from the fact that drought stressed plants also have an increase in freezing tolerance similar to the levels seen by treatment with ABA (Mäntylä et al. 1995).

Analysis of High-Throughput Data Provides New Insights into Cold Acclimation

It appears that there is substantial integration of multiple signals which converge in regulating the process of cold-acclimation both through the CBF pathway and possible CBF-independent pathways. One method of analyzing the networks that compose the cold response is to perturb the system one variable at a time, changing either the functional components of known pathways of cold response, or altering the environmental signals which may be involved in regulating this response. Quantitative analysis of the effects each perturbation has on the system of cold response will be important for building accurate networks of signal transduction as qualitative changes maybe masked by redundancy. Fortunately, methods exits for sensitive analysis of such changes on a global scale. The primary focus here is on global transcriptional changes, however advances in the analysis of proteins, metabolites, ions, and other cellular components on a global scale is also improving and will add significantly to our understanding of the cold response.

The analysis of the effects of different growth conditions in publically available cold-response studies is already yielding interesting results. Comparison of the cold-induced transcripts from several labs revealed an enhancement of circadian-regulated genes in those considered to be cold-induced. Further analysis of these transcripts revealed that the large change in these cold and circadian transcripts was likely due to changes in output from the circadian clock. Therefore many transcripts were considered to be cold regulated because the phase of their circadian regulation shifted in response to low temperature. However, this was not a universal effect on all clock-regulated transcripts as both the phase and amplitude of some transcripts were maintained in response to low temperature (Blasing et al. 2005). Also by comparing the cold-treatment conditions of various labs and linking this to changes in cold-regulated gene expression Blasing et al. found that sugar signaling was also an important component of regulation of cold-responsive transcripts.

Analysis of the representation of previously described promoter elements and transcription factor binding sites in the promoters of cold-regulated transcripts indicates that there is extensive combinatorial regulation by trans-acting factors on cold-responsive genes. Each transcription factor family is predicted to interact with multiple other transcription factors, each interaction resulting in the regulation of a subset of cold-responsive target genes (Chawade et al. 2007). However, many cold-responsive transcripts are also regulated in response to other stresses. Therefore, the presence of multiple motifs on a single promoter could be a mechanism of regulation by independent transcription factors as downstream signals originating from distinct stresses and may not necessarily represent combinatorial control of the transcript in response to cold stress. Analysis of the response of these transcripts across multiple stresses using bi-clustering methods, which allow for the representation of a transcript into multiple clusters, will further divide cold regulated transcripts into patterns of regulation across multiple stresses. Analysis of the overrepresented promoter elements in these refined classes may provide additional insights into the role of these promoter elements in regulation of these transcripts to the various environmental stimuli to which they respond.

Analysis of transcriptional or metabolite data on a genome-wide scale in response to perturbations in the genome or environment during cold acclimation has the potential to provide a wealth of information on networks involved in the cold-response. Changes in gene expression can be correlated with different environmental factors or regulatory genes. However, for this information to be translatable into an improvement in ability of plants to survive low temperature, it will be important to establish a link between changes in the networks and the phenotype of freezing tolerance. Current methods of measuring freezing tolerance are incomplete, as they lack the capacity for quantitative assessment of the complex phenotype of freezing tolerance in a high-throughput manner. Methods such as electrolyte leakage analysis or thermal imaging, while quantitative, measure only one aspect of a complex response. In some cases the results of the measurements of these specific components of freezing tolerance are at odds with the overall phenotype. For example, Zat12 overexpressing plants are more freezing tolerant when measured by whole plant freeze tests, yet have a lower electrolyte leakage measure (Vogel et al. 2005, Vogel, unpublished). Whole plant freeze tests are not an ideal answer either because, as a qualitative measure, they are not sensitive enough to pick up changes in phenotype which maybe subtle due to compensation by multiple pathways of cold-acclimation. Since wild-type plants are able to survive after cold acclimation, it is difficult to quantify an improvement above complete survival. Similarly, as basal acclimation is not sufficient for survival of wild-type plants, non-acclimated wild-type plants do not survive freezing. Therefore, it is not possible to measure a decrease in basal acclimation ability, as mutations resulting in a decrease in basal freezing tolerance will show the same phenotype after freezing tolerance as wild-type plants, non-survival. To fully take advantage of the powerful tools of high-throughput analysis of the transcriptome and metabolome which are becoming available in plants, it will be necessary to develop a quantitative, high-throughput method for measuring freezing tolerance, possibly by incorporating measurements of several components of freezing tolerance.

Interaction between Cold and Development

Low temperature treatment plays a significant role in the regulation of many other processes. Cold-entrainment of the circadian clock is an important factor in the regulation of many circadian transcripts (Michael et al. 2008). This effect has physiological significance; plants grown in warm nights and cold days have decreased growth rates (Thingnaes et al. 2003). Many cold-regulated transcripts are also circadian regulated and their low-temperature regulation involves changes in the amplitude of the circadian clock in response to cold (Bieniawska et al. 2008). Further understanding of how cold contributes to these changes in the circadian regulation of target genes can help identify CBF-independent mechanisms for regulation of cold-responsive transcripts.

Low-temperature treatment also has an effect on development; short term cold treatment causes a delay in flowering. However, long-term cold treatment promotes flowering by activating the vernalization pathway. In this process, exposure to low temperatures for extended periods, results in repression of FLC, a negative regulator of flowering. This repression of FLC is maintained through epigenetic modifications to the FLC locus (Sung and Amasino 2005).

Integration of multiple signals, including cold, is also important for germination. Environmental cues of light, temperature, and water availability must all be analyzed in the decision to germinate. It will be interesting to see if the mechanisms of integration of these environmental signals at this stage of development are similar to the mechanisms of integration during cold acclimation.

Developmental State Affects Low Temperature Responses

The range to which an individual species can adapt to freezing depends on other environmental factors and the developmental state of the plant. Some environmental factors that have been shown to affect the process of cold acclimation include altitude, exposure to growth hormones, light quality and light quantity (Zarter et al. 2006; Franklin and Whitelam 2007; Soitamo et al. 2008). Development as well plays a role in cold acclimation levels. During the switch to a reproductive state from a vegetative state by induction of the vernalization pathway, rye and wheat have a reduction in their ability to induce cold-regulated transcripts and a loss in their ability to cold acclimate (Fowler et al. 1996). Other developmental factors including sex in dimorphous plant species has an effect on freezing tolerance (Li et al. 2005). Environmental cues have an effect on freezing tolerance. *Arabidopsis*, a model plant examined for freezing tolerance, has a dramatic increase in freezing tolerance when grown under short days compared to continuous light (Doherty, unpublished). The mechanisms that result in this increase in freezing tolerance remain unknown. One mechanism may be the gating of the cold response by day-length signals ensuring that the full cold response occurs only during short day conditions. Another possible mechanism for this increase in freezing tolerance seen in short day grown plants may be due to differences in developmental stage between short day and continuous light grown plants, if the continuous light grown plants have already made the switch to reproductive phase, their ability to cold acclimate maybe reduced.

The roles of CAMTAs, calmodulin regulated transcriptional activators, in regulating CBFs are discussed in this thesis. CAMTA regulation of CBF could serve as the integration point of calcium into the cold-response pathway. The second question of the role of CBF in the cold-acclimation process is addressed using a dominant negative version of CBF. Identifying a potential integration point for calcium signaling in the cold response and understanding the requirement for the CBF based pathway in the ability of *Arabidopsis* to cold acclimate will serve as important contributions to our knowledge of how plants interact with their environment. Additionally, these contributions may lay the groundwork for understanding how plants are able to integrate multiple signals in order to adapt to the complex environments in which they grow. With increased environmental changes on the near horizon due to increased atmospheric CO2 concentrations, it is of vital importance to understand how plants integrate complex environmental signals and the limits of the incredible plasticity plants demonstrate in responding to their ever-changing environment.

Literature Cited

Agarwal, M., Y. Hao, et al. (2006). "A R2R3 Type MYB Transcription Factor Is Involved in the Cold Regulation of CBF Genes and in Acquired Freezing Tolerance." J. Biol. Chem. **281**(49): 37636-37645.

Allen, G. J., K. Kuchitsu, et al. (1999). "Arabidopsis abi1-1 and abi2-1 Phosphatase Mutations Reduce Abscisic Acid–Induced Cytoplasmic Calcium Rises in Guard Cells." Plant Cell **11**(9): 1785-1798.

Ballou, S. M., K. Y. Yun, et al. (2007). "Cold Sensitivity Gradient in Tuber-Bearing Solanum Based on Physiological and Transcript Profiles." Crop Science **47**(5): 2027.

Bieniawska, Z., C. Espinoza, et al. (2008). "Disruption of the Arabidopsis Circadian Clock Is Responsible for Extensive Variation in the Cold-Responsive Transcriptome." Plant Physiol. **147**(1): 263-279.

Blasing, O. E., Y. Gibon, et al. (2005). "Sugars and circadian regulation make major contributions to the global regulation of diurnal gene expression in Arabidopsis." Plant Cell **17**: 3257 - 3281.

Cao, S., M. Ye, et al. (2005). "Involvement of GIGANTEA gene in the regulation of the cold stress response in Arabidopsis." Plant Cell Reports **24**(11): 683-690.

Chawade, A., M. Brautigam, et al. (2007). "Putative cold acclimation pathways in Arabidopsis thaliana identified by a combined analysis of mRNA co-expression patterns, promoter motifs and transcription factors." BMC Genomics **8**(1): 304.

Chinnusamy, V., M. Ohta, et al. (2003). "ICE1: a regulator of cold-induced transcriptome and freezing tolerance in Arabidopsis." Genes & Development **17**(8): 1043.

Chinnusamy, V., J. Zhu, et al. (2007). "Cold stress regulation of gene expression in plants." Trends in Plant Science **12**(10): 444-451.

Dong, C.-H., M. Agarwal, et al. (2006). "The negative regulator of plant cold responses, HOS1, is a RING E3 ligase that mediates the ubiquitination and degradation of ICE1." Proceedings of the National Academy of Sciences **103**(21): 8281-8286.

Fowler, D. B., L. P. Chauvin, et al. (1996). "The regulatory role of vernalization in the expression of low-temperature. induced genes in wheat and rye." Theor Appl Genet **93**: 554-559.

Fowler, S. and M. F. Thomashow (2002). "Arabidopsis Transcriptome Profiling Indicates That Multiple Regulatory Pathways Are Activated during Cold Acclimation in Addition to the CBF Cold Response Pathway." Plant Cell: tpc.003483.

Fowler, S. G., D. Cook, et al. (2005). "Low Temperature Induction of Arabidopsis CBF1, 2, and 3 Is Gated by the Circadian Clock." Plant Physiol **137**(3): 961 - 968.

Franklin, K. A. and G. C. Whitelam (2007). "Light-quality regulation of freezing tolerance in Arabidopsis thaliana." Nat Genet **39**(11): 1410-1413.

Gilmour, S. J., D. G. Zarka, et al. (1998). "Low temperature regulation of theArabidopsisCBF family of AP2 transcriptional activators as an early step in cold-inducedCORgene expression." The Plant Journal **16**(4): 433-442.

Harmer, S. L., J. B. Hogenesch, et al. (2000). "Orchestrated Transcription of Key Pathways in Arabidopsis by the Circadian Clock." Science **290**(5499): 2110-2113.

Hescheler, J., G. Mieskes, et al. (1988). "Effects of a protein phosphatase inhibitor, okadaic acid, on membrane currents of isolated guinea-pig cardiac myocytes." Pflügers Archiv European Journal of Physiology **412**(3): 248-252.

Jaglo-Ottosen, K. R., S. J. Gilmour, et al. (1998). "Arabidopsis CBF1 Overexpression Induces COR Genes and Enhances Freezing Tolerance." Science **280**(5360): 104-106.

Kim, H.-J., Y.-K. Kim, et al. (2002). "Light signalling mediated by phytochrome plays an important role in cold-induced gene expression through the C-repeat/dehydration responsive element (C/DRE) in Arabidopsis thaliana." The Plant Journal **29**(6): 693-704.

Knight, H., A. J. Trewavas, et al. (1996). "Cold Calcium Signaling in Arabidopsis Involves Two Cellular Pools and a Change in Calcium Signature after Acclimation." Plant Cell **8**(3): 489-503.

Kuo, A., S. Cappelluti, et al. (1996). "Okadaic Acid, a Protein Phosphatase Inhibitor, Blocks Calcium Changes, Gene Expression, and Cell Death Induced by Gibberellin in Wheat Aleurone Cells." Plant Cell **8**(2): 259-269.

Lang, V., E. Mantyla, et al. (1994). "Alterations in Water Status, Endogenous Abscisic Acid Content, and Expression of rab18 Gene during the Development of Freezing Tolerance in Arabidopsis thaliana." Plant Physiology **104**(4): 1341.

Lee, B.-h., H. Lee, et al. (2002). "A Mitochondrial Complex I Defect Impairs Cold-Regulated Nuclear Gene Expression." Plant Cell **14**(6): 1235-1251.

Li, C., Y. Yang, et al. (2005). "Sexual differences in cold acclimation and freezing tolerance development in sea buckthorn (Hippophae rhamnoides L.) ecotypes." Plant Science **168**(5): 1365-1370.

Mäntylä, E., V. Lång, et al. (1995). "Role of abscisic acid in drought-induced freezing tolerance, cold acclimation, and accumulation of LTI78 and RAB18 proteins in Arabidopsis thaliana." Plant Physiol **107**: 141-148.

Michael, T. P., T. C. Mockler, et al. (2008). "Network Discovery Pipeline Elucidates Conserved Time-of-Dayâ€"Specific cis-Regulatory Modules." PLoS Genetics **4**(2): e14.

Monroy, A. F. and R. S. Dhindsa (1995). "Low-temperature signal transduction: induction of cold acclimation-specific genes of alfalfa by calcium at 25 degrees C." Plant Cell **7**(3): 321-31.

Mori, I. C. and J. I. Schroeder (2004). "Reactive Oxygen Species Activation of Plant Ca2+ Channels. A Signaling Mechanism in Polar Growth, Hormone Transduction, Stress Signaling, and Hypothetically Mechanotransduction." Plant Physiol. **135**(2): 702-708.

Novillo, F., J. M. Alonso, et al. (2004). "CBF2/DREB1C Is a Negative Regulator of CBF1/DREB1B and CBF3/DREB1A Expression and Plays a Central Role in Stress Tolerance in Arabidopsis." Proceedings of the National Academy of Sciences of the United States of America **101**(11): 3985-3990.

Novillo, F., J. Medina, et al. (2007). "Arabidopsis CBF1 and CBF3 have a different function than CBF2 in cold acclimation and define different gene classes in the CBF regulon." Proceedings of the National Academy of Sciences **104**(52): 21002.

Soitamo, A., M. Piippo, et al. (2008). "Light has a specific role in modulating Arabidopsis gene expression at low temperature." BMC Plant Biology **8**(1): 13.

Sung, S. and R. M. Amasino (2005). "REMEMBERING WINTER: Toward a Molecular Understanding of Vernalization." Annual Review of Plant Biology **56**(1): 491-508.

Thingnaes, E., S. Torre, et al. (2003). "Day and Night Temperature Responses in Arabidopsis: Effects on Gibberellin and Auxin Content, Cell Size, Morphology and Flowering Time." Ann Bot **92**(4): 601-612.

Thomashow, M. F. (1999). "PLANT COLD ACCLIMATION: Freezing Tolerance Genes and Regulatory Mechanisms." Annual Review of Plant Physiology and Plant Molecular Biology **50**(1): 571-599.

Thomashow, M. F. (2001). "So What's New in the Field of Plant Cold Acclimation? Lots!" Plant Physiol. **125**(1): 89-93.

Townley, H. E. and M. R. Knight (2002). "Calmodulin as a Potential Negative Regulator of Arabidopsis COR Gene Expression." Plant Physiol. **128**(4): 1169-1172.

Viswanathan, C. and J. K. Zhu (2002). "Molecular genetic analysis of cold-regulated gene transcription." Philosophical Transactions of the Royal Society B: Biological Sciences **357**(1423): 877.

Vogel, J. T., D. G. Zarka, et al. (2005). "Roles of the CBF2 and ZAT12 transcription factors in configuring the low temperature transcriptome of Arabidopsis." The Plant Journal **41**(2): 195-211.

Xin, Z. and J. Browse (1998). "eskimo1 mutants of Arabidopsis are constitutively freezing-tolerant." Proceedings of the National Academy of Sciences **95**(13): 7799-7804.

Xin, Z., A. Mandaokar, et al. (2007). "Arabidopsis ESK1 encodes a novel regulator of freezing tolerance." The Plant Journal **49**(5): 786-799.

Yan, Y., C.-l. Wei, et al. (2006). "Cross-talk between calcium and reactive oxygen species signaling." Acta Pharmacologica Sinica **27**(7): 821-826.

Zarka, D. G., J. T. Vogel, et al. (2003). "Cold induction of Arabidopsis CBF genes involves multiple ICE (inducer of CBF expression) promoter elements and a cold-regulatory circuit that is desensitized by low temperature." Plant Physiol **133**: 910 - 918.

Zarter, C. R., W. W. Adams, et al. (2006). "Winter acclimation of PsbS and related proteins in the evergreen Arctostaphylos uva-ursi as influenced by altitude and light environment." Plant, Cell & Environment **29**(5): 869-878.

Zhang, X., S. G. Fowler, et al. (2004). "Freezing-sensitive tomato has a functional CBF cold response pathway, but a CBF regulon that differs from that of freezing-tolerant Arabidopsis." The Plant Journal **39**(6): 905-919.

Zhu, J., P. E. Verslues, et al. (2005). "HOS10 encodes an R2R3-type MYB transcription factor essential for cold acclimation in plants." Proceedings of the National Academy of Sciences **102**(28): 9966-9971.

CHAPTER TWO

Roles for Arabidopsis CAMTA Transcription Factors in Cold-Regulated Gene Expression and Freezing Tolerance

SUMMARY

The ability of some plants to cold-acclimate is an important survival trait that increases both the seasonal and geographic range of plant growth. Understanding the process by which plants are able to cold acclimate will allow us to extend the growing season and available land for crop growth. The CBF cold-response pathway is an important component of the cold acclimation process. To understand how plants are able to turn on the CBF pathway in response to low temperature we analyzed the *CBF2* promoter in Arabidopsis. This analysis led to the identification of a 27 bp region of the *CBF2* promoter, the CM1/CM2-Box that is sufficient for cold responsiveness. We identified CAMTA3 as a positive trans-acting regulator of *CBF1* and *CBF2* in response to low temperature. Loss of CAMTA 3 results in a reduced cold-induction of *CBF1* and *CBF2*. Loss of both CAMTA1 and CAMTA3 results in reduced ability of Arabidopsis to cold acclimate. These results indicate that the CAMTA 1 and CAMTA3 transcription factors are components of the cold acclimation response in Arabidopsis.

INTRODUCTION

Many plants from temperate regions increase in freezing tolerance upon exposure to low non-freezing temperatures through a process known as cold acclimation. One major pathway involved in cold acclimation is the *CBF* cold-response pathway. In Arabidopsis, the pathway includes three genes, *CBF1*, *CBF2* and *CBF3*, also known as *DREB1b*, *DEB1c* and *DREB1a*, respectively, whose transcript levels increase within 15 minutes of exposure to low temperature (Gilmour et al. 1998). The *CBF* genes encode three closely related members of the AP2/ERF domain family of transcription factors. CBF proteins regulate the transcription of many downstream target genes, known as the CBF regulon, by binding to CRT/DRE (C-repeat/dehydration responsive element) DNA regulatory element found in the promoters of these genes (Stockinger et al. 1997; Liu et al. 1998). Expression of the CBF regulon, which includes *COR* and other cold-responsive genes, results in an increase in freezing tolerance (Jaglo-Ottosen et al. 1998). This increase in freezing tolerance involves a variety of biochemical and physiological changes that are mediated by changes in expression of CBF regulon genes, including the accumulation of compatible solutes such as raffinose, sucrose and proline and the production of cryoprotective polypeptides such as *COR15a* (Gilmour, Zarka et al. 1998).

While the CBF pathway has been demonstrated to have a major role in plant cold acclimation, relatively little is known about the mechanism(s) that induces *CBF1-3* gene expression in response to low temperature. Zarka et al. (Zarka et al. 2003) identified a 155-bp region of the *CBF2* promoter that is capable of driving cold-induced transcription and found that within this promoter fragment that there are two short sequences, ICEr1 and ICEr2 (Inducer of CBF expression region 1 and 2), that contribute to cold-induction. Zhu and colleagues have identified two transcription factors involved in *CBF* gene expression (Chinnusamy et al. 2003; Agarwal et al. 2006). One of these, MYB15, appears to act as a negative regulator of *CBF1-3* expression; loss of *MYB15* function does not affect *CBF1-3* expression at warm temperature, but results in a detectible increase in *CBF1-3* expression at low temperature. The other transcription factor, ICE1, is a MYC-like bHLH protein that binds to MYC recognition sites in the *CBF3* promoter. A dominant-negative mutation of ICE1 was isolated, *ice1*, that nearly eliminates cold-induction *CBF3,* indicating the protein acts as a positive regulator *CBF3* expression. The *ice1* mutation, however, has little effect on cold-induced expression of *CBF1* and *CBF2* indicating that there are differences in the specific transcription factors that regulate expression of *CBF1-3*.

Upon exposure to low temperature there is a transient increase in cytosolic calcium levels. A vacuolar release of calcium in response to low temperature has been demonstrated in Arabidopsis through use of aequorin, a bioluminescent calcium reporter (Knight et al. 1996). This rapid influx of calcium into the cytosol is required for proper regulation of *KIN1*, a target of CBF, in response to low temperature. Similarly, in alfalfa, regulation of two cold acclimation specific (cas) genes, cas15 and cas18 was shown to involve calcium signaling (Monroy and Dhindsa 1995). These results point to the importance of calcium in the response of plants to low temperature. However, the molecular mechanism connecting the calcium increase and cold-regulated gene expression is not known.

Here the goal was to further an understanding of the *cis*-acting DNA regulatory elements and *trans*-acting factors involved in regulation of the CBF cold response pathway. A cold-regulatory element overlapping the ICEr2 region was identified in the *CBF2* promoter that is sufficient for cold-induction of a reporter construct. Additionally, it is shown here that members of the CAMTA (calmodulin-binding transcription factors) family of transcription factors can bind to this region of the *CBF2* promoter; CAMTA3 is a positive regulator of *CBF2* expression; and that the increase in freezing tolerance that occurs with cold acclimation involves concerted action of CAMTA1 and CAMTA3. These results identify new *cis*-acting cold-regulatory elements, establish a role for CAMTA proteins in cold-acclimation, and provide a possible point of integrating calcium into the cold response pathway.

RESULTS

A 27 bp Sequence from the *CBF2* Promoter is Sufficient to Impart Cold-Induced Transcription

We recently identified seven DNA motifs, 6 to 9 nt in length, that are present in both the 155 bp cold-responsive promoter fragment of *CBF2* and a 224 bp region of the *ZAT12* promoter that is sufficient to impart cold-induced transcription (Van Buskirk, in preparation). One of these sequences, the CM2-box, overlaps the ICEr2 element. To determine whether this region of the *CBF2* promoter was sufficient to impart cold-induced gene expression, a 27 bp region of the *CBF2* promoter (Fig 2.1A) that included the ICEr2 sequence, as well as the overlapping CM2-box and the CM1-box (another of the seven conserved DNA motifs), was fused as a tetramer to the GUS reporter gene and the construct tested for expression in Arabidopsis. The results indicated that the 27 bp fragment could indeed impart cold-induced gene expression (Fig 2.1B,C).

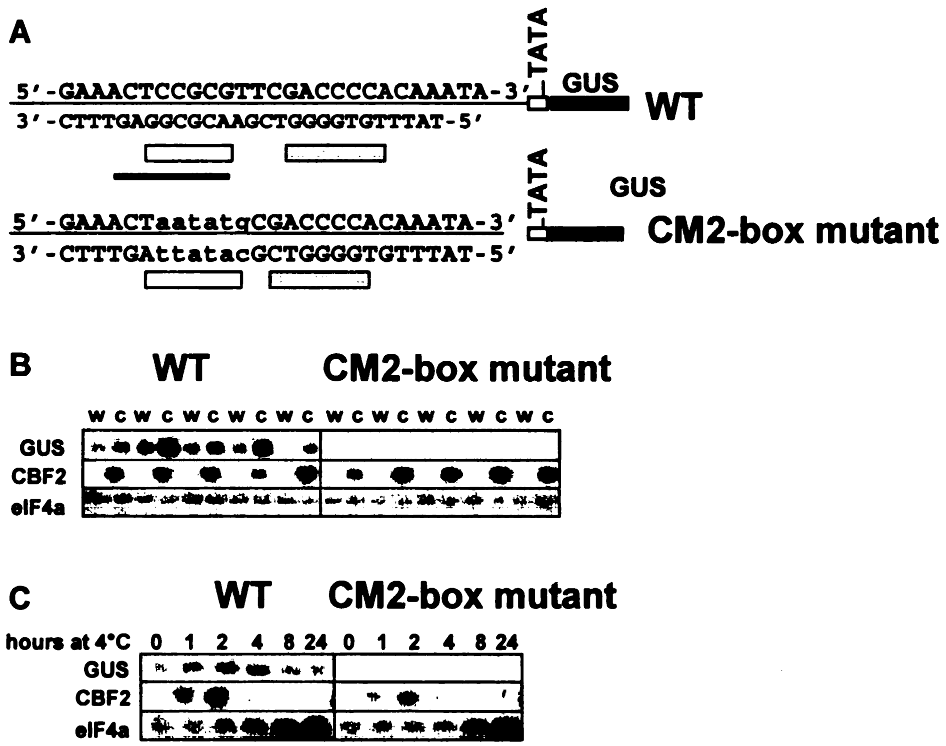


Figure 2.1 Tetramer Containing CM2-box and CM1-box Is Able to Drive Transcription of GUS Reporter Gene in Response to Treatment at 4°C.
(A) Sequence of conserved region defining CM2-box (clear) and CM1-box (shaded) and mutated version of CM2-box. ICER2 sequence is indicated by bar.
(B) Northern blot analysis of plants expressing either WT version of the CM2/CM1-box tetramer or plants expressing a reporter gene with a mutated CM2-box sequence. Plants were harvested after growth in the (W) warm or (C) after treatment at 2h4°C. Five independent transgenic lines of the reporter construct are shown for WT and CM2-box mutant.
(C) Induction of GUS reporter and CBF2 in response to treatment at 4°C of one line shown in B.

In addition, it was found that nucleotide substitutions that resulted in the elimination of the CM2-box resulted in a DNA fragment that could not impart cold-inducible gene expression (Fig. 2.1B,C). These results indicated that the 27 bp sequence can impart cold-inducible transcription and that this requires the CM2-box sequence.

CAMTA Proteins Bind the CM2-box Sequence

The CAMTA family of calmodulin-binding transcription factors comprises six members in Arabidopsis (Bouche et al. 2002; Yang and Poovaiah 2002). Each protein includes a DNA binding domain referred to as the CG-domain which binds to a core consensus sequence, vCGCGb, referred to as the CG-1 element (de Costa e Silva 1994). This sequence matches the CM2-box sequence, ACGCGG, and overlaps the ICEr1 element (Figure 2.2A). Thus, the possibility raised was that one or more of the CAMTA proteins have a role regulating *CBF2* expression.

As a first test of this possibility, we asked whether the CG-1 DNA binding domain of the CAMTA proteins could bind to the CM2-box sequence (Figure 2.2B). Specifically, the CG-1 DNA-binding domains of CAMTA1, 2, 3 and 5 (CAMTA4 and 6 were not tested) were fused to GST, expressed in *E. coli*, and soluble protein extracts were assayed for DNA binding using the electro-mobility shift assay (EMSA). No shift was detected when lysates from *E. coli* lacking the expression vector or expressing a recombinant protein consisting of GST fused to a disrupted version of the CAMTA5 CG-1 domain with a premature early stop codon. In contrast, a band shift occurred with proteins comprised of the CAMTA1, 2, 3 or 5 CG-1 domains fused to GST. The binding could be competed off with unlabeled DNA containing the wild-type CM2-box sequence, but not with unlabeled DNA containing a mutation in the CM2-box sequence indicating specificity of protein-DNA binding.

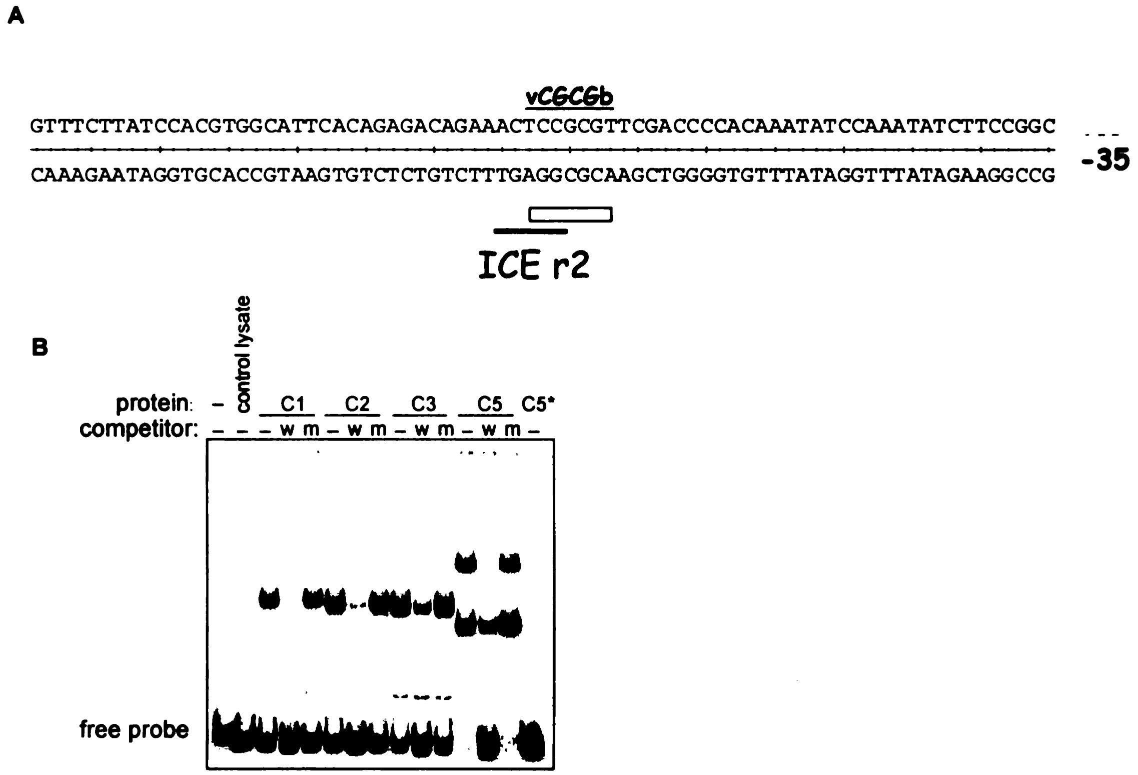


Figure 2.2 CAMTAs Bind CM2-Box Region of CBF2 Promoter
(A) Overlap of CM2-Box (open box), ICEr2 region, and putative CG-1 DNA recognition sequence.
(B) EMSA of CM2/CM1-Box by CAMTA CG-1 DNA binding domain. Probe is the CM2/CM1-Box sequence as in Figure 1A. Lanes are lysate from non-vector containing E.coli (lysate), E.coli expressing CG-1 DNA binding domains from CAMTA1 (C1), CAMTA2(C2), CAMTA3(C3), and CAMTA5(C5), and a scrambled version of the CAMTA5 CG-1DNA binding domain(C5*). Competition with WT CM2/CM1-Box (w) and with CM2-Box mutant (m) sequence as in Figure 1A

CAMTA3 has a Role in Cold-Induced Expression of *CBF2*

To further test the possibility that one or more of the CAMTA proteins have a role in *CBF2* expression, we identified homozygous T-DNA insertion lines for each *CAMTA* gene that resulted in undetectable transcripts for each corresponding gene (Figure 2.3). Each *camta* mutant plant was tested for differences in *CBF2* expression from wild-type plants after 2h0°C treatment. Initial northern analysis suggested that the *camta3* mutation resulted in a reduction of cold-induced accumulation of *CBF2* transcripts (Figure 2.4A). This was confirmed by quantitative RT-PCR analysis; *CBF2* transcript levels in cold-treated *camta3* plants were reduced to about 50% of the level found in cold-treated wild-type plants (Figure 2.4B). No statistically significant reduction in *CBF2* expression was observed in *camta1, 2, 4, 5* or *6* at a p-value <0.05.

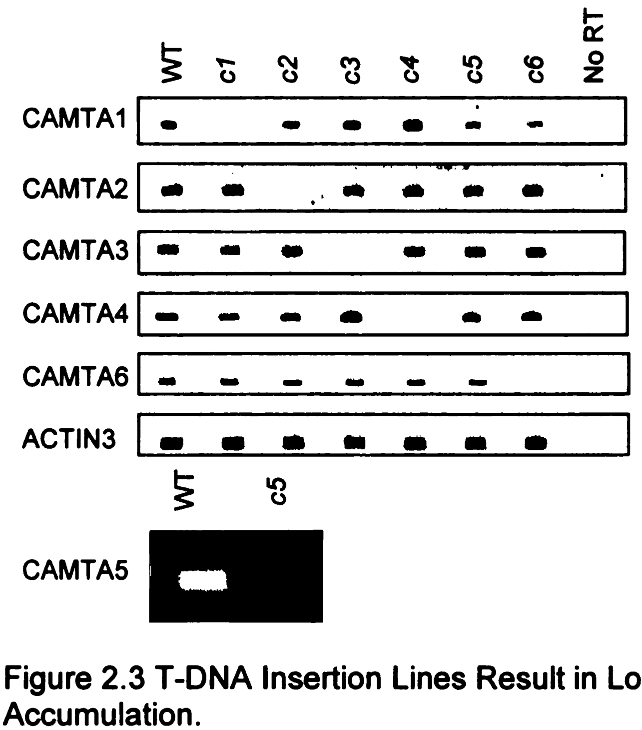


Figure 2.3 T-DNA Insertion Lines Result in Loss of CAMTA Transcript Accumulation.
RT-PCR analysis of CAMTA T-DNA insertion lines *camta1(c1), camta2(c2), camta3(c3), camta5(c5), camta6(c6)* shows a loss of transcript in each T-DNA insertion line compared to WT (Col-0)

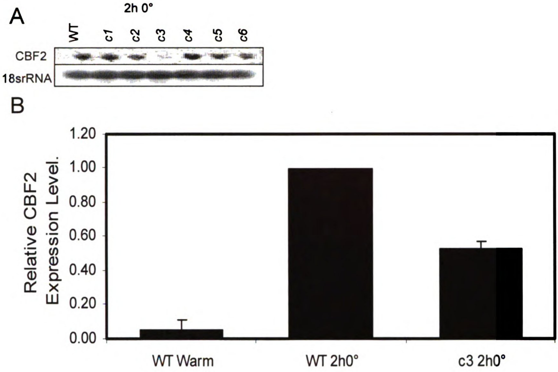


Figure 2.4 CBF2 Levels Are Reduced in *camta3* Plants
(A) RNA gel blot analysis for cold-induced CBF2 expression. Transcript levels of CBF2 gene were examined by gel blot analysis using total RNA prepared from seedlings of WT and all *camta* T-DNA insertion lines grown for 2h at 0°C. The same blot probed for 18s rRNA is shown for a loading control.
(B) QPCR analysis. Relative expression level of CBF2 transcript in terms of WT 2h0°C. Error bars indicate SE. The averages for WT warm and *camta3* 2h0°C are significantly different than WT 2h0°C (p-value <0.0001) (n=6 for WT warm, n=12 for WT 2h0°C, n=12 for *camta3* 2h0°C).

In growing the plants for these experiments, it was noted that those carrying either *camta1, 2 , 3 ,4 or 5* mutations showed no obvious abnormalities in growth and development when their life cycle was carried out at either warm (22°C) or cold (4°C) temperatures. The *camta6* mutant plants displayed yellowing of the veins and midrib at warm temperature, which was suppressed in new leaves produced at low temperatures (Figure 2.5). We have not yet tried to suppress this phenotype by overexpressing *CAMTA6*, therefore we cannot rule out the possibility that this phenotype was due to a separate mutation unlinked to *camta6*.

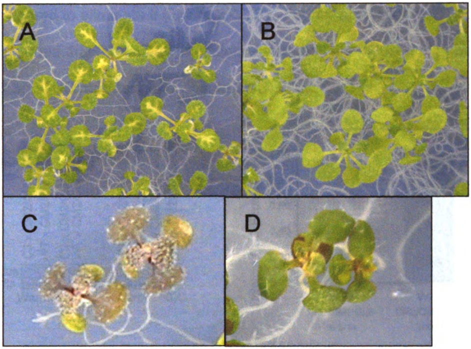


Figure 2.5 *camta6* Growth Phenotype
(A) *camta6* T-DNA insertion line showed a yellowing of the veins when grown at 22°C.
(B) WT Col-0 plants grown at 22°C
(C) *camta6* germinated and grown for 40d at 4°C did not show this phenotype
(D) WT Col-0 plants germinated and grown for 40d at 4°C

The CM2/CM1-box Sequence is a Site of CAMTA3 Action *In Planta*

The results presented above indicated that the 27 bp fragment of the *CBF2* promoter that includes both the CM2-box and CM1-box imparts cold-induced gene expression, and that this required the CM2-box sequence to which the CAMTA3 protein can bind. If the CAMTA3 protein contributes to *CBF2* cold-induction through binding to the CM2-box *in planta,* then it would be anticipated that the camta3 mutation would affect cold-induction of the GUS reporter gene driven by the 27 bp promoter fragment. This was the case.

Northern analysis indicated that whereas the *camta1* mutation had no detectable effect on cold-induction of the reporter gene, the *camta3* mutation appeared to eliminate it (Figure 2.6A,B). Further analysis by qRT-PCR confirmed that the *camta3* mutation eliminated cold-induced expression of the 27 bp::GUS reporter gene (Figure 2.6C).

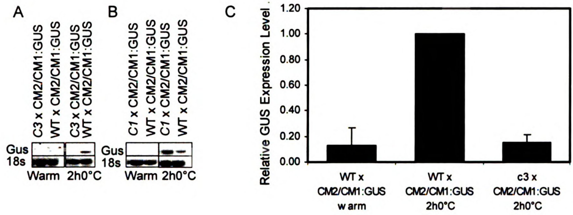


Figure 2.6 CM2/CM1-Box:GUS mRNA Levels Are Reduced in *camta3* Plants
(A) Northern analysis of *camta3*T-DNA insertion lines crossed to the CM2/CM1-Box:GUS reporter construct lines (*C3* x CM2/CM1:GUS)revealed a reduction in cold-induced GUS mRNA levels compared to WT plants crossed to the CM2/CM1-Box:GUS lines (WT x CM2/CM1:GUS).
(B) Northern analysis of *camta1* T-DNA insertion lines crossed to the CM2/CM1-Box:GUS reporter construct lines (*C1* x CM2/CM1:GUS) showed no reduction in cold-induced GUS mRNA levels.
(C) QPCR analysis. Relative expression level of GUS transcript in terms of WT 2h0°C. Error bars indicate SE. The average Gus level in the *camta3* background at 2h0°C are significantly different than WT 2h0°C (p-value <0.0001) (n=2 for WT warm, n=6 for WT 2h0°C, n=6 for *camta3* 2h0°C).

Histochemical staining of lines carrying the 27bp::GUS reporter indicated that GUS activity was dramatically reduced in plants carrying the *camta3* mutation whether they were grown at warm or cold-temperature (Figure 2.7A). The small amount of GUS staining in the *camta3* plants was limited to the roots of the plants, indicating that CAMTA3 may only be responsible for regulation of the CM2/CM1-Box in shoot tissue. The staining of the wild-type plants grown under warm conditions was presumably due to the low level expression of the reporter gene observed at warm temperature (Figure 2.1B,C). Transformation of these plants with the wild-type *CAMTA3* gene under control of the 35*S* CaMV promoter resulted in recovery of staining confirming that the *camta3* mutation was responsible for the lack of reporter gene expression (Figure 2.7A). Recovery of *CBF2* expression levels was also observed in the *camta3* plants transformed with the wild-type CAMTA3 gene (Figure 2.7B). Finally, the levels of expression of the transgenic CAMTA3 gene were near equal to or less that the level of the endogenous CAMTA3 gene in wild-type plants (Figure 2.8).

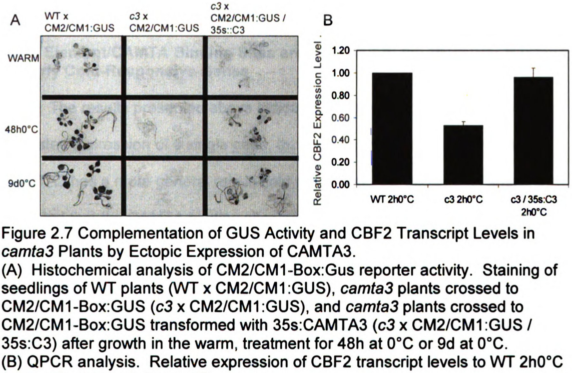


Figure 2.7 Complementation of GUS Activity and CBF2 Transcript Levels in *camta3* Plants by Ectopic Expression of CAMTA3.
(A) Histochemical analysis of CM2/CM1-Box:Gus reporter activity. Staining of seedlings of WT plants (WT x CM2/CM1:GUS), *camta3* plants crossed to CM2/CM1-Box:GUS (*c3* x CM2/CM1:GUS), and *camta3* plants crossed to CM2/CM1-Box:GUS transformed with 35s:CAMTA3 (*c3* x CM2/CM1:GUS / 35s:C3) after growth in the warm, treatment for 48h at 0°C or 9d at 0°C.
(B) QPCR analysis. Relative expression of CBF2 transcript levels to WT 2h0°C (n=3). Error bars indicate SE.

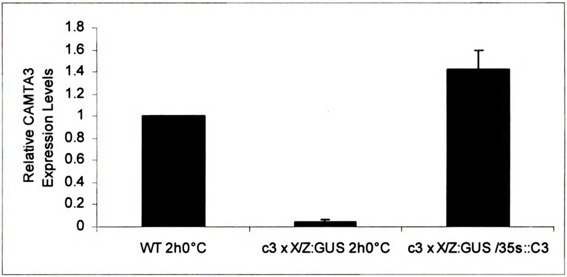


Figure 2.8 Recovery of WT Levels of CAMTA3 in *camta3 x* CM2/CM1-Box:GUS Plants by Ectopic Expression of CAMTA3.
QPCR analysis of relative expression levels of CAMTA3 in terms of WT level of expression in *camta3* plants crossed to CM2/CM1-Box:GUS (*c3* x CM2/CM1:GUS) and *camta3* plants crossed to CM2/CM1-Box:GUS transformed with 35s:CAMTA3 (*c3* x CM2/CM1:GUS / 35s:C3) after treatment for 2h0°C. Error bars indicate SE.

CG-1 Element/CAMTA Binding Sites are Enriched in the Promoter Regions of Early Cold-Responsive Genes

The results presented above indicated that CAMTA3 has a role in cold-regulated expression of a single gene, thus raising the question of whether CAMTA3 has a more general role in cold regulation. Computational analysis indicated that the CAMTA binding sites are enriched in the promoters of cold-regulated genes. A search of publicly available microarray data (Vogel et al. 2005; Kilian et al. 2007) identified 46 genes having an expression pattern that highly correlated with that of *CBF2,* with a minimum Pearson's correlation of 0.85 (Table 2.1). These 46 genes were analyzed for the presence of the CG-1 binding site using Promomer (Toufighi et al. 2005). Nineteen of these genes had at least one vCGCGb sequence present in the upstream 500 bp sequence

(TAIR7). Hypergeometric testing, a statistical method to determine if an event is overrepresentation in a subset of a population, was used to determine if the CG-1 element was overrepresented. Analysis of the presence of the CG-1 element in this set of cold-regulated transcripts compared to its presence in the 500 bp upstream sequences of all sequences indicated that the CG-1 element was highly enriched in the promoters of these cold-responsive genes (p-value, $<10^{-5}$).

To test if the CG-1 sequence was overrepresented in early cold-induced genes, transcripts were selected that are up-regulated early in response to low temperature, regardless of their overall correlation with *CBF2* expression and were tested for enrichment of the CG-1 sequence. Publicly available microarray data were analyzed to create a list of genes that responded rapidly to low temperature. Thirty genes (Table 2.2) were selected that were up-regulated two-fold in wild-type plants in Vogel et al. and were also up 2 fold at 1 h and 3h in ATGenExpress cold-treated samples (Vogel et al. 2005; Kilian et al. 2007). Of these 30 genes, 12 contained at least on CG-1 element in the promoter region 500 bp upstream of the start site (TAIR7). Hypergeometric testing showed the occurrence of the element in these genes was enriched as compared to the rest of the genome ($p<0.001$). Together, these results indicate that the CG-1 sequence is overrepresented in early cold-responsive transcripts and those with a pattern of expression at low temperature similar to *CBF2.*

Table 2.1 Transcripts with a Pattern of Induction in the Cold Similar to CBF2
Forty-six transcripts that have a pattern of induction similar to that of CBF2 in response to treatment at 4°C. Those in bold contain the sequence vCGCGb in the region 500bp upstream of the 5'UTR (TAIR7).

Transcripts with pattern of induction similar to CBF2 in cold microarray experiments	
AGI	
At1g09070	**Soybean Regulated by Cold SRC2**
At1g13260	RAV1
At1g21010	Unknown Protein
At1g27730	ZAT10
At1g61340	F-box family protein
At1g65390	ATPP2-A5
At1g69570	Dof-type zinc finger domain-containing protein
At1g70420	Unknown Protein
At1g74450	**Unknown Protein**
At1g75020	LPAT4
At1g75180	**Unknown Protein**
At2g27260	**Similar to Hydroxyproline-Rich Glycoprotein Family Protein**
At2g36220	Unknown Protein
At2g37970	**SOUL-1**
At2g38790	Unknown Protein
At3g04640	Glycine-Rich Protein
At3g23170	similar to AtBET12
At3g28340	**GATL10**
At3g48520	CYP94B3
At3g51920	**CAM9**
At3g52800	Zinc finger (AN1-like) family protein
At4g16780	ATHB-2
At4g18280	Glycine-Rich Cell Wall Protein
At4g25470	**CBF2**
At4g25480	CBF3
At4g25490	CBF1
At4g29190	Zinc Finger (CCCH-type) Family Protein
At4g29610	Putative Cytidine Deaminase
At4g32020	**Unknown Protein**
At4g33920	**Protein Phosphatase 2C Family Protein**
At4g36500	Putative Protein
At4g37260	MYB73
At5g04340	CZF2
At5g10695	**Unknown Protein**
At5g20230	**Blue Copper Binding Protein**
At5g39785	**Unknown Protein**
At5g46710	Zinc Binding Family Protein
At5g47230	ATERF5
At5g54470	**Zinc Finger (B-box type) Family Protein**
At5g58650	**PSY1**
At5g59730	**A member of EXO70 gene family**
At5g59820	**Zat12**
At5g60680	Unknown Protein
At5g62460	**Zinc Finger (C3HC4-type RING finger) Family Protein**
At5g63130	**Octicosapeptide/Phox/Bem1p (PB1) Domain-Containing Protein**
At5g66070	**Zinc Finger (C3HC4-type RING finger) Family Protein**

Table 2.1 Transcripts with a Pattern of Induction in the Cold Similar to CBF2

Transcripts up early in response to low temperature	
AGI	Description
At1g19050	ARR7
At1g21050	Unknown Protein
At1g21910	Encodes a Member of the DREB subfamily A-5 of ERF/AP2 transcription factor family.
At1g27730	ZAT10
At1g68840	RAV2
At1g74890	ARR15
At1g76600	**Unknown Protein**
At1g80440	**Kelch Repeat-containing F-box Family Protein**
At1g80840	WRKY40
At2g25900	**CTH**
At2g26530	Unknown Protein
At2g38470	WRKY33
At3g15450	Unknown Protein
At3g48100	**ARR5**
At3g48360	BT2
At3g55980	**Zinc Finger (CCCH-type) Family Protein**
At4g01250	WRKY22
At4g24570	**Mitochondrial Substrate Carrier Family Protein;**
At4g25470	**CBF2**
At4g25480	CBF3
At4g29780	**Unknown Protein**
At4g34150	**C2 Domain-containing Protein**
At4g36040	DNAJ heat shock N-terminal domain-containing protein
At4g37610	BT5
At5g20230	**Blue Copper Binding Protein**
At5g28770	bZIP 63
At5g37260	**CIR1**
At5g57560	**Cell wall-modifying enzyme**
At5g59820	Zat12
At5g62920	ARR6

Table 2.2 Transcripts up Early in Response to Low Temperature
Thirty transcripts up-regulated at 1h in response to treatment at 4°C. Those in bold contain the sequence vCGCGb in the region 500bp upstream of the 5'UTR (TAIR7).

A Role for CAMTA3 in Cold-Induction of *CBF1*, *ZAT12* and *GOLS3*

Like *CBF2*, the promoter regions (1kb region upstream of start codon) of *CBF1* and *ZAT12* include a CG-1 sequence whereas that of *CBF3* does not. It was therefore of interest to determine whether any of the *camta* mutations affected cold-induced expression of these genes. The results indicated that the *camta3* mutation reduced *CBF1* transcript levels by about 40% (Figure 2.9A) and reduced *ZAT12* transcript levels by about 50% (Figure 2.9B). Although a slight decrease in *CBF3* expression was suggested, there was great variability over repeated experiments indicating that this was not statistically significant at a p-value <0.05 (Figure 2.9C).

One downstream target of CBF, *GOLS3*, was reduced about 40% in the *camta3* plants (Figure 2.10). The effect on *GOLS3* levels indicates that the reduction in *CBF1* and *CBF2* levels seen in *camta3* plants may have an effect on downstream cold responses.

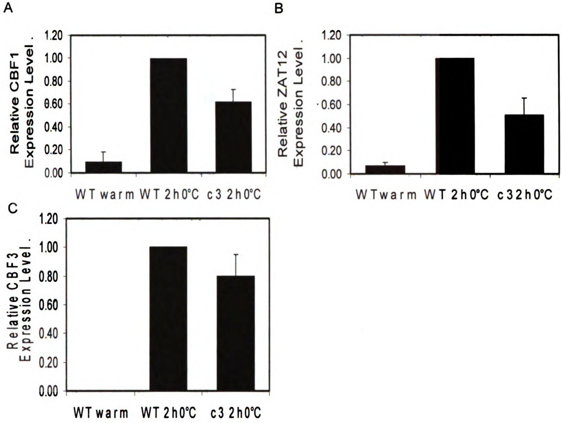


Figure 2.9 *CBF1, ZAT12*, and *CBF3* Levels in *camta3* Plants
(A)QPCR analysis shows relative expression of *CBF1* transcript levels in terms of WT2h0°C. Error bars indicate SE. The averages for WT warm and *camta3* 2h0°C are significantly different than WT 2h0°C (Student's t-test p-value <0.01) (n=10 for WT warm, n=12 for WT and *camta3* 2h0°C)
(B)Relative expression of *Zat12* transcript levels in terms of WT2h0°C. Error bars indicate SE. The averages for WT warm and *camta3* 2h0°C are significantly different than WT 2h0°C (Student's t-test p-value <0.01) (n=10 for WT warm, n=12 for WT and *camta3* 2h0°C)
(C)Relative expression of *CBF3* transcript levels in terms of WT2h0°C. Error bars indicate SE. The average for *camta3* 2h0°C is not significantly different than WT 2h0°C (Student's t-test cutoff p-value <0.05) (n=2 for WT warm, n=4 for WT and *camta3* 2h0°C)

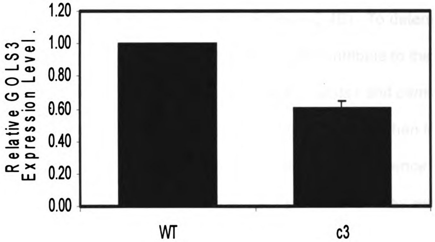


Figure 2.10 *Galactinol synthase 3* Levels Are Reduced in *camta3* Plants
QPCR analysis shows relative expression levels of galactinol synthase3 are significantly reduced in 24h0°C *camta3* plants compared to WT at 24h0°C (Student's t-test p-value <0.01, n=4).

Concerted Action of both *CAMTA1* and *CAMTA3* Are Required to Attain Full Levels of Freezing Tolerance

The results presented above establish that the *camta3* mutation caused about a 40-50% decrease in cold-induced accumulation of transcripts for *CBF1, CBF2*, *ZAT12,* and one of the CBF targets, *GOLS3*. It was therefore of interest to determine whether the *camta3* mutation had an effect on freezing tolerance. The results of both electrolyte leakage (Figure 2.11A) and whole plant freeze tests (not shown) did not reveal any difference in freezing tolerance wild-type and *camta3* mutant plants grown at either warm temperature or cold-acclimated for 7d4°C. Additional testing indicated that the *camta1, 2, 4, 5, and 6* mutants were also not affected in freezing tolerance (not shown).

The CAMTA1, 2 and 3 proteins have similar protein structure and thus might have overlapping functions (ref; Figure 2.4B). To determine if redundancy between CAMTAs 1 and 3 might contribute to the lack of freezing tolerance seen in the *camta3* mutant plants, *camta1* and *camta3* mutants were crossed to obtain a *camta1/3* double mutant which was then tested for freezing tolerance (Figure 2.11B). There was no significant difference in freezing tolerance between the *camta1/3* and WT plants when they were grown at 22°C, but there was a considerable difference after cold acclimation. A 7 day period of cold acclimation at 4°C resulted in about a 5°C increase in freezing tolerance in WT plants, but the *camta1/3* plants only increased about 2°C in freezing tolerance. Thus, for Arabidopsis to attain full levels of freezing tolerance, it needs the concerted action of both the *CAMTA1* and *CAMTA3* genes. The basis for this requirement is not known, but the *camta1/3* plants displayed outward phenotypic differences that might have accounted, at least in part, for the differences in freezing tolerance. The *camta1/3* plants were smaller than WT plants when grown at 22°C and showed an increase in chlorosis compared to WT plants (Figure 2.12A). Moreover, both of these phenotypes were more pronounced when grown at 4°C (Figure 2.12B). The CBF2 levels in response to 2h0°C treatment were near WT levels in *camta1/camta3* double mutants (Figure 2.13 A,B).

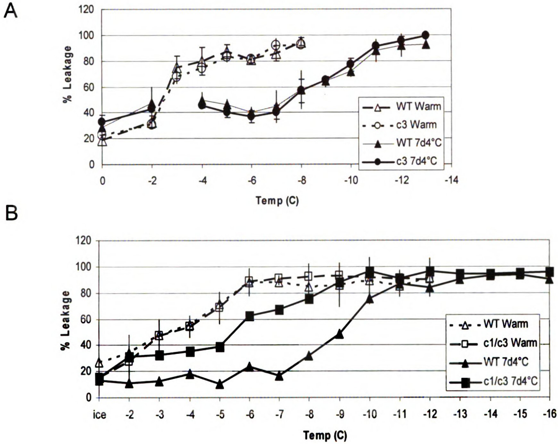


Figure 2.11 Effect of *camta3* and *camta1/3* Mutations on Freezing Tolerance
(A) Electrolyte leakage assay of non-acclimated WT (dashed lines, open triangles), non-acclimated *camta3*(c3) (dotted lines, open circle), WT acclimated for 7d4°C (solid line, filled triangles) and camta3(c3) acclimated for 7d4°C(solid line, filled circles).
(B) Electrolyte leakage assay of non-acclimated WT (dashed lines, open triangles), non-acclimated *camta1/camta3* (c1/c3) (dotted lines, open squares), WT acclimated for 7d4°C (solid line, filled triangles), and *camta1/camta3* (c1/c3) acclimated for 7d4°C (solid line, filled squares)

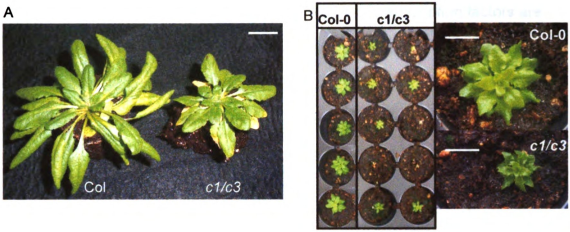


Figure 2.12 Effect of *camta1/3* Mutations on Development
(A) Warm grown Columbia-0 (Col) and *camta1/3 (c1/c3)* double mutants. Bar indicates 18mm.
(B) Colombia-0 (Col) and *camta1/3(c1/c3)* double mutants grown at 4°C

DISCUSSION

CAMTA3 Regulates *CBF2* Expression in Response to Low Temperature Through the CM2-Box Region of the *CBF2* Promoter.

This study took an in depth look at the *CBF2* promoter and identified multiple elements present in the promoter that are involved in regulation of *CBF2* transcription. Conserved regions in the similarly regulated *ZAT12* promoter were identified and through mutational analysis we determined that a 27bp sequence, the CM2/CM1-box is necessary for cold-induced regulation of a reporter construct. This result is consistent with the overlap between the CM2-box region and the previously identified ICEr2 region (Zarka et al. 2003). Additionally, four copies of the CM2-box, in combination with the region downstream, the CM1-box, was sufficient to induce cold-transcription of a reporter gene in a manner dependent on an intact CM2-box sequence. We showed that several members of the CAMTA family of transcription factors are able to bind specifically to this region of the *CBF2* promoter.

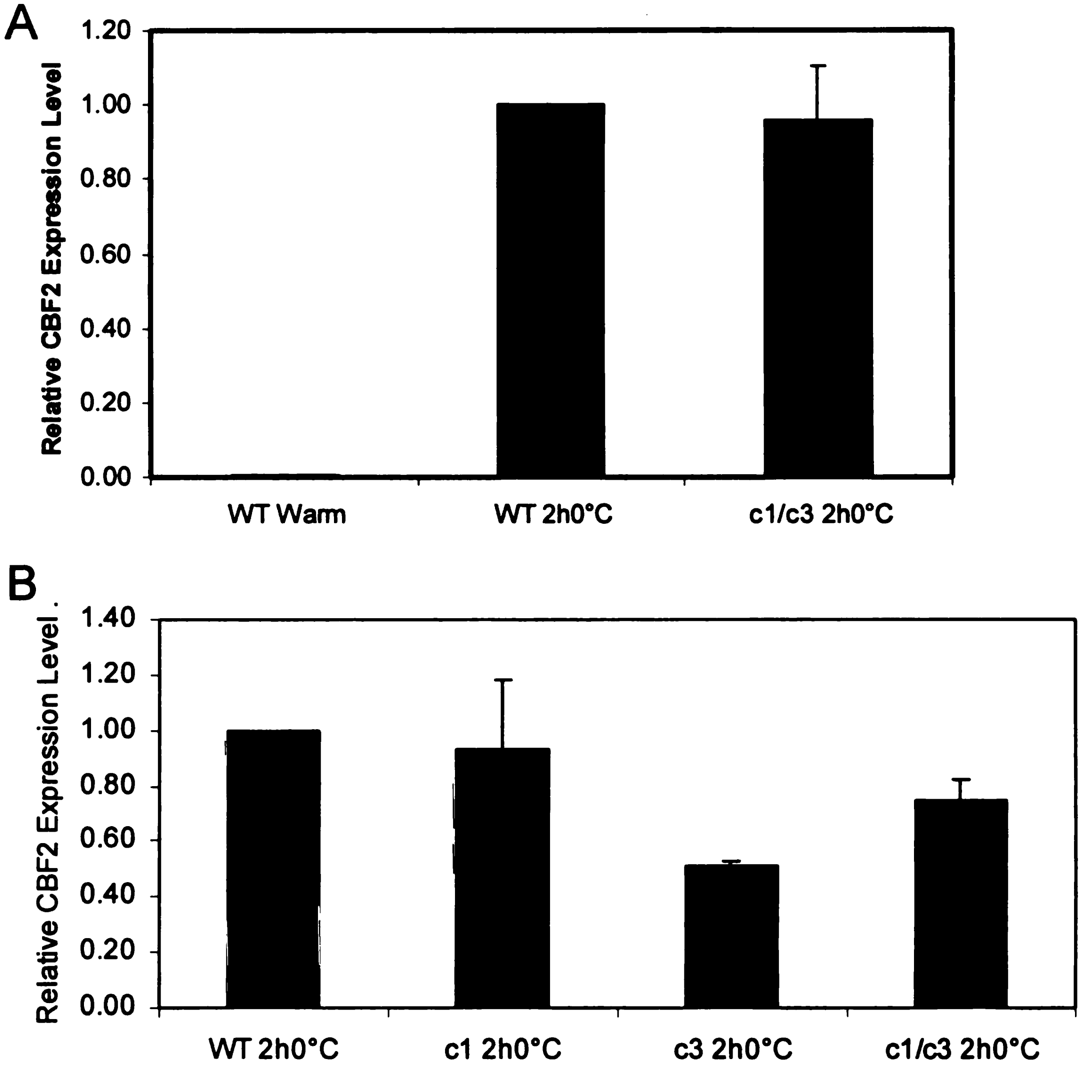


Figure 2.13 *CBF2* Levels Are Near Wild-Type Levels in *camta1/3* Double Mutants
(A) QPCR analysis shows relative expression levels of CBF2 in camta1/ camta3 double mutants (c1/c3) are similar to WT levels in plants treated with 2h0°C
(B) QPCR analysis shows that relative expression levels of CBF2 are significantly reduced in *camta3* mutants(c3) compared to WT, but not *camta1* or *camta1/3* double mutants (c1/c3)

Analysis of T-DNA insertion lines in each of the CAMTA family members revealed a functional role for CAMTA3 in the regulation of *CBF2* in response to low temperature. Loss of CAMTA3 reduced *CBF2* levels in response to low temperature. This was shown to be through the CM2/CM1-box region of the *CBF2* promoter (Figure 2.14). Complementation of the *camta3* T-DNA insertion line recovered *CBF2* expression. This effect was not limited to the *CBF2* cold-response, the CAMTA transcription factor family has been shown to bind DNA containing a sequence vCGCGb (Bouche et al. 2002; Yang and Poovaiah 2002). We have shown that this binding plays an important role in regulation of cold-responsive transcripts, particularly *CBF1*, *CBF2* and *Zat12*.

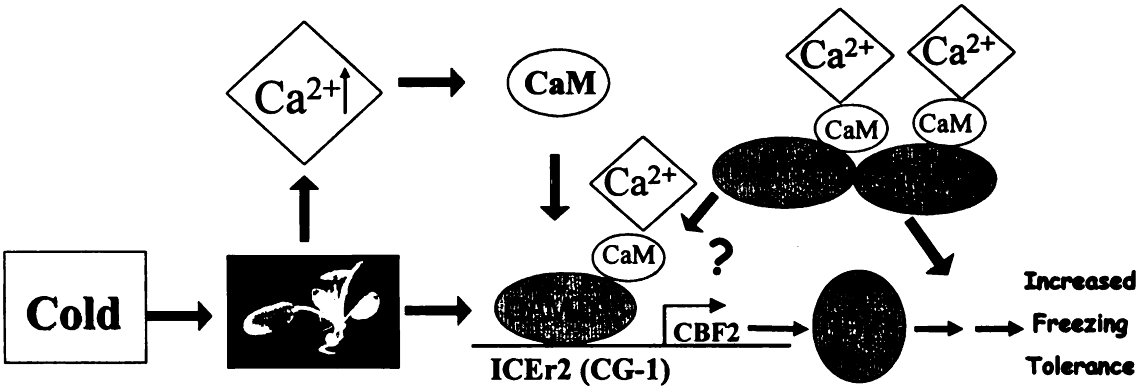


Figure 2.14 Model of CAMTA3 Regulation of *CBF* Expression in Response to Low Temperature

The effect on *CBF3* mRNA levels was not statistically significant across repeated experiments. *CBF3* lacks a CAMTA DNA binding site in the 1KB upstream promoter region and thus may not be regulated by CAMTAs. The slight effect seen in the *CBF3* transcript could be due to the proximity of the three cold-induced CBFs. Since these three genes are located in tandem on chromosome 4, the effects seen in *CBF1* and *CBF2* may perpetuate along the chromosome extending their effects to *CBF3* perhaps through changes in chromatin modification. The differences in regulation of *CBFs 1, 2*, and *3* are consistent with results from studies of the *ice1* mutant (Chinnusamy et al. 2003). The distinct regulation mechanisms for these key cold response regulators *CBF2* and *CBF3* may have functional importance to cold response, providing a redundant mechanism for the induction of the CBF cold response pathway in response to low temperature stress.

CAMTAs Are Potential Regulator of Cold Acclimation in *Arabidopsis*

Three lines of evidence support a model that CAMTA proteins, in particular, CAMTA3, may be important regulators of the response to cold stress in *Arabidopsis.* First CAMTA3 appears to be a positive regulator of the induction of *CBF1, CBF2*, *ZAT12*, and *GOLS3* in response to low temperature. Secondly, *camta1/3* double mutants have a significant reduction in freezing tolerance compared to wild-type plants. Finally, CAMTA binding sites are over-represented in the promoters of early cold responsive genes. The effect of *camta3* on *CBF1*, *CBF2,* and known CBF2 target gene, *GOLS3* indicates that at least part of this response is through the CBF cold-acclimation pathway.

The significant reduction in freezing tolerance seen in the *camta1/3* double mutants while *CBF2* levels are unaffected in the *camta1* mutant suggests that CAMTAs may also play a role in CBF independent cold-acclimation pathways. It will be interesting to examine the global effects of the loss of CAMTA3 and CAMTA1/3 on the cold-regulated transcriptome.

The recovery of the CBF2 levels in the *camta1/3* double mutants suggests that interaction between the CAMTA proteins may be complex. In Drosophila, which contains only one copy of a CAMTA transcription factor, the dimerization domain is required for nuclear localization and proper function of the CAMTA protein (Gong et al. 2007). This dimerization domain is conserved in the plant CAMTA transcription factors. The possibility exists that in Arabidopsis this family can form heterodimers or heteroligomers and that the loss of CAMTA3 impairs this multimerization, thus explaining the significant effects seen in *camta3* plants.

Analysis of CAMTA proteins in Arabidopsis and other organisms indicates that CAMTAs can act both positively and negatively on targets and through two distinct mechanisms (Choi et al. 2005). In Drosophila dCAMTA regulates transcription of a gene containing an F box motif through direct activation through a CGCG box region of the promoter (Han et al. 2006). However, in mammals CAMTA2 is brought to the promoter through interaction with another DNA binding protein (Song et al. 2006). Transcriptional activity of CAMTA2 in mammals is inhibited by interaction with HDACs. This inhibition is relieved by protein kinase C ε (PKCε) and protein kinase D (PKD) (Song, Backs et al. 2006). This study provides evidence that CAMTA3 interacts with the CBF2 promoter in a mechanism similar to that seen in the Drosophila CAMTA functions. It will be interesting to see if this is the predominant mode of action of CAMTAs in Arabidopsis or if there are some promoter / CAMTA protein combinations that act in concert with additional transcription factors as seen in mammals.

Potential Role of CAMTAs in Early Responses to Abiotic and Biotic Stresses

Transcripts containing the putative CG-1 DNA binding site within 500 bp upstream of their ATG are overrepresented in early cold-responsive genes and in transcripts that show a pattern of induction similar to *CBF2*. This suggests that perhaps CAMTA transcription factors may have a larger role in early responses to low temperature. Recently, Walley et al. identified a novel cis-element overrepresented in the promoters of wound-induced genes and postulated that this could be an early signaling response to many biotic and abiotic stresses (Walley et al. 2007). This element contains the recognition sequence for CAMTA binding. Therefore, it will be interesting to see if the CAMTAs bind to this overrepresented early stress response element and if they are primary regulators of the signaling response for multiple abiotic and biotic stresses. A recent report identified CAMTA3 as a suppressor of defense responses (Galon et al. 2008). My work contributes to the potential role of CAMTAs as early components of abiotic stress responses, showing the role for CAMTA3 as a positive regulator of early cold responses.

Consistent with the role of CAMTAS in the stress responsive regulation of hypertrophic cardiac growth in mammals and the recently demonstrated role for CAMTA3 in response to biotic stress, my study provides evidence that

CAMTAs are important signaling components of cold-stress response in Arabidopsis. The high conservation of CAMTA proteins and their target binding sites across distantly related species, Arabidopsis, rice, Drosophila, & mammals suggests that this family of transcription factors may be and ancient tool for response to environmental stresses on an organism.

MATERIALS AND METHODS

Plant Material and Growth Conditions

All plants were stratified for 3-5 days in the dark at 4°C then transferred to constant illumination at 24°C for 10 days prior to treatment. Cold treatment consisted of moving the plates to a 4°C or 0°C chamber with constant light at a reduced level (approximately 35 μ mol $m^{-2}sec^{-1}$). For experiments with the CAMTA mutants and complimented lines, plants were grown on Gamborg's B5 nutrients (Caisson Laboratories, www.caissonlabs.com) and 0.8% phytagar (Caisson Laboratories, www.caissonlabs.com) without sucrose. Experiments on the reporter constructs from *CBF2* and *Zat12* were grown on the same medium with the addition of 0.2% sucrose. The experiments testing for the complementation of CAMTA3 by overexpression in the *camta3* background were performed on shoot tissue, all other experiments were done on whole seedlings.

T-DNA insertion mutants were identified using SIGnAL database (Alonso et al. 2003) and obtained from ABRC for *camta1*(Salk_008187), *camta2*(Salk_007027), *camta3*(Salk_001152), *camta4*(Salk_013723), and *camta6*(Salk_078900). *camta5* was obtained from GABI-Kat Line ID 815B08 (Rosso et al. 2003).

The CM2/CM1-box:Gus plasmids were transformed in Arabidopsis ecotype WS using standard procedures. Selected lines of homozygous CM2/CM1-box:Gus plants were crossed into *camta* mutant lines. Seven independent T4 lines of *camta3-/-* CM2/CM1-box:GUS +/+ and four lines of *camta1 -/-* CM2/CM1-box:GUS +/+ were analyzed for Gus expression level by northern analysis (two representative lines shown in manuscript). Two lines of *camta3-/-* CM2/CM1-box:GUS were transformed with 35s::CAMTA3. T2 lines were analyzed by histochemical staining for GUS and selected T3 homozygous lines were analyzed for *GUS, CBF2*, and *CAMTA3* mRNA levels by qRT-PCR.

EMSA

EMSA analysis was performed by Dr. Heather Van Buskirk. The protein coding regions of the DNA binding domain for CAMTAs 1, 2, 3, and 5 were expressed in *E. coli.* and lysate was harvested. CM2/CM1-box and mutated CM2/CM1-box sequences were generated and labeled with [ά-32P]dCTP. Probe was incubated with protein and unlabeled competitor, if indicated and then resolved on a 5% polyacrylimide gel. The gel was dried and exposed to a phosphorimager screen.

RNA isolation and Analysis

Total RNA was extracted from plant material with the use of RNeasy Plant Mini kits (Qiagen, Valencia, CA) with modifications as described (Zarka, Vogel et al. 2003). Northern transfers were prepared and hybridized as described (Hajela et al. 1990) and washed with high-stringency conditions (Stockinger, Gilmour et al. 1997).

For RT-PCR cDNA sythesis was performed using Promega Reverse Transcription system according to the manufacuturer's directions using random primers with the following modifications. Total reaction volumes were doubled and starting RNA was adjusted. Starting RNA was 0.01ug for warm and 2h0°C samples for analysis of *CBF1,2,3*, and *ZAT12* mRNA and 0.1ug for all 24h0°C samples and for warm and 2h0°C samples for analysis of *GUS* and *CAMTA3* mRNA. cDNA was diluted five-fold with water and 3uL was used as a template for quantitative real-time PCR (qRT-PCR). qRT-PCR using SYBR Green was performed using the Applied Biosystems 7500 real-time PCR system in Standard Mode with SYBR Green PCR Core Reagents Mix (Applied Biosystems). qRT-PCR was performed according to manufacturers protocols with the following modification. Reactions were performed in a 30µL volume. For *CBF2* mRNA analysis, the annealing/extension temperature was 62°C. Serial dilutions of 2h0°C WT samples were performed to determine the efficiencies of the primers for *CBF1,2,3, ZAT12, GUS*, and *ACTIN3(ACT3)*. Serial dilutions of 24h0°C samples were performed to determine the efficiencies of *GOLS3* and *ACT3* primers. Reactions were performed in triplicate and products checked by melting curve analysis. The abundance of transcripts was analyzed with the relative standard curve method normalizing to the reference transcript, *ACT3* (AT3G53750). The primers used for amplification were *CBF1*

(CGACTATCGAATATTAGTAACTCCAAAGCGACACG-3' and 5'GGAGACAATGTTTGGGATGC-3'), *CBF2* (5'-GGA TGCTCATGGTCTTGACAT-3' and 5'-TCTTCATCCATATAAAACGCATCTTG-3'), *CBF3* (5'-CAACAAACTCGGCATCTCAA-3' and 5'-GGCGTTTCAGGATGAGATGT-3'), *ZAT12* (5'-CCTTAGGAGGTCACCGTGC-3' and 5'- CAAGCCACTCTCTTCCCACT-3'), *ACT3* (5'-GGTCGTACTACTGGTATTGTGCT-3' and 5'-TGACAATTTCACGCTCAGCT-3'), *GOLS3* (5'-GGAGTGGTTGGTCTGGCTAA-3' and 5'-TTGGTTATCCGGTGGGTAAA-3'), *CAMTA1* (5'-CTGTCAGAAGCCCAACACAG-3' and 5'-CCTTGAGCTTCTCATGAGCTTCTC-3'), *CAMTA2* (5'-GGCAAGGAGCACATGAAAAT-3' and 5'-TAAGATCCTCGGGGCCTAAT-3'), *CAMTA3* (5'-CAACGACATCCAAGAAAGCA-3' and 5'-TGAGGACATAGGCAACATCAA-3'), *CAMTA4* (5'-TTTGGAAAGGGCAGGAACTA-3' and 5'-TTTGGTAACCTCGCACATGA-3'), *CAMTA5* (5'-ATCGCGAGACACATGAGGTT-3' and 5'- GACTGTTGCTCCGCACTGTA-3'), *CAMTA6* (5'-TTGTCTTCAGGGACGGTCTT-3' and 5'-TGGAGTCTACCGTTGCATCA-3').

Statistical Analysis

Experiments consisting of three or more conditions were tested for statistical significance using two way ANOVA followed by a protected t-test. Experiments with only two comparisons were tested for significance using Student's t-test.

Staining for GUS activity

Gus activity was analyzed as described by Zarka et al. (2003).

Overrepresentation of Motif Analysis

CEL files were obtained from TAIR for warm and cold treated shoot samples by Kilian et al. (2007) and warm and cold treated samples by Vogel et al. (2005). RMA normalization was performed using AFFY package for Bioconductor (Irizarry et al. 2003, www.bioconductor.org). Resulting data was analyzed through BAR expression Angler (Toufighi et al.2005) and selected for genes with a minimum Pearson's correlation coefficient to *CBF2* of 0.85. The 500 kb region upstream of the transcriptional start site (TAIR7) was analyzed for the presence of vCGCGb using Promomer (Toufighi et al. 2005). The number of genes that contained this motif was compared to the number in the entire genome (3794).

A list of transcripts that responded rapidly to low temperature was identified as those that were differentially regulated at one or three hours at 4°C (Kilian et al. 2007) or one hour in Vogel et al. (2005) by ANOVA with a multiple testing correction of 0.05 FDR and were two fold or more induced in these conditions. These were analyzed for the presence of the vCGCGb sequence in their promoter using Promomer and compared to the promoters of the entire genome.

Whole Plant Freeze Test

Whole plant freeze tests were performed essentially as previously described (Vogel et. al. 2005).

Electrolyte Leakage Freeze Test

Electrolyte leakage freeze tests were performed essentially as described (Gilmour et al. 2000) with minor modifications. The SAS system was not used to aid in randomization

Accession Numbers

The Arabidopsis Genome Initiative locus identifiers for the CAMTAs are as follows: CAMTA1 AT5G09410, CAMTA2 AT5G64220, CAMTA3 AT2G22300, CAMTA4 At1G67310, CAMTA5 AT5G16150, and CAMTA6 At3G16940.

Literature Cited

Agarwal, M., Y. Hao, et al. (2006). "A R2R3 Type MYB Transcription Factor Is Involved in the Cold Regulation of CBF Genes and in Acquired Freezing Tolerance." J. Biol. Chem. **281**(49): 37636-37645.

Alonso, J. M., A. N. Stepanova, et al. (2003). "Genome-Wide Insertional Mutagenesis of Arabidopsis thaliana." Science **301**(5633): 653-657.

Bouche, N., A. Scharlat, et al. (2002). "A Novel Family of Calmodulin-binding Transcription Activators in Multicellular Organisms." J. Biol. Chem. **277**(24): 21851-21861.

Chinnusamy, V., M. Ohta, et al. (2003). "ICE1: a regulator of cold-induced transcriptome and freezing tolerance in Arabidopsis." Genes Dev. **17**(8): 1043-1054.

Choi, M. S., M. C. Kim, et al. (2005). "Isolation of a Calmodulin-binding Transcription Factor from Rice (Oryza sativa L.)." J. Biol. Chem. **280**(49): 40820-40831.

de Costa e Silva, O. (1994). "CG-1, a parsley light-induced DNA binding protein." Plant Molecular Biology **25**: 921-924.

Galon, Y., R. Nave, et al. (2008). "Calmodulin-binding transcription activator (CAMTA) 3 mediates biotic defense responses in Arabidopsis." FEBS Letters **582**(6): 943-948.

Gilmour, S. J., A. M. Sebolt, et al. (2000). "Overexpression of the Arabidopsis CBF3 Transcriptional Activator Mimics Multiple Biochemical Changes Associated with Cold Acclimation." Plant Physiol. **124**(4): 1854-1865.

Gilmour, S. J., D. G. Zarka, et al. (1998). "Low temperature regulation of theArabidopsisCBF family of AP2 transcriptional activators as an early step in cold-inducedCORgene expression." The Plant Journal **16**(4): 433-442.

Gong, P., J. Han, et al. (2007). "A Potential Dimerization Region of dCAMTA Is Critical for Termination of Fly Visual Response." J. Biol. Chem. **282**(29): 21253-21258.

Hajela, R. K., D. P. Horvath, et al. (1990). "Molecular Cloning and Expression of cor (Cold-Regulated) Genes in Arabidopsis thaliana." Plant Physiol. **93**(3): 1246-1252.

Han, J., P. Gong, et al. (2006). "The Fly CAMTA Transcription Factor Potentiates Deactivation of Rhodopsin, a G Protein-Coupled Light Receptor." Cell **127**(4): 847-858.

Jaglo-Ottosen, K. R., S. J. Gilmour, et al. (1998). "Arabidopsis CBF1 Overexpression Induces COR Genes and Enhances Freezing Tolerance." Science **280**(5360): 104-106.

Kilian, J., D. Whitehead, et al. (2007). "The AtGenExpress global stress expression data set: protocols, evaluation and model data analysis of UV-B light, drought and cold stress responses." The Plant Journal **50**(2): 347-363.

Knight, H., A. J. Trewavas, et al. (1996). "Cold Calcium Signaling in Arabidopsis Involves Two Cellular Pools and a Change in Calcium Signature after Acclimation." Plant Cell **8**(3): 489-503.

Liu, Q., M. Kasuga, et al. (1998). "Two Transcription Factors, DREB1 and DREB2, with an EREBP/AP2 DNA Binding Domain Separate Two Cellular Signal Transduction Pathways in Drought- and Low-Temperature-Responsive Gene Expression, Respectively, in Arabidopsis." Plant Cell **10**(8): 1391-1406.

Monroy, A. F. and R. S. Dhindsa (1995). "Low-Temperature Signal Transduction: Induction of Cold Acclimation-Specific Genes of Alfalfa by Calcium at 25[deg]C." Plant Cell **7**(3): 321-331.

Rosso, M. G., Y. Li, et al. (2003). "An Arabidopsis thaliana T-DNA mutagenized population (GABI-Kat) for flanking sequence tag-based reverse genetics." Plant Molecular Biology **53**(1): 247-259.

Song, K., J. Backs, et al. (2006). "The Transcriptional Coactivator CAMTA2 Stimulates Cardiac Growth by Opposing Class II Histone Deacetylases." Cell **125**(3): 453-466.

Stockinger, Eric J., Sarah J. Gilmour, et al. (1997). "Arabidopsis thaliana CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit." Proceedings of the National Academy of Sciences **94**(3): 1035-1040.

Toufighi, K., S. M. Brady, et al. (2005). "The Botany Array Resource: e-Northerns, Expression Angling, and promoter analyses." The Plant Journal **43**(1): 153-163.

Vogel, J. T., D. G. Zarka, et al. (2005). "Roles of the CBF2 and ZAT12 transcription factors in configuring the low temperature transcriptome of Arabidopsis." The Plant Journal **41**(2): 195-211.

Walley, J. W., S. Coughlan, et al. (2007). "Mechanical Stress Induces Biotic and Abiotic Stress Responses via a Novel cis-Element." PLoS Genetics **3**(10): e172.

Yang, T. and B. W. Poovaiah (2002). "A calmodulin-binding/CGCG Box DNA-binding protein family involved in multiple signaling pathways in plants." J. Biol. Chem.: M207941200.

Zarka, D. G., J. T. Vogel, et al. (2003). "Cold Induction of Arabidopsis CBF Genes Involves Multiple ICE (Inducer of CBF Expression) Promoter Elements and a Cold-Regulatory Circuit That Is Desensitized by Low Temperature." Plant Physiol. **133**(2): 910-918.

CHAPTER THREE

Role of CBF in the Regulation of Cold-Responsive Transcripts

Introduction

Large changes in gene expression occur in response to low temperature treatment, and many of these changes in transcription are controlled by the CBF family of transcription factors, however, the extent to which CBFs are required for the process of cold acclimation is not known. Clearly, these transcription factors do play an important role because constitutive expression of *CBFs 1, 2,* and *3*, which are cold-inducible themselves, is sufficient to confer freezing tolerance to Arabidopsis plants without the period of cold-acclimation required by WT plants. Previous work by Vogel et al identified CBF sufficient transcripts, those which were up-regulated by both cold-treated wild type plants and warm-grown plants overexpressing *CBF2* (Vogel et al. 2005). This analysis identified a CBF regulon of 85 up- and eight down- regulated transcripts. Over 80% of the identified cold-regulated transcriptome was not regulated by *CBF2* overexpression (Vogel, Zarka et al. 2005). It is of interest to know if these transcripts that are not up-regulated by *CBF* overexpression contribute to the ability of Arabidopsis to cold-acclimate and if so, what controls their regulation in response to low temperature.

The regulation of these additional transcripts may be due to many possible sources. One possibility is that CBF1 or CBF3 are responsible for the regulation of these transcripts. Novillo et al. recently demonstrated that *CBF1* and *3* are expressed in different tissues than *CBF2* and that the loss of either CBF1 or CBF3 individually or in combination through miRNA targeting showed specificity in their effects on certain cold-induced transcripts (Novillo et al. 2007). This information refines the CBF expression network and identifies that each CBF has a specific role in the regulation of different CBF-regulon transcripts. Analysis of *CBF1, 2* and *3* overexpression lines revealed extensive overlap in the transcripts regulated by these three CBFs (Fowler and Thomashow 2002). This supports the idea that the specificity of the CBF cold-induction pathway likely comes from controlled expression of the three cold-induced CBF transcripts with respect to tissue and timing. However, since the effects seen in plants constitutively expressing *CBF1* or *CBF3* were very similar to the effect of constitutive expression of *CBF2*, including the transcripts differentially regulated by the overexpression of each CBF, the specificity of the CBFs for their targets is likely involved in the complex regulation of the CBF regulon genes and does not contribute much of an effect on the CBF-independent transcripts. Therefore, while the specificity of the three cold-inducible CBFs is important for establishing the network cascade of regulation of CBF-regulon transcripts, CBF1 and CBF3 are not likely to be major contributors to the regulation of the CBF-independent transcripts.

Another possible explanation for the regulation of these CBF-independent transcripts is that their differential expression is due to disruption of the circadian clock at low temperature (Bieniawska et al. 2008). Comparative analysis by Bieniawska et al. showed that many of the transcripts identified as differentially regulated in response to low temperature in several studies appear to be expressed at different levels from warm due to a shift in the circadian clock in response to low temperature. Many output genes from the clock show a dampening or loss of their cyclical expression in low temperature. It is possible that the CBF-independent transcripts are comprised mostly of transcripts regulated by the clock, whose change in response to low temperature is due to disruption of cycling. However, in experiments where plants are grown in constant light and shifted to low temperature in constant light, there are still a large number of CBF-independent transcripts that are cold-regulated. These results indicate that while the effects of the cold on the clock may explain the cold-regulation on some CBF-independent transcripts, it does not account for the regulation of all CBF-independent transcripts.

Two additional explanations for the regulation of these CBF-independent transcripts are that either they are truly CBF-independent and are induced in response to low temperature by a parallel pathway to CBF or perhaps these transcripts are regulated by CBF but require some additional factor for their regulation that is not present in warm grown CBF overexpressing plants. Identifying if these transcripts are indeed CBF-independent will help to establish the role of CBFs in response to low temperature. Additionally, while it is known that CBF is sufficient for the regulation of a set of cold-regulated genes, it is not known if CBF is required. In order to establish which transcripts, if any, are CBF-independent and to identify the extent to which CBFs are required for cold acclimation in Arabidopsis, we attempted to identify plants that were incapable of inducing the CBF regulon in response to low temperature. Elimination of CBFs 1, 2, and 3 was attempted initially by using an RNAi construct or the addition of an EAR domain to the CBF protein, turning CBF from a transcriptional activator to a repressor (Hiratsu et al. 2003). However, a dominant negative version of the CBF protein was identified that was successful in inhibiting the response of the CBF regulon to low temperature.

A dominant negative version of the CBF protein, CBF2ΔC, was identified that affects the regulation of many CBF regulon transcripts in response to low temperature. Use of this mutated version of CBF2 enabled us to identify 398 up-regulated transcripts (60% of the cold induced transcriptome) that are CBF independent. These transcripts are not enriched for circadian regulation and the promoters of these transcripts are overrepresented for the TBX (telo-box) element, suggesting a novel method of cold-induction of these genes. Several transcripts which were induced in the warm by CBF2 overexpression and were identified as CBF sufficient were still able to be induced in CBF2ΔC plants in response to low temperature indicating that there are multiple mechanisms for inducing these transcripts in response to low temperature. The induction of 40% of the cold-induced transcripts was affected in CBF2ΔC plants, indicating a larger role for CBF in the cold-acclimation pathway than previously thought. The transcripts down-regulated in response to low temperature were largely unaffected in the CBF2ΔC plants. Ninety percent of these down-regulated transcripts were still repressed in CBF2ΔC plants, indicating that either down-regulation of these transcripts in response to low temperature is independent of

CBF or that an intact activation domain of CBF is not required for their down-regulation. CBF2ΔC plants showed a decrease in the freezing tolerance as well as a reduction of the basal freezing tolerance. However, CBF2ΔC plants were still able to cold acclimate suggesting an important role for both the CBF-independent and CBF co-regulated transcripts in the process of cold acclimation.

Results

Identification of a Dominant Negative Version of CBF2

Overexpression of *CBF1,2*, or *3* results in a dwarf phenotype (Gilmour et al. 2004). A population of *CBF2* overexpressing plants was mutagenized using EMS. Offspring were selected that were larger than CBF2 overexpressing plants, indicating a suppression of the CBF size phenotype. One identified mutant showed a recovery of WT size (Figure 3.1A). This mutant was tested for expression of CBF2 target gene COR15. Northern analysis of these genes indicated that the expression levels of both *COR15* and *COR78* mRNA levels were reduced in both warm grown plants and plants grown for 7d4°C (Figure 3.1B). Sequencing of the CBF2 transgene in these plants revealed a point mutation converting glutamine 136 to a stop codon positioned just past the AP2 DNA binding domain and prior to the activation domain (Figure 3.1C, Figure 3.2). This truncated version of CBF2 was called CBF2ΔC.

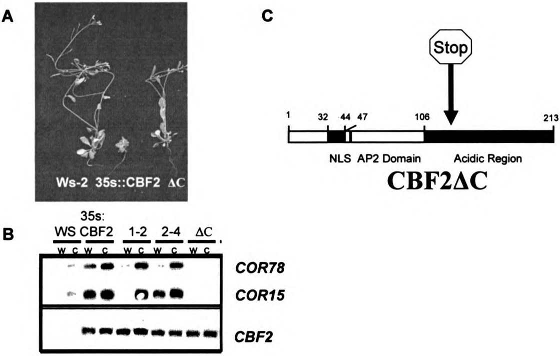


Figure 3.1 Identification of CBF2ΔC, a Truncated Version of CBF2 That Suppresses the 35s::CBF2 Phenotype
(A) CBF2ΔC (ΔC) plants show a recovery of WT (Ws-2) phenotype compared to their 35s::CBF2 parents
(B) Northern analysis of COR78 and COR15 levels in WT(WS), 35s::CBF2, CBF2ΔC (ΔC) plants and two other mutants (1-2) and (2-4) after growth in warm (w) and treatment for 24h4°C (C)
(C) Sequencing of the CBF2 transgene in CBF2ΔC plants revealed a stop codon just past the DNA binding domain

CBF2ΔC Effectively Inhibits Induction of GOLS3, a CBF Regulon Transcript.

We hypothesized that CBF2ΔC might be acting as a dominant negative version of the CBF proteins. The CBF2ΔC protein, with its intact DNA binding domain, may recognize and bind the C-repeat (CRT) cis-element, RCCGAC, in the promoters of the CBF target genes. However, with the stop codon upstream of the activation domain, CBF2ΔC would not be able to activate transcription of these genes. Thus, the presence of the CBF2ΔC transgene may block access of the endogenous CBFs to the CRT elements in the CBF target genes. If CBF2ΔC is functioning as a dominant negative version of CBF2, this construct could serve as a tool to improve our understanding of the role of CBF in low temperature responses.

CBF2ΔC Mutation
1
MNSFSAFSEMFGSDYESPVSSGGDYSPKLATSCPKKPAGRKKFRETRHPI

51 AP2 domain
YRGVRQRNSGKWVCELREPNKKTRIWLGTFQTAEMAARAHDVAAIALRGR

101
SACLNFADSAWRLRIPESTCAKEIQKAAAEAALNF**Q**DEMCHMTTDAHGLD

151
MEETLVEAIYTPEQSQDAFYMDEEAMLGMSSLLDNMAEGMLLPSPSVQWN

201
YNFDVEGDDDVSLWSY

Figure 3.2 CBF2ΔC Is a Glutamine-to-stop Codon Mutation
Sequence of CBF2 highlighting the glutamine that is converted to a stop codon in CBF2ΔC.

To confirm that CBF2ΔC was functioning as a dominant negative protein we cloned CBF2ΔC and transformed it into wild type plants under control of the 35s CaMV promoter. These CBF2ΔC-containing plants were then tested for the expression of Galactinol Synthase 3 (GOLS3), one of the most highly induced transcripts in plants constitutively expressing CBF2 (Vogel et al. 2005). There was an 80% reduction in the cold-induced accumulation of *GOLS3* transcript in the CBF2ΔC plants after 24h4°C treatment (Figure 3.3). The reduction seen in the expression level of *GOLS3*, a CBF regulon transcript, indicates that the presence of the CBF2ΔC construct is responsible for the inhibition of the induction of CBF target genes in response to low temperature.

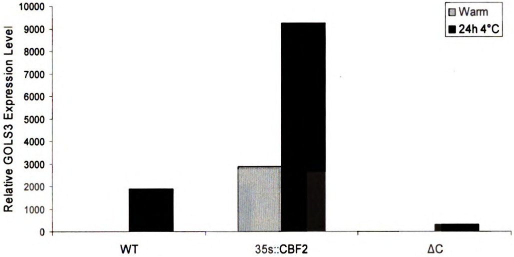


Figure 3.3 GOLS3 mRNA Levels Are Reduced in CBF2ΔC Plants
QPCR analysis of exprssion of GOLS3 levels in WT, 35s::CBF2, and CBF2ΔC (ΔC) lines relative to WT warm levels in either warm grown (grey) or 24h4°C treatment (black).

CBF2ΔC Functions as a Dominant Negative Protein

The effect of the CBF2ΔC construct in preventing the large fold increase of *GOLS3* mRNA accumulation in response to low temperature and the increase in plant size seen in the original mutant suggest that CBF2ΔC is acting as a dominant negative protein and preventing the access of wild-type(WT) CBF proteins to their targets. To test the ability of CBF2ΔC to block WT-CBF2 proteins we transformed plants containing 35s::CBF2 with the 35s::CBF2ΔC construct. If CBF2ΔC functions as a dominant negative protein, when mRNA levels of CBF2ΔC and WT-CBF2 accumulate to equal levels, CBF2ΔC should inhibit the induction of CBF target genes by the WT-CBF2 construct in warm grown plants, where the expression and activity of the endogenous CBFs should be minimal compared to the WT-CBF2 transgene. Primers were designed which could amplify transcripts originating from the vector expressing

CBF2, but do not amplify CBF2ΔC transcripts. Three lines were identified which did not show silencing of the CBF2 transcript (2ΔC 6-4, 8-3, and 9-4). These lines expressed transcripts of the WT-CBF2 construct at levels similar to the parent plant (Figure 3.4A).

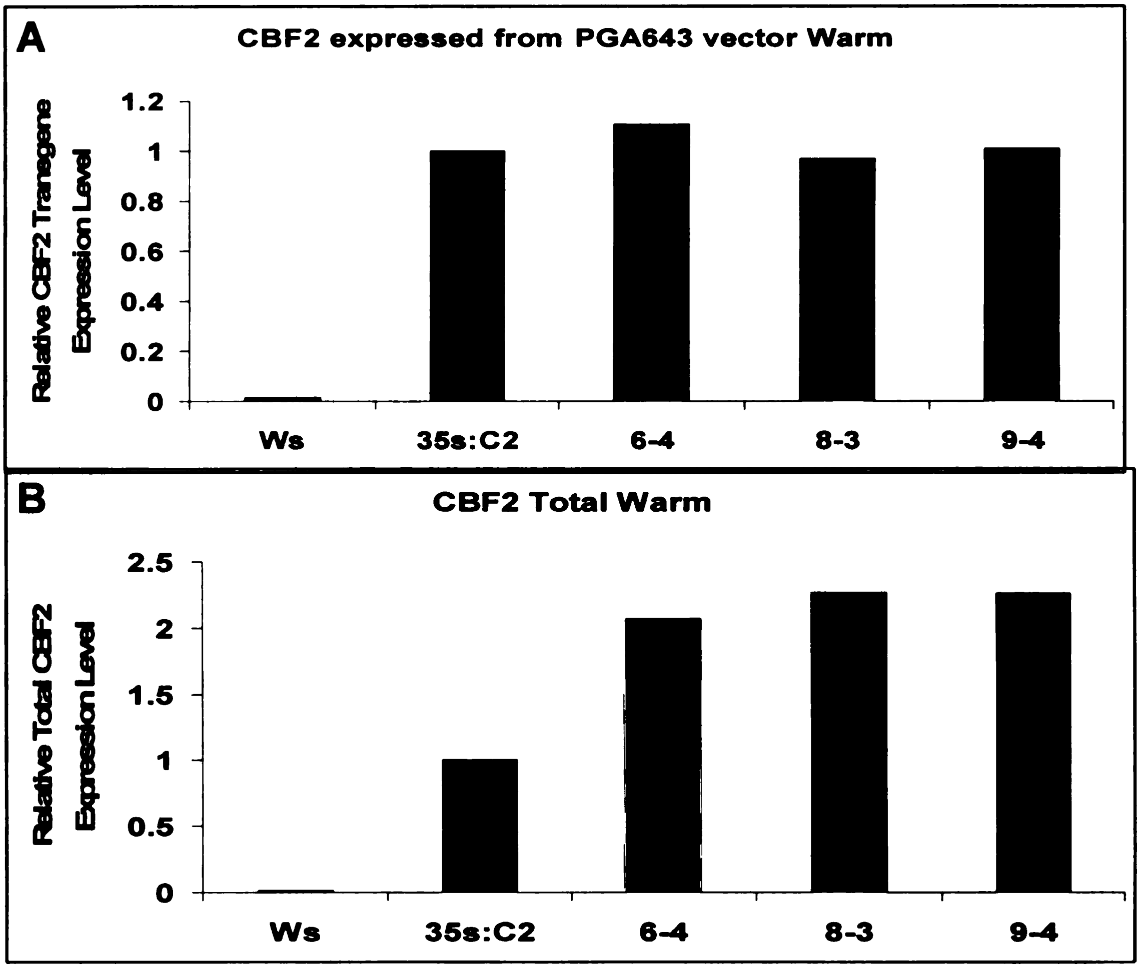


Figure 3.4 CBF2 Levels in Plants Expressing 35s::CBF2 and 35s::CBF2ΔC Transgenes

(A) QPCR analysis of CBF2 transgene levels in WT, 35s::CBF2 (35s:C2), and 35s::CBF2 x 35s::CBF2ΔC (6-4, 8-3, and 9-4) plants. Expression is relative to levels in 35s::CBF2 plants (35s:C2).

(B) QPCR analysis of total CBF2 levels in WT, 35s::CBF2 (35s:C2), and 35s::CBF2 x 35s::CBF2ΔC (6-4, 8-3, and 9-4) plants. Expression is relative to levels in 35s::CBF2 plants (35s:C2).

Analysis of total CBF expression levels with primers that would amplify either CBF2 or CBF2ΔC indicated that, after subtraction of WT-CBF2 levels from total CBF levels, CBF2ΔC levels were expressed at approximately equivalent levels as WT-CBF2 (Figure 3.4B). After identifying these three lines which showed approximately equal expression of WT-CBF2 and CBF2ΔC transcripts, we examined the expression level of two CBF regulon genes, *COR15* and *GOLS3*.

Analysis of two CBF target genes COR15a and GOLS3 indicated that the CBF2ΔC construct was able to greatly reduce the expression of these transcripts by WT-CBF2 although the *CBF2ΔC* mRNA accumulates to only equal levels as the *WT-CBF2* transgene mRNA levels (Figure 3.5 A, B). This is consistent with the possibility that CBF2ΔC is acting as a dominant negative protein and that when *CBF2ΔC* is expressed at levels similar to *WT-CBF2* it is able to inhibit expression of two CBF regulon genes. When expressed in WT background, the *CBF2ΔC* transcript accumulates to even higher levels, accumulating to over 15 times the level of the endogenous *CBF2* transcripts at 2h4°C (Figure 3.6).

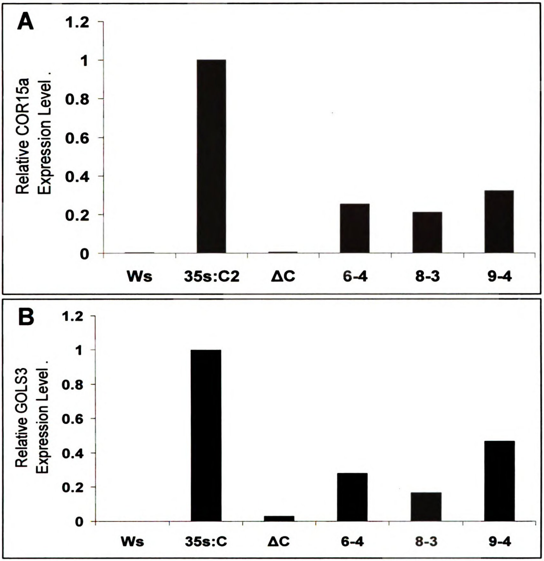


Figure 3.5 Expression of CBF Target Genes in Plants Expressing 35s::CBF2 and 35s::CBF2 ΔC Transgenes
(A) QPCR analysis of COR15 levels in WT, 35s::CBF2 (35s:C2), 35s::CBF2ΔC (ΔC), and 35s::CBF2 x 35s::CBF2ΔC (6-4, 8-3, and 9-4) plants. Expression is relative to levels in 35s::CBF2 plants (35s:C2).
(B) QPCR analysis GOLS3 levels in WT, 35s::CBF2 (35s:C2), 35s::CBF2ΔC (ΔC), and 35s::CBF2 x 35s::CBF2ΔC (6-4, 8-3, and 9-4) plants. Expression is relative to levels in 35s::CBF2 plants (35s:C2).

Since CBF2ΔC is able to inhibit the targets of constitutively expressed WT-CBF2 when both transgenes are expressed at the same level, it would be expected that the effects of CBF2ΔC in wild-type plants will be even more profound since *CBF2ΔC* mRNA levels accumulate to a higher level than the endogenous *CBF* mRNA levels. For *GOLS3* transcripts, the level of induction by WT-CBF2 transgene is reduced about 50% in plants containing both the WT-CBF2 transgene and the CBF2ΔC transgene. In agreement with the dosage effect predicted on the endogenous transcripts, cold-induction of *GOLS3* mRNA at 24h is reduced about 80% in the CBF2ΔC plants compared to WT plants (Figure 3.5 B). The ability of CBF2ΔC to block the access of the endogenous CBFs to their targets in its role as a dominant negative form of CBF makes it a potential tool to assess the requirement and involvement of CBFs in the process of cold acclimation.

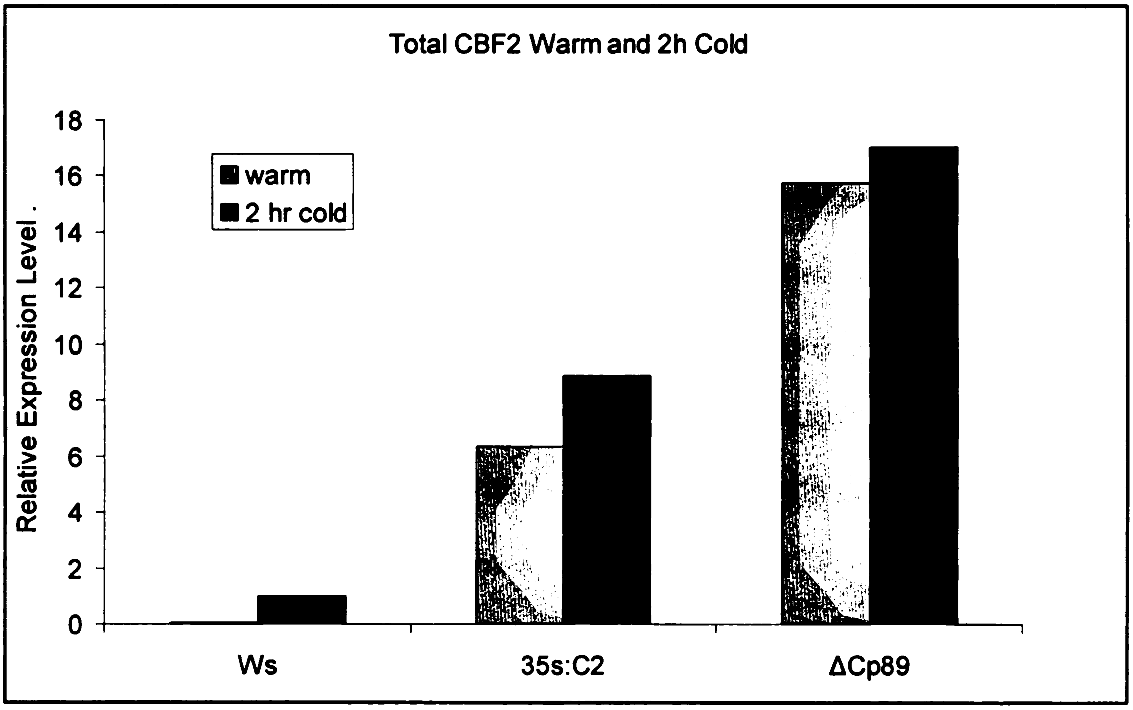


Figure 3.6 Levels of *CBF2ΔC* mRNA Accumulate to Higher Levels than Endogenous *CBF2* Levels
QPCR analysis of total CBF2 levels in WT, 35s::CBF2 (35s:C2), and 35s::CBF2ΔC (ΔC) plants grown in warm (grey) or cold-treated, 2h4°C (black). Expression is relative to WT cold levels.

Analysis of cold regulated transcripts in CBF2ΔC

These results indicate that CBF2ΔC construct is effectively acting as a dominant negative protein, blocking the function of the endogenous CBFs. This suggests that in the presence of the CBF2ΔC construct, these plants are not able to mount a functional CBF pathway-based response to low temperature. To determine the full effects of an inhibited CBF pathway on the cold-regulated transcriptome, Affymetrix expression arrays were performed on WT and CBF2ΔC plants cold treated for 24h4°C.

Analysis was focused on a robust cold–regulated set of transcripts in order to minimize the effects of growth conditions and to minimize the selection of circadian-regulated transcripts identified as differentially regulated due to cold-induced changes in the clock (Ramos et al. 2005; Bieniawska, Espinoza et al. 2008). RankProd, a Rank Product method was used to identify transcripts that were cold-regulated at 24h across three different experiments in two different growth conditions, 12/12 day/night cycles and constant light (Breitling et al. 2004; Vogel, Zarka et al. 2005; Hong et al. 2006; Kilian et al. 2007). This analysis identified 2368 probesets that were robustly cold regulated. To further refine this list we selected those that were cold regulated in both WS and Col-0 ecotypes at 24h4°C, resulting in 1279 probesets regulated by low temperature treatment in two cold-acclimating ecotypes (Appendix Table A.1).

	WS 0h A	WS 0h B	ΔC 0h A	ΔC 0h B	CBF2 0h A	CBF2 0h B	ΔC 24h A	[illegible] 24h B	[illegible] 24h C	[illegible] 24
Model_1	0	0	1	1	0	0	0	0	0	0
Model_2	0	0	1	1	0	0	0.1	0.1	0.1	0.1
Model_3	0	0	1	1	0	0	0.5	0.5	0.5	0.5
Model_4	0	0	1	1	0	0	1	1	1	1
Model_5	0	0	1	1	0.5	0.5	0	0	0	0
Model_6	0	0	1	1	0.5	0.5	0.1	0.1	0.1	0.1
Model_7	0	0	1	1	0.5	0.5	0.5	0.5	0.5	0.5
Model_8	0	0	1	1	0.5	0.5	1	1	1	1
Model_9	0	0	1	1	1	1	0	0	0	0
Model_10	0	0	1	1	1	1	0.1	0.1	0.1	0.1
Model_11	0	0	1	1	1	1	0.5	0.5	0.5	0.5
Model_12	0	0	1	1	1	1	1	1	1	1
Model_13	1	1	0	0	0	0	0	0	0	0
Model_14	1	1	0	0	0	0	0.1	0.1	0.1	0.1
Model_15	1	1	0	0	0	0	0.5	0.5	0.5	0.5
Model_16	1	1	0	0	0	0	1	1	1	1
Model_17	1	1	0	0	0.5	0.5	0	0	0	0
Model_18	1	1	0	0	0.5	0.5	0.1	0.1	0.1	0.1
Model_19	1	1	0	0	0.5	0.5	0.5	0.5	0.5	0.5
Model_20	1	1	0	0	0.5	0.5	1	1	1	1
Model_21	1	1	0	0	1	1	0	0	0	0
Model_22	1	1	0	0	1	1	0.1	0.1	0.1	0.1
Model_23	1	1	0	0	1	1	0.5	0.5	0.5	0.5
Model_24	1	1	0	0	1	1	1	1	1	1

Table 3.1 Predetermined models of expression used for Haystack analysis of cold-regulated transcripts. Samples compared are WS_0h and 24h, 35s::CBF2ΔC (ΔC) 0h and 24h, 35s::CBF2 (CBF2) 0h. Each row represents a potential model of expression for a transcript. Transcripts whose relative pattern of expression across all samples closely has a Pierson's correlation of >0.80 to a particular model were considered matches of that model.

To identify groups of genes with patterns of expression across the various genotypes Haystack analysis was employed (Mockler et al. 2007). Haystack uses a pattern matching algorithm that compares the expression pattern of each transcript across treatments to user-defined models of expression. Analysis of the expression pattern of these 1279 probesets was compared to 24 predefined models of expression (Table 3.1) across the following samples: WT 0h, WT24h, 35s::CBF2 0h, 35s::CBF2ΔC 0h and

35s::CBF2ΔC 24h. Of these 1279 probesets 1129 had a correlation coefficient greater than 0.80 to one of 24 predefined models of expression, 1086 of these are predicted to bind to a unique transcript. These included 642 transcripts up-regulated in WT at 24h4°C and 444 down-regulated transcripts.

Effect of 35s::CBF2ΔC on cold-induced transcripts

Ten percent (63) of the up-regulated transcripts matched a pattern where the expression at 24h4°C in 35s::CBF2ΔC plants was similar to wild type warm plants, corresponding to models 1,2,5,6,9, or 10 (Table 3.1, Figure 3.7A). These genes were classified as requiring CBF. Over 60 percent, 398, of cold-induced transcripts in WT plants were classified as CBF independent; these transcripts were still induced to their WT levels in 35s::CBF2ΔC plants, corresponding to models 4,8, or 12 (Table 3.1, Figure 3.7B). The final group of 181 transcripts were induced in both WT and 35s::CBF2ΔC plants, however, their level in 35s::CBF2ΔC was significantly reduced from the WT level of expression at 24h4°C, corresponding to models 3,7, or 11 (Table 3.1, Figure 3.7C).

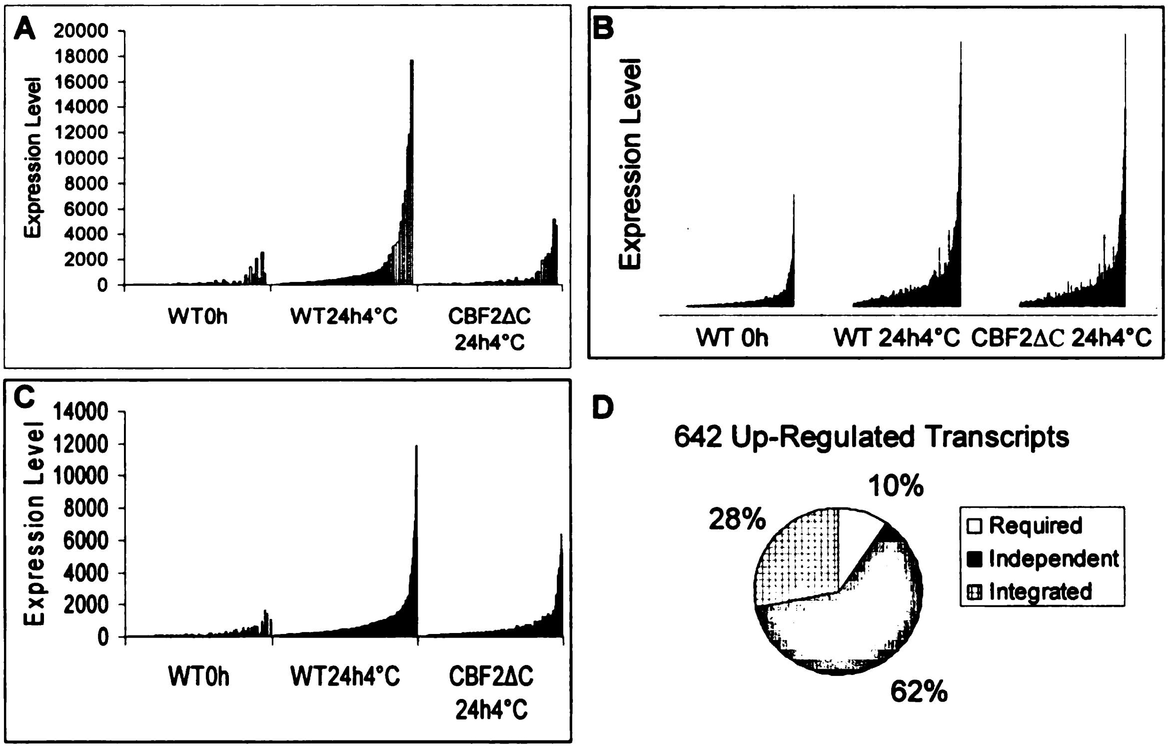


Figure 3.7 Cold-Induced Transcripts

(A) Expression level of 63 transcripts that are upregulated in WT plants, but are not significantly upregulated at 24h4°C in CBF2ΔC plants.

(B) Expression level of 398 transcripts that are upregulated in WT plants, and are expressed to a level equal to that of WT in CBF2ΔC plants at 24h4°C

(C) Expression level of 181 transcripts that are still induced in CBF2 ΔC plants, but are not induced to their full WT level.

(D) Pie-chart showing the distribution of each category of transcripts based on their requirement for CBF, required (A), independent (B), integrated(C)

Some transcripts previously identified as CBF-regulated due to their induction by CBF2 overexpression in warm-grown plants were still found to be induced to their full wild-type level in CBF2ΔC plants, suggesting that there are multiple mechanisms for inducing these transcripts in response to low-temperature. Additionally, this analysis indicates that there is a larger role for CBF than previously identified by analysis of warm grown CBF overexpressing plants suggesting that CBF maybe involved in the regulation of up to 40% of the cold responsive transcripts.

Constitutive Expression of CBF2 is also Sufficient for Most CBF Dependent Transcripts

Of the 63 transcripts identified as requiring CBF for their cold-induction, 54 were induced by constitutive expression of CBF2 in warm-grown plants, matching models 9 or 10. This indicates that CBF expression is both sufficient and required for the expression of these transcripts (Figure 3.8). As would be expected for a group of genes that are regulated by CBF, the presence of the CRT element to which CBF binds, RCCGAC is overrepresented in the promoters region, with 44 of these 54 genes containing at least one CRT element in the 1KB upstream promoter region, p-value<10^{-10}. All but eight of these transcripts contained the core element of the CRT, CCGAC. The remaining 8 transcripts lack a known CBF binding site in the 1KB upstream region and maybe regulated by CBF through an indirect mechanism.

A subgroup of the genes whose induction was inhibited by the presence of CBF2ΔC was not induced by overexpression of CBF2 in the warm. These 9 transcripts matched haystack model 2, where their induction in response to low temperature was inhibited in the presence of CBF2ΔC; however, expression of CBF2 was not sufficient for their expression in the warm. For this subgroup perhaps there is a requirement for an additional factor, present only at low temperatures, to induce their transcription (Figure 3.9). Promoters of these transcripts are overrepresented for the presence of the CG-1 binding element (ATSR1) vCGCGb, p-value <10^{-3} (Yang and Poovaiah 2002). This element was shown to be important for the induction of CBF2 in response to low temperature and is overrepresented in early-cold responsive transcripts (Doherty, unpublished).

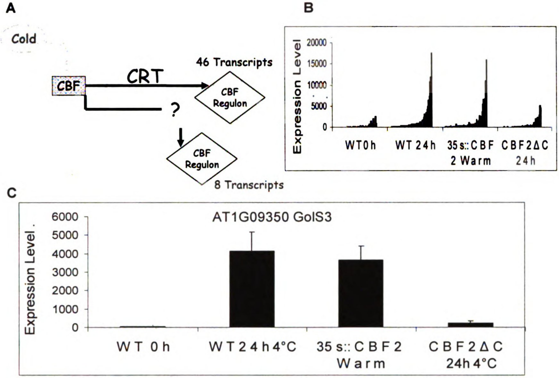


Figure 3.8 CBF Sufficient and Required Transcripts
(A) Model of regulation for 54 transcripts identified as CBF sufficient and required
(B) Expression level of 54 transcripts in WT warm, WT 24h4°C, 35s:CBF2 warm and 35s:CBF2ΔC 24h4°C
(C) Representative transcript expression level for one transcript *GOLS3* for samples in (B)

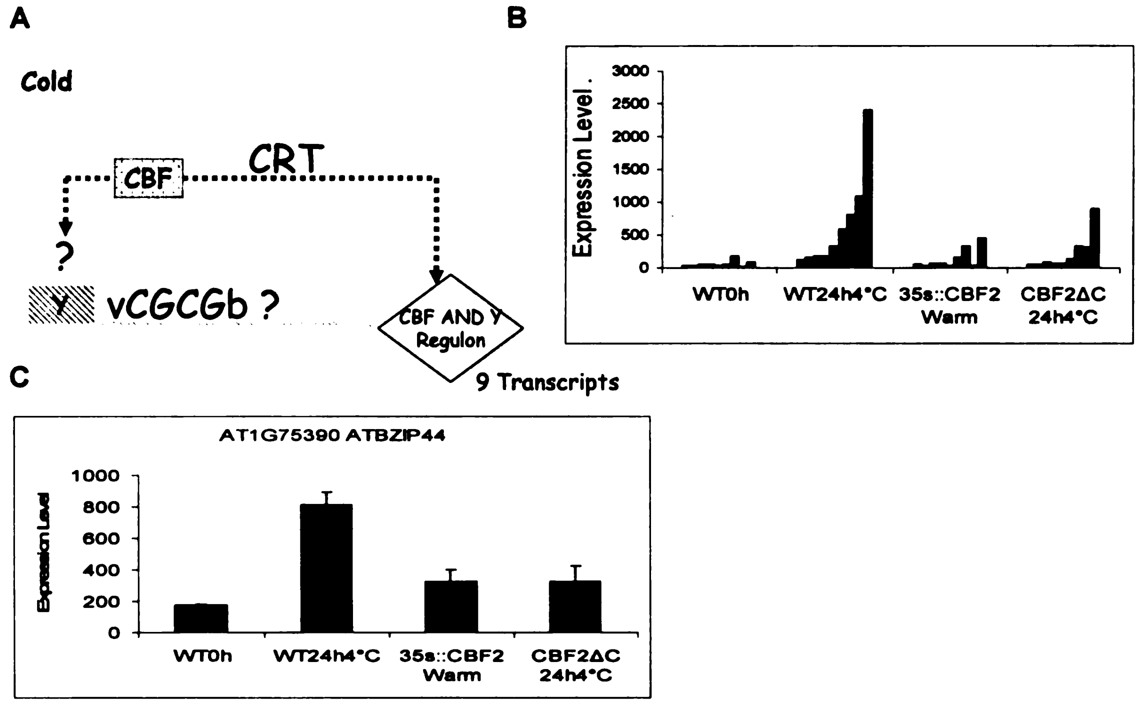


Figure 3.9 CBF is Not Sufficient Yet Is Required for Induction of Nine Transcripts
(A) Model of regulation for 9 transcripts identified as CBF required but not sufficient
(B) Expression level of 9 transcripts in WT warm, WT 24h4°C, 35s:CBF2 warm and 35s:CBF2ΔC 24h4°C
(C) Representative transcript expression level for one transcript, *ATBZIP44* for samples in (B)

TBX Element is Present in Promoters of many CBF-Independent Transcripts

The induction of 398 transcripts in CBF2ΔC plants at 24h4°C was similar to the level of induction in wild-type plants in response to low temperature (Figure 3.7 B) indicating that a functional CBF pathway is not required for their induction. The majority of these probesets, 370, matched model 4, in that they were not up-regulated in response to overexpression of CBF2 in the warm, suggesting that they are entirely independent of CBF (Figure 3.10).

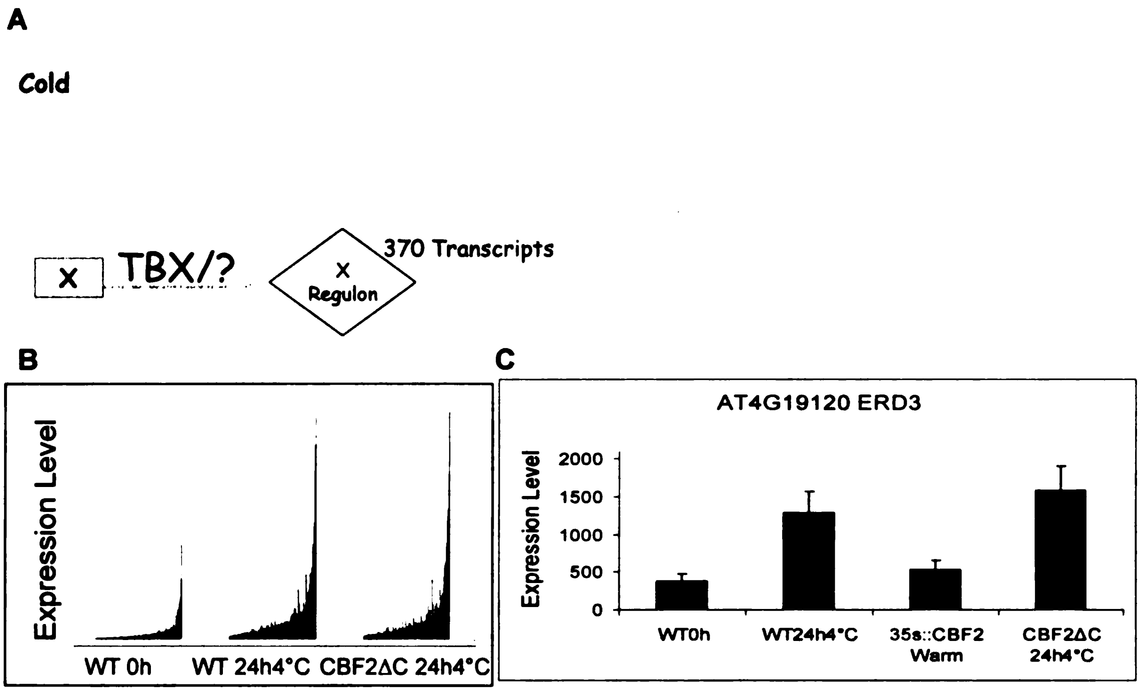


Figure 3.10 CBF is Neither Sufficient Nor Required for Induction of 370 Transcripts
(A) Model of regulation for 370 transcripts where CBF is neither sufficient nor required
(B) Expression level of 370 transcripts in WT warm, WT 24h4°C, and 35s:CBF2ΔC 24h4°C
(C) Representative transcript expression level for one transcript, *ERD3* for samples in WT warm, WT 24h4°C, 35s:CBF2 warm and 35s:CBF2ΔC 24h4°C

The 1KB upstream promoter region of these CBF independent transcripts was enriched for the Telo-box binding element, AAACCCTAA, p-value $<10^{-10}$. The telo-box element is a cis-element known to be involved in the regulation of gene expression in root meristems, *eEF1A* gene expression, and *rp40* expression (Manevski et al. 2000; Tremousaygue et al. 2003). The core telo-box sequence, ACCCTA, was present in the 1kb upstream promoter region of 144 of these transcripts. This element was previously identified as being enriched in a midnight specific module of circadian regulated transcripts (Michael et al. 2008). However, the set of transcripts with the TBX element present in the 1KB upstream region of their promoter was enriched for those not matching any circadian pattern in the 5 experiments analyzed by Michael et al., p-value <0.01. The overrepresentation of the TBX element in the promoters in these non-circadian regulated transcripts suggests that there may be an additional role for the TBX in regulating response to low temperature, in addition to circadian rhythms.

A subset of these transcripts that were induced to near their full wild-type level in CBF2ΔC plants corresponded to models 8 or 12, indicating that they were up-regulated in response to CBF2 overexpression. Overexpression of CBF2 is sufficient for induction of these 28 transcripts, yet they are unaffected by the CBF2ΔC transgene (Figure 3.11). Promoters of 21 of these 28 transcripts contain the core CRT, p-value $<10^{-6}$. The overrepresentation of the CBF binding site in the promoters of these transcripts supports their induction by CBF2 overexpression in warm-grown plants. However, no other known elements are overrepresented in this set of transcripts to explain their induction in the presence of CBF2ΔC. This could indicate that either the truncated version of CBF2 is enough for induction of these transcripts or that there are independent pathways that are sufficient for their induction.

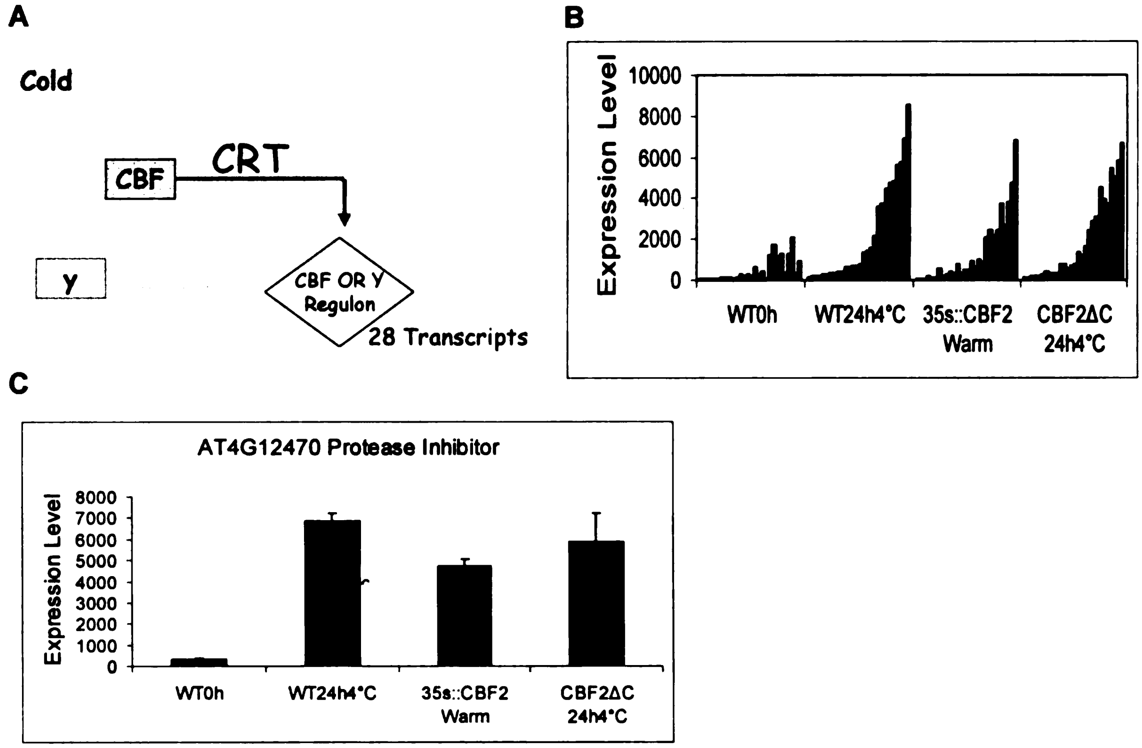


Figure 3.11 CBF is Sufficient But Not Required for Induction of 28 Transcripts
(A) Model of regulation for 28 transcripts identified as CBF sufficient but not required
(B) Expression level of 28 transcripts in WT warm, WT 24h4°C, 35s:CBF2, and 35s:CBF2ΔC 24h4°C
(C) Representative transcript expression level for one transcript, AT4G12470 for samples in (B)

CBF-Integrated Transcripts are Induced in 35s::CBF2ΔC Plants, but not to Their WT Levels

A large number of transcripts, 181, were still induced in response to low temperature in the CBF2ΔC expressing plants; however, their expression level was reduced when compared to WT plants. This suggests that while CBF is not required for the low-temperature induction of these transcripts, CBF is required for them to reach their full level of induction after 24h of cold treatment (Figure 3.12). Constitutive expression of CBF2 was sufficient for induction of 64 of these transcripts, matching haystack models 7 and 11. As would be expected for transcripts that can be induced by overexpression of CBF, the CRT element was overrepresented in the 1KB upstream region of these transcripts, p-value 10^{-10} with 54 of these transcripts containing the core CRT element. Additionally, the promoters of these transcripts were also overrepresented, p-value 10^{-7}, for the presence of an ABRE-like element BACGTGKM, with 31 of the 64 promoters containing this element (Shinozaki and Yamaguchi-Shinozaki 2000). The pattern of regulation of these transcripts and overrepresentation of both of these elements suggest a model where CBF is sufficient for induction of these transcripts through the CRT element, however, in the CBF2ΔC plants, these transcripts can be activated via another mechanism, although not to the same level as they reach in WT plants. The overrepresentation of both the CRT element and the ABRE-like element in the promoters of these transcripts indicates that perhaps ABA might play a role in the induction of these transcripts in the CBF2ΔC plants. In support of this model, GO annotation of these transcripts shows that they are enriched for genes which respond to ABA, p-value 10^{-7}. Precedence for such a cooperative effect between CBF and an ABRE-like binding protein has been demonstrated with the RD29a promoter where CRT elements and ABRE elements are interdependent for expression in response to ABA treatment (Narusaka et al. 2003).

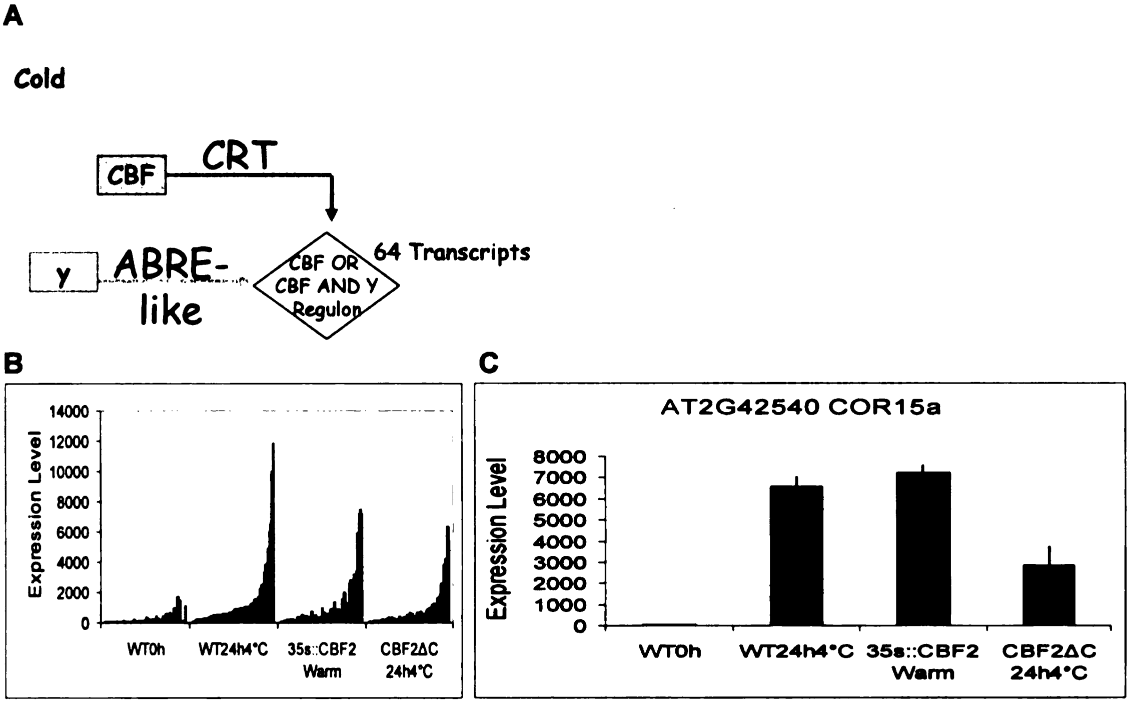


Figure 3.12 CBF Is Sufficient and Partially Required For the Induction of 64 CBF Integrated Transcripts
(A) Model of regulation for 64 transcripts identified as CBF-Integrated, sufficient and partially required.
(B) Expression level of 64 CBF-Integrated transcripts in WT warm, WT 24h4°C, 35s:CBF2, and 35s:CBF2ΔC 24h4°C
(C) Representative transcript expression level for one transcript, *COR15A* for samples in (B)

The majority of these transcripts, 117, matched haystack model 3 and were not induced in response to constitutive expression of CBF2. For these transcripts, while CBF is required for their full, wild-type level of induction, CBF2 alone is not sufficient for induction of these transcripts in the warm (Figure 3.13). The CRT element was overrepresented in the promoters of these transcripts, p-value 10^{-10}, perhaps contributing to the quantitative effect seen in the CBF2ΔC plants. The ABRE-like element, was also overrepresented (p-value, 10^{-7}), perhaps indicating a role for ABA in the induction of these transcripts in response to low temperature in the absence of CBF.

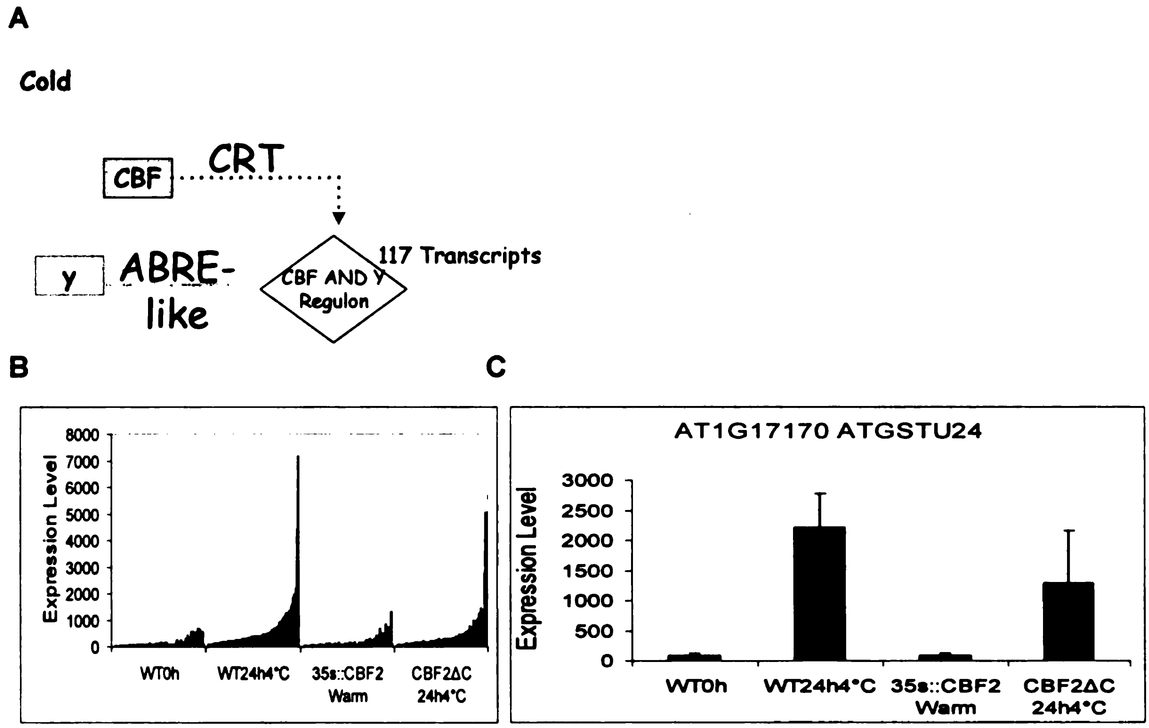


Figure 3.13 CBF Is Not Sufficient Yet Is Partially Required For the Induction of 117 CBF Integrated Transcripts
(A) Model of regulation for 117 transcripts identified as CBF-Integrated, not sufficient and partially required.
(B) Expression level of 117 CBF-Integrated transcripts in WT warm, WT 24h4°C, 35s:CBF2, and 35s:CBF2ΔC 24h4°C
(C) Representative transcript expression level for one transcript, *ATGSTU24* for samples in (B)

No Down-Regulated Transcripts Are Completely Dependent on CBF

None of the 444 transcripts down-regulated in WT plants in response to low temperature completely lost their repression in response to low temperature in 35s::CBF2ΔC plants. However 29 of these transcripts were not repressed to the same extent in 35s::CBF2ΔC plants as they were in WT plants (Figure 3.14 A).

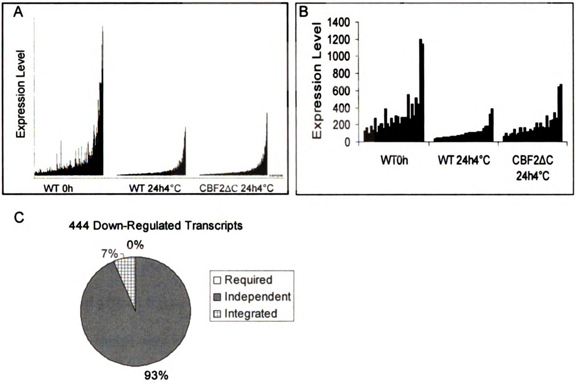


Figure 3.14 Cold-Repressed Transcripts
(A) Expression level of 415 transcripts that are repressed in WT plants and are also reduced to a level equal to that of WT in CBF2ΔC plants at 24h4°C
(B) Expression level of 29 transcripts that are still repressed in CBF2 ΔC plants, but are not reduced to their full WT level.
(C) Pie-chart showing the distribution of each category of transcripts based on their requirement for CBF, independent (A) and integrated (B).

The repression level of the remaining 415 transcripts was unaffected by the presence of the 35s::CBF2ΔC construct (Figure 3.14 B). This suggests that either CBF is not required for the down-regulation of transcripts or that the DNA binding domain, still intact in the CBF2ΔC constructs is sufficient for the repressive function of CBF.

Of the 415 transcripts whose repression in response to low temperature was not affected by the presence of the CBF2ΔC construct, 194 were repressed in warm grown plants constitutively expressing CBF2, indicating that while wild-type CBF is sufficient for the down-regulation of these transcripts, a full-length CBF protein is not required for their repression (Figure 3.15). The CBF binding sequence, the CRT, was not overrepresented in the promoters of these transcripts; only 34 of the 194 contained this CRT in the 1KB upstream region, p-value 0.66. Therefore, either direct binding of CBF through the CRT element is not a likely mechanism of repression for these transcripts or the regulatory regions for these genes is not in the upstream 1KB region. A possible regulatory mechanism is through the I-Box, which is present in the promoters of 108 of these 194 transcripts, p-value 10^{-10}. In tomato a MYB transcription factor was identified as a potential binding factor to the I-Box (Rose et al. 1999).The I-Box motif, GATAAG, is also enriched in the promoters of light-regulated genes (Giuliano et al. 1988). Mutation of this sequence in the context of the ribulose-1,5-bisphosphate carboxylase small subunit promoter sequence causes reduced expression of a reporter gene (Donald and Cashmore 1990). In agreement with this group of I-Box enriched transcripts being involved in light regulation, these transcripts were also enriched for the GO cellular component ontology term, chloroplast (10^{-7}). In addition to the I-Box, a second cis-element, a MYC recognition sequence, CACATG present in the promoter of RD22, was also overrepresented in these promoters, with 92 of these 194 containing at least one copy of this element, p-value 10^{-7}. Cooperation between MYC and MYB binding elements has previously been demonstrated for the RD29a stress responsive promoter (Abe et al. 1997). Perhaps the overrepresentation of the MYC element in this set of genes indicates cooperative regulation of the MYC element and the potential MYB binding site in the I-Box.

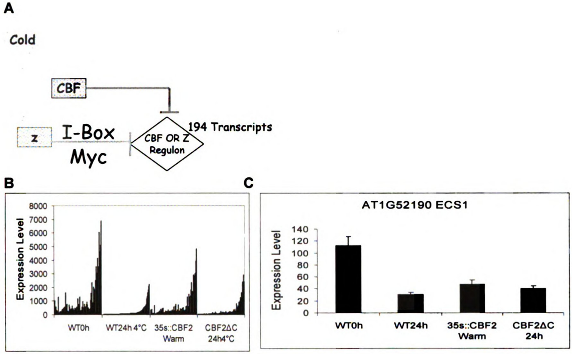


Figure 3.15 CBF Is Sufficient But Is Not Required For the Repression of 194 Transcripts
(A) Model of regulation for 194 transcripts identified as CBF sufficient but not required.
(B) Expression level of these 194 transcripts in WT warm, WT 24h4°C, 35s:CBF2, and 35s:CBF2ΔC 24h4°C
(C) Representative transcript expression level for one transcript, *ECS1* for samples in (B)

Both the I-Box and the same MYC recognition sequence were overrepresented in the CBF independent down-regulated transcripts, p-value 10^{-10} and 10^{-7} respectively. These 221 transcripts were reduced in CBF2ΔC plants to the same level as WT plants after 24h4°C, and were not reduced in 35s::CBF2 plants in the warm indicating that they are entirely CBF independent (Figure 3.16). As would be expected for CBF independent transcripts, the CBF recognition sequence, CRT, is not overrepresented in the promoters of these transcripts

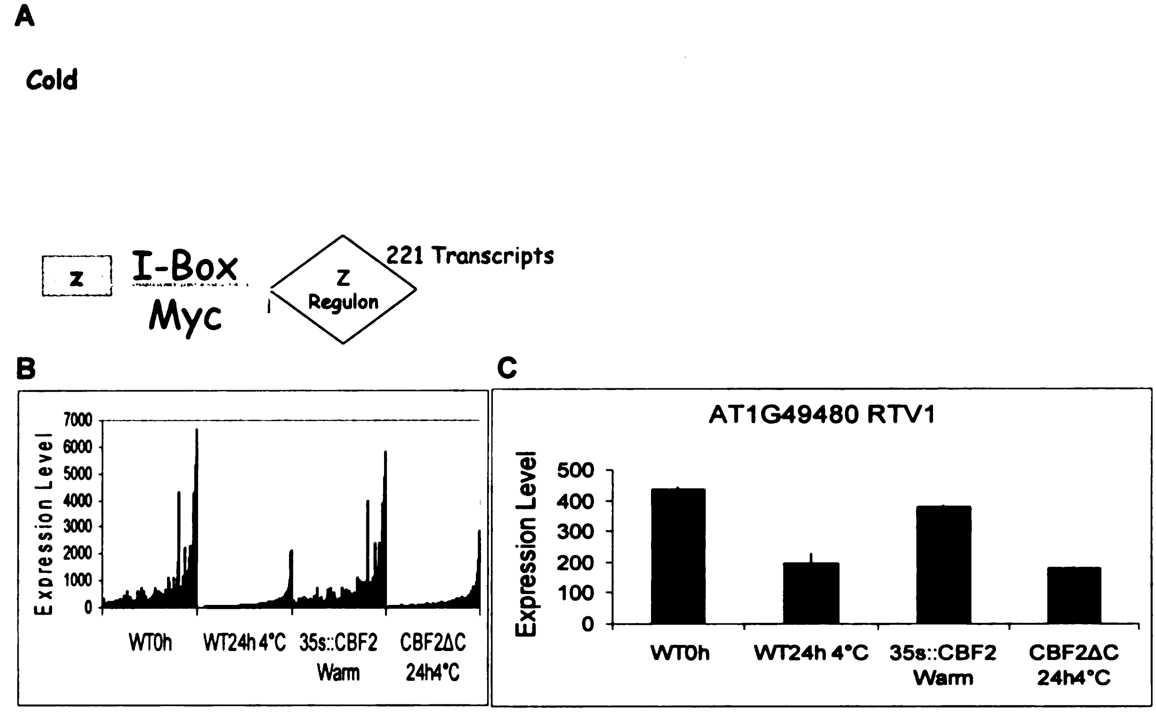


Figure 3.16 CBF Is Neither Sufficient Nor Required For the Repression of 221 Transcripts
(A) Model of regulation for 221 transcripts identified as CBF Independent.
(B) Expression level of these 221 transcripts in WT warm, WT 24h4°C, 35s:CBF2, and 35s:CBF2ΔC 24h4°C
(C) Representative transcript expression level for one transcript, *RTV1* for samples in (B)

The 29 transcripts that are quantitatively affected in their level of down-regulation in CBF2ΔC expressing plants at low temperature suggest a mechanism of regulation where CBF is not required for their repression; yet, achieving complete reduction to the level seen in WT plants is dependent on CBF. Most of these transcripts, 23, that are no longer repressed to their WT level, are also not repressed in 35s::CBF2 plants in the warm, indicating that CBF is not sufficient for their repression, but is required for achieving the full, WT level of repression (Figure 3.17). No known motifs showed any overrepresentation in the promoters of these transcripts. Six transcripts that were qualitatively affected were repressed in 35s::CBF2 plants in the warm, demonstrating that CBF is sufficient for their repression, and is required for their being reduced to their WT level (Figure 3.18). There was no overrepresentation for any known cis-elements in this group, however, this could be due to the small sample size, for example, the I-Box, which is present in 5 of the 6 promoters, is not statistically overrepresented in this set of transcripts, p-value 0.01.

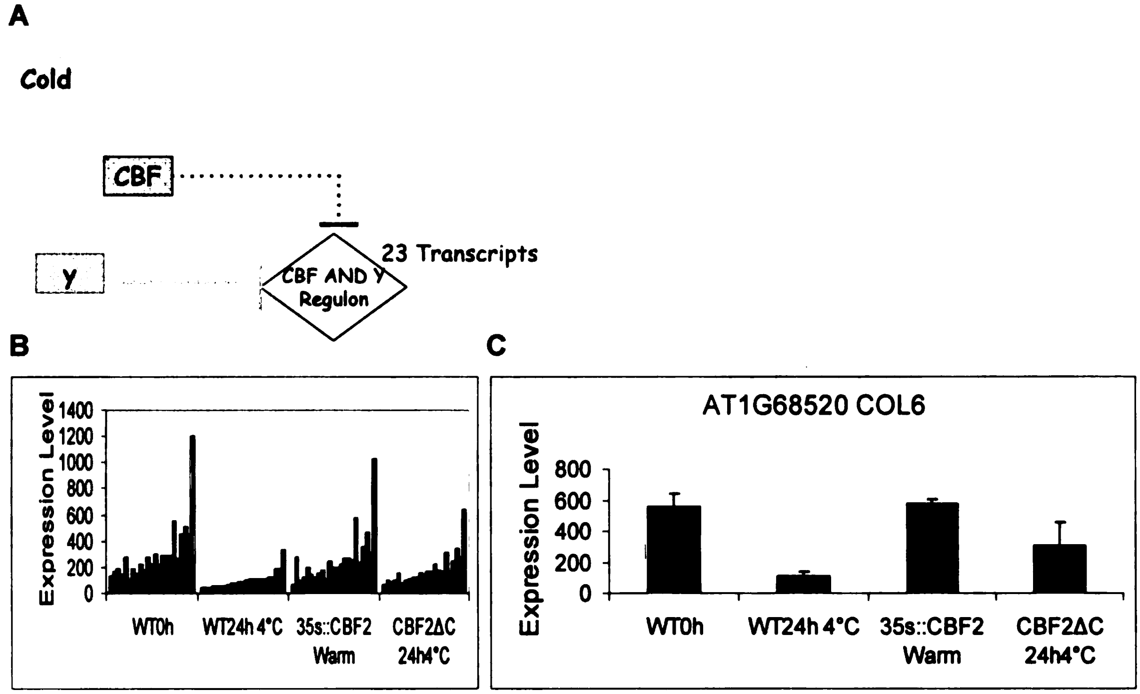


Figure 3.17 CBF Is Not Sufficient But Is Partially Required for the Repression of 23 CBF-Integrated Transcripts
(A) Model of regulation for 23 CBF-Integrated Transcripts.
(B) Expression level of these 23 transcripts in WT warm, WT 24h4°C, 35s:CBF2, and 35s:CBF2ΔC 24h4°C
(C) Representative transcript expression level for one transcript, *COL6* for samples in (B)

Effects on Transcript Levels Are Reflected in Metabolite Profiles

Transcript levels are not always reflective of the protein levels, enzyme activity, or phenotypic activity of the organism. To determine if the transcript changes seen in the 35s::CBF2ΔC plants was reflective of downstream effects we analyzed changes in metabolites in response to low temperature in both WT and 35s::CBF2ΔC plants. In previous work Cook et al. showed that the metabolite profile of plants overexpressing CBF2 at warm temperature resembled that of cold-treated plants(Cook et al. 2004). With the changes seen in the transcript of CBF2ΔC plants we would expect that there would be an effect on metabolite levels, particularly of raffinose. GOLS3 is one of the most highly up-regulated transcripts in CBF2 overexpression and its level of induction is drastically reduced in 35s::CBF2ΔC plants (Figure 3.3, 3.8 C). GOLS3 is one member of the six gene galactinol synthase family and is a component of the raffinose metabolism pathway. Therefore, if this change in transcript level is reflective of a change in the metabolism of the plant, we would expect there to be a dramatic reduction in raffinose levels. When metabolite levels of WT warm and cold plants were compared to cold-treated 35s::CBF2ΔC plants, there was in fact a dramatic reduction of raffinose levels in the cold-treated 35s::CBF2ΔC plants compared to WT indicating that the changes in the transcript level in this case are reflective of changes in the metabolic profile of the plant (Figure 3.18).

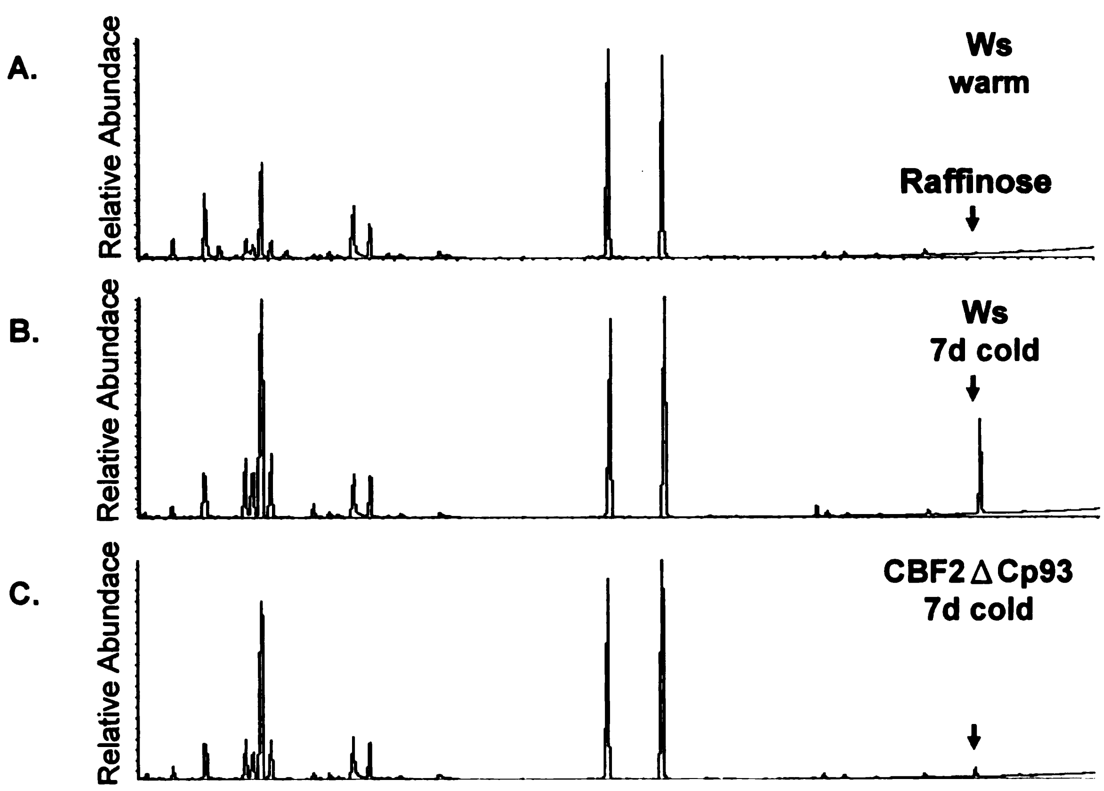


Figure 3.18 GC-MS Metabolite Profiles of Warm and Cold Treated Plants. Arrow indicates raffinose peak. X-axis indicates retention time in two minute increments beginning at 20.00 min and continuing through 48.00 min.
(A) WT warm-grown plants,
(B) WT plants after treatment at 4°C for 7d
(C) CBF2ΔC plants after 7d4°C treatment.

CBF2ΔC Plants Cold Acclimate, but at a Drastically Reduced Level Compared to WT Plants

The results presented here indicate that CBF2ΔC construct is effectively acting as a dominant negative gene blocking the function of the endogenous CBFs. The presence of the CBF2ΔC construct drastically reshapes the cold-regulated transcriptome at both a quantitative and qualitative level. To determine if these plants with an inhibited CBF response are still able to cold acclimate, three lines of CBF2ΔC plants were tested for their ability to cold acclimate by whole plant freeze tests and electrolyte leakage assays. The CBF2ΔC plants were able to cold acclimate as analyzed by a whole plant freeze test (Figure 3.19).

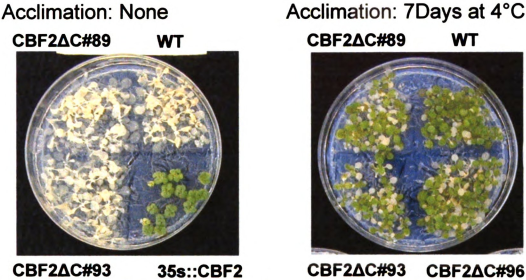


Figure 3.19 CBF2ΔC Survives Whole Plant Freeze Tests After Acclimation Plants were treated for 1h at -10°C either without acclimation or after acclimation of 7d at 4°C.

However, when measured by electrolyte leakage assay, CBF2ΔC were not able to acclimate to the same degree as WT plants (Figure 3.20). EL50 values for WT plants were -10.5°C after cold acclimation while EL50 of CBF2ΔC plants only reaches -8.5°C after acclimation. Interestingly, CBF2ΔC plants also had a lower basal level of freezing tolerance than WT plants.

DISCUSSION

Previous work suggested that CBF was not sufficient for regulation of all cold-responsive transcripts, and that CBF independent pathways for cold acclimation may exist (Vogel et al. 2005). However, it was not known if CBF was required for the induction of these transcripts, and to what extent Arabidopsis can cold-acclimate without a functional CBF pathway.

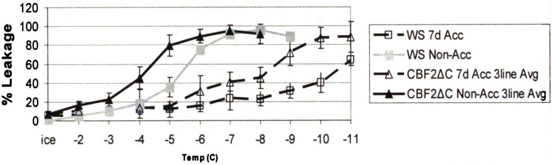


Figure 3.20 Electrolyte Leakage Assays of WT and CBF2ΔC Plants Before and After Acclimation.
Percent electrolyte leakage of non-acclimated WT (solid squares,solid lines), non-acclimated CBF2ΔC (solid triangles,solid lines), acclimated WT (open squares, dashed lines), and acclimated CBF2ΔC (open triangles, dashed lines). Cold acclimation was for 7d 4°. Data for CBF2ΔC plants is an average of three independent transgenic lines.

This work has further refined the CBF regulon, adding two new categories of CBF-regulated transcripts: those where CBF is required but not sufficient for their regulation and those where CBF is not required for their response to low temperature, but is required for their reaching their wild-type level of induction or repression. Comparison of warm-grown and 24h4°C WT plants with 35s::CBF2ΔC warm-grown and 24h4°C and with warm-grown 35s::CBF2 plants revealed several complicated models of regulation for cold-induced transcripts. The two groups of transcripts, those where CBF is sufficient, but not required and those where CBF2ΔC results in a quantitative, but not absolute loss of induction comprise one third of the cold-induced transcripts. The evidence for combinatorial regulation seen here for these modules is in agreement with predictions of large combinatorial regulation in response to low temperature based on motif analysis of cold-regulated genes (Chawade et al. 2007).

The further refinement of the CBF-independent transcripts through the removal from this group of those transcripts where CBF is not sufficient, but is required, should provide a more cohesive group for regulatory element analysis. In fact, analysis of the promoters of these more narrowly defined CBF-independent transcripts identified the telo-box (TBX) as a highly overrepresented element in this group of transcripts. This element has been previously identified as a "midnight element" overrepresented in transcripts peaking at midnight in the circadian cycle (Michael et al. 2008). One possible interpretation of the strong presence of the TBX could be that the effect of cold on the circadian clock has caused this group of transcripts to be shifted from their normal expression pattern (Bieniawska et al. 2008). However, preliminary analysis of this CBF-independent group showed that these transcripts were not over-represented for circadian peak at midnight (or any time point) and in fact the transcripts containing the TBX element were overrepresented for those that did not match any tested model of circadian induction in five different circadian analysis conditions (Michael et al. 2008).

One possible interpretation of this result is that the TBX represents a cold-responsive element that has been adapted for cold-entrainment of the circadian clock in a subset of transcripts. This could be one mechanism for the described effect on many clock output transcripts by low temperature (Bieniawska et al. 2008).

Down-regulation of transcripts in response to abiotic stress in general, and in particular in response to cold- treatment, has not been examined as thoroughly as regulation of induced transcripts. Like induced transcripts, there is evidence for cooperativity in the repression of these transcripts (Chawade et al. 2007). The quantitative effect seen in CBF2ΔC plants supports this idea of cooperativity in the down-regulation of cold-responsive genes. While no repressed transcripts were entirely dependent on CBF for their repression, a large number did not reach their wild-type level of repression in CBF2ΔC plants, suggesting that CBF and another factor or factors may contribute cooperatively to their repression.

Additionally, by refining the cold-repressed transcripts into those that are repressed in both WS and Col and those that are independent of CBF in their repression, a potentially important cis-element for cold-responsive repression, the I-Box, was identified. The I-Box is highly overrepresented in transcripts that are down-regulated in response to low temperature and are CBF not affected by the presence of the CBF2ΔC construct.

The large number of transcripts that were still reduced to their wild-type levels in CBF2ΔC plants could indicate that either CBF is not involved in the repression of these transcripts in response to low temperature or that the CBF DNA binding domain is sufficient to initiate repression in these transcripts. In support of the latter idea, the CBF DNA binding domain was shown to interact with the co-activator protein ADA2a (Mao et al. 2006). However, the CRT element was not present in the promoters of many of these genes indicating that if the CBF DNA binding domain is sufficient for this repression, it is likely recruited to these genes through a novel mechanism.

Importantly, this work identified the importance of the CBF pathway for the ability of Arabidopsis to cold-acclimate. Without a fully-functional CBF pathway, plants containing the CBF2ΔC transcript, while still able to acclimate, are not able to reach the freezing tolerance capabilities of their WT counterparts. Interestingly, even the basal freezing tolerance was affected in CBF2ΔC plants. The expression of CBFs in response to circadian rhythms may play some role in the basal freezing tolerance (Harmer et al. 2000). Further combination of CBF2ΔC with other factors known or identified as important in freezing tolerance will narrow down the set of transcripts that are responsible for contributing to freezing tolerance and distinguish those that are actively contributing to increase freezing tolerance and those that are responding to the change in temperature.

The CBF-independent transcripts identified here, which may contribute to this remaining ability to cold acclimate, can now be used as a tool to work upstream and identify potential cold sensors and regulators, providing additional targets for improving cold tolerance in plants.

MATERIALS AND METHODS

EMS Mutagenesis

EMS Mutagenesis of 35s::CBF2 overexpressing plants, E2, was performed essentially as described in Kim et al. (2005). Mutants of interest were selected from M2 populations based on size; gene expression analysis was performed on M3 plants.

Plant Material and Growth Conditions

All plants were stratified for 3-5 days in the dark at 4°C then transferred to constant illumination at 24°C for 10 days prior to treatment. Cold treatment consisted of moving the plates to a 4°C chamber with constant light at a reduced level (approximately 35 μ mol $m^{-2}sec^{-1}$). Plants were grown on Gamborg's B5 nutrients (Caisson Laboratories, www.caissonlabs.com) and 0.8% phytagar (Caisson Laboratories, www.caissonlabs.com) without sucrose. All experiments were performed on tissue from whole seedlings. 35s::CBF2 plants were as described in Gilmour et al. (2004).

The plasmid containing 35s::CBF2ΔC in the PGA643 vector was transformed into Arabidopsis ecotype WS using standard procedures (Gilmour et. al. 2004). Three transformant lines (89, 93, and x96) were selected based on their level of CBF2ΔC expression in warm grown conditions and were taken to homozygosity.

RNA isolation and Analysis

Total RNA was extracted from plant material with the use of RNeasy Plant Mini kits (Qiagen, Valencia, CA) with modifications as described (Zarka et al. 2003).

Northern transfers were prepared and hybridized as described (Hajela et al. 1990) probes prepared, and membranes washed with high-stringency conditions (Stockinger et al. 1997).

For RT-PCR cDNA synthesis was performed using Promega Reverse Transcription system according to the manufacturer's directions using random primers with the following modifications. Total reaction volumes were doubled and starting RNA was adjusted. Starting RNA was 0.01ug for warm and 2h0°C samples for analysis of CBF1, 2, 3, and Zat12 mRNA and 0.1ug for all 24h0°C samples. cDNA was diluted five-fold with water and 3uL was used as a template for quantitative real-time PCR (qRT-PCR). qRT-PCR using SYBR Green was performed using the Applied Biosystems 7500 real-time PCR system in Standard Mode with SYBR Green PCR Core Reagents Mix (Applied Biosystems). qRT-PCR was performed according to manufacturer's protocols with the following modification. Reactions were performed in a 30μL volume. For CBF2 mRNA analysis, the annealing/extension temperature was 62°C. Serial dilutions of 2h0°C WT samples were performed to determine the efficiencies of the primers for CBF2, transgeneCBF2, and Actin3. Serial dilutions of 24h0°C samples were performed to determine the efficiencies of COR15, GOLS3, and Actin3 primers. Reactions were performed in triplicate and products checked by melting curve analysis. The abundance of transcripts was analyzed with the relative standard curve method normalizing to the reference transcript, Actin3 (AT3G53750). The primers used for amplification were CBF2 (5'-GGA TGCTCATGGTCTTGACAT-3' and 5'-

TCTTCATCCATATAAAACGCATCTTG-3'), transgeneCBF2 (5'-GCTCGTTAACGGTACCATCG-3' and 5'-GTAATCACCGCCTGAGGAAA-3'), ACTIN3 (5'-GGTCGTACTACTGGTATTGTGCT-3' and 5'-TGACAATTTCACGCTCAGCT-3'), GOLS3 (5'-GGAGTGGTTGGTCTGGCTAA-3' and 5'- TTGGTTATCCGGTGGGTAAA-3'), COR15 (5'-ATGGCTTCTTCTTTCCACAGC-3' and 5'-GAAGCTTTCTTTGTGGCCTC-3'),

Affymetrix GeneChip Hybridization and Data Collection

Biotinylated target RNA was prepared from 15ug of total RNA equally pooled from three plates. Labeling was performed using Affymetrix IVT labeling kit according to manufacturer's directions (Affymextrix, Santa Clara, CA, USA). The samples were hybridized to the Affymetrix Arabidopsis ATH1 GeneChip. Two biological replicates were analyzed for CBF2ΔC warm, WS 24h, Col 24h, and four biological replicates were analyzed for CBF2ΔC 24h.

Affymetrix GeneChip Data Analysis

Publically available microarray data available from ATGE and Vogel et al was used in addition to new chips (Vogel, Zarka et al. 2005; Kilian, Whitehead et al. 2007). Each experiment was maintained as an individual unit and normalized using RMA in the AFFY package for Bioconductor (Irizarry et al. 2003; Vincent et al. 2004). RankProduct analysis was performed with the RankProd package (Hong, Breitling et al. 2006). Cold-regulated transcripts were selected as those with a fold change greater than 2 and significance cutoff of p value <0.01 (FDR corrected) in any of the three selected time points (1h, 24h, or 7d). Differentially expressed transcripts between WS and Col at 24h4°

and 24h0° were determined using limma with a 2 fold cutoff and FDR corrected p-value of 0.05 (Smyth 2004). These transcripts were removed from the analysis of cold-regulated genes. CBF2 was removed from all ensuing analysis.

Overrepresentation of Motif Analysis

Prior to promoter analysis probe sets known to hybridize to multiple genes were removed. Probesets that do hybridize multiple genes remain in the total numbers of each category and are listed in tables as "multiple". Analysis for overrepresentation of known elements was performed using Athena (O'Connor et al. 2005). Frequency of occurrence for modifications of known elements was performed using Promomer and hypergeometric testing performed using R (Toufighi et al. 2005; Team 2008).

Overrepresentation of GO Terms

Each subgroup of genes was analyzed for enrichment of GO terms using GOStats and Athena (O'Connor, Dyreson et al. 2005; Falcon and Gentleman 2007).

Extraction of Polar Metabolites

100mg (warm) or 25mg (cold treated) of powdered tissue was extracted in 700μL cold methanol with 10μM ribitol. 130 uL of methanol/water phase was dried under vacuum.

Derivatization and Analysis

50μL of methoxyamine hydrochloride (20 mg/mL) was added to extraction and incubated for 120 min at 37°C while shaking. 80μL MSTFA was added and incubated 30 min at 37°C while shaking. Incubate 120 min at 22°C. 100µL was transferred to GC/MS vial. 50µL of derivitized metabolites were injected on a 30m HP5 ramping from 80°C to 325°C at 2.5°C / min, then 3 min at 325°C. The injection was split for cold samples. Peaks were identified by retention time referenced to a standard mix. Quantification was analyzed based on the ribitol internal standard.

Whole Plant Freeze Test

Whole plant freeze tests were performed essentially as previously described (Vogel et. al. 2005).

Electrolyte Leakage Freeze Test

Electrolyte leakage freeze tests were performed essentially as described (Gilmour et al. 2000) with minor modifications. The SAS system was not used to aid in randomization

LITERATURE CITED

Abe, H., K. Yamaguchi-Shinozaki, et al. (1997). "Role of Arabidopsis MYC and MYB homologs in drought-and abscisic acid-regulated gene expression." Plant Cell **9**(10): 1859-1868.

Bieniawska, Z., C. Espinoza, et al. (2008). "Disruption of the Arabidopsis Circadian Clock Is Responsible for Extensive Variation in the Cold-Responsive Transcriptome." Plant Physiol. **147**(1): 263-279.

Breitling, R., P. Armengaud, et al. (2004). "Rank products: a simple, yet powerful, new method to detect differentially regulated genes in replicated microarray experiments." FEBS Letters **573**(1-3): 83-92.

Chawade, A., M. Brautigam, et al. (2007). "Putative cold acclimation pathways in Arabidopsis thaliana identified by a combined analysis of mRNA co-expression patterns, promoter motifs and transcription factors." BMC Genomics **8**(1): 304.

Cook, D., S. Fowler, et al. (2004). "From The Cover: A prominent role for the CBF cold response pathway in configuring the low-temperature metabolome of Arabidopsis." Proceedings of the National Academy of Sciences **101**(42): 15243.

Donald, R. G. K. and A. R. Cashmore (1990). "Mutation of either G box or I box sequences profoundly affects expression from the Arabidopsis rbcS-1A promoter." EMBO J **9**(6): 1717-1726.

Falcon, S. and R. Gentleman (2007). "Using GOstats to test gene lists for GO term association." Bioinformatics **23**(2): 257-258.

Fowler, S. and M. F. Thomashow (2002). "Arabidopsis Transcriptome Profiling Indicates That Multiple Regulatory Pathways Are Activated during Cold Acclimation in Addition to the CBF Cold Response Pathway." Plant Cell: tpc.003483.

Gilmour, S. J., S. G. Fowler, et al. (2004). "Arabidopsis Transcriptional Activators CBF1, CBF2, and CBF3 have Matching Functional Activities." Plant Molecular Biology **54**(5): 767-781.

Gilmour, S. J., A. M. Sebolt, et al. (2000). "Overexpression of the Arabidopsis CBF3 Transcriptional Activator Mimics Multiple Biochemical Changes Associated with Cold Acclimation." Plant Physiol. **124**(4): 1854-1865.

Giuliano, G., E. Pichersky, et al. (1988). "An Evolutionarily Conserved Protein Binding Sequence Upstream of a Plant Light-Regulated Gene." Proceedings of the National Academy of Sciences **85**(19): 7089-7093.

Hajela, R. K., D. P. Horvath, et al. (1990). "Molecular Cloning and Expression of cor (Cold-Regulated) Genes in Arabidopsis thaliana." Plant Physiol. **93**(3): 1246-1252.

Harmer, S. L., J. B. Hogenesch, et al. (2000). "Orchestrated Transcription of Key Pathways in Arabidopsis by the Circadian Clock." Science **290**(5499): 2110-2113.

Hiratsu, K., K. Matsui, et al. (2003). "Dominant repression of target genes by chimeric repressors that include the EAR motif, a repression domain, in Arabidopsis." The Plant Journal **34**(5): 733-739.

Hong, F., R. Breitling, et al. (2006). "RankProd: a bioconductor package for detecting differentially expressed genes in meta-analysis." Bioinformatics **22**(22): 2825.

Irizarry, R. A., B. Hobbs, et al. (2003). "Exploration, normalization, and summaries of high density oligonucleotide array probe level data." Biostat **4**(2): 249-264.

Kilian, J., D. Whitehead, et al. (2007). "The AtGenExpress global stress expression data set: protocols, evaluation and model data analysis of UV-B light, drought and cold stress responses." The Plant Journal **50**(2): 347-363.

Manevski, A., G. Bertoni, et al. (2000). "In synergy with various cis-acting elements, plant insterstitial telomere motifs regulate gene expression in Arabidopsis root meristems." FEBS Letters **483**(1): 43-46.

Mao, Y., K. A. Pavangadkar, et al. (2006). "Physical and functional interactions of Arabidopsis ADA2 transcriptional coactivator proteins with the acetyltransferase GCN5 and with the cold-induced transcription factor CBF1." Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression **1759**(1-2): 69-79.

Michael, T. P., T. C. Mockler, et al. (2008). "Network Discovery Pipeline Elucidates Conserved Time-of-Dayâ€"Specific cis-Regulatory Modules." PLoS Genetics **4**(2): e14.

Mockler, T. C., T. P. Michael, et al. (2007). "The Diurnal Project: Diurnal and Circadian Expression Profiling, Model-based Pattern Matching, and Promoter Analysis." Cold Spring Harbor Symposia on Quantitative Biology **72**(1): 353-363.

Narusaka, Y., K. Nakashima, et al. (2003). "Interaction between two cis-acting elements, ABRE and DRE, in ABA-dependent expression of Arabidopsis rd29A gene in response to dehydration and high-salinity stresses." The Plant Journal **34**(2): 137-148.

Novillo, F., J. Medina, et al. (2007). "Arabidopsis CBF1 and CBF3 have a different function than CBF2 in cold acclimation and define different gene classes in the CBF regulon." Proceedings of the National Academy of Sciences **104**(52): 21002.

O'Connor, T. R., C. Dyreson, et al. (2005). "Athena: a resource for rapid visualization and systematic analysis of Arabidopsis promoter sequences." Bioinformatics **21**(24): 4411-4413.

Ramos, A., E. PÃ©rez-SolÃ-s, et al. (2005). "Winter Disruption of the Circadian Clock in Chestnut." Proceedings of the National Academy of Sciences of the United States of America **102**(19): 7037-7042.

Shinozaki, K. and K. Yamaguchi-Shinozaki (2000). "Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways." Current Opinion in Plant Biology **3**(3): 217-223.

Smyth, G. K. (2004). "Linear models and empirical Bayes methods for assessing differential expression in microarray experiments." Statistical Applications in Genetics and Molecular Biology **3**(1).

Stockinger, Eric J., Sarah J. Gilmour, et al. (1997). "Arabidopsis thaliana CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit." Proceedings of the National Academy of Sciences **94**(3): 1035-1040.

Team, R. D. C. (2008). R: A Language and Environment for Statistical Computing.

Toufighi, K., S. M. Brady, et al. (2005). "The Botany Array Resource: e-Northerns, Expression Angling, and promoter analyses." The Plant Journal **43**(1): 153-163.

Tremousaygue, D., L. Garnier, et al. (2003). "Internal telomeric repeats and 'TCP domain' protein-binding sites co-operate to regulate gene expression in Arabidopsis thaliana cycling cells." The Plant Journal **33**(6): 957-966.

Vincent, R. C. G. a., J. C. a. D. M. B. and, et al. (2004). "Bioconductor: Open software development for computational biology and bioinformatics." Genome Biology **5**: R80.

Vogel, J. T., D. G. Zarka, et al. (2005). "Roles of the CBF2 and ZAT12 transcription factors in configuring the low temperature transcriptome of Arabidopsis." The Plant Journal **41**(2): 195-211.

Yang, T. and B. W. Poovaiah (2002). "A calmodulin-binding/CGCG box DNA-binding protein family involved in multiple signaling pathways in plants." J Biol Chem **277**: 45049 - 45058.

Zarka, D. G., J. T. Vogel, et al. (2003). "Cold Induction of Arabidopsis CBF Genes Involves Multiple ICE (Inducer of CBF Expression) Promoter Elements and a Cold-Regulatory Circuit That Is Desensitized by Low Temperature." Plant Physiol. **133**(2): 910-918.

CHAPTER FOUR

Integration of Cold Response with Other Environmental Responses

Introduction

The overall goal of this project is to build a descriptive network of the regulation of the response to low temperature and cold acclimation in *Arabidopsis*. The creation of such a network would allow for identification of key regulatory steps in the response to low temperature. These key regulatory steps are potential hubs of cold-response and would then be excellent targets for further study to improve tolerance of plants to low temperatures. This work has provided additional information in two aspects of this network. First, CAMTA3 has been identified as an upstream regulator of the CBF cold responsive pathway. Secondly, transcriptome analysis of a dominant negative version of CBF2, CBF2ΔC, provided a refined classification of cold-regulated transcripts and demonstrated the importance of CBF on both basal and acclimated freezing tolerance levels.

The identification of CAMTA3 as a positive regulator of *CBF2* induction in response to low temperature provides insight into a possible link between calcium signaling and the cold-response. Additional experiments have shown that CAMTAs are also involved in the regulation of *CBF2* in response to mechanical stimulus and cycloheximide treatment. These results indicate that perhaps CAMTAs are integration points for multiple environmental signals. As potential hubs for integration points of multiple environmental inputs, further understanding of the role of CAMTAs in stress response may provide mechanisms for improving stress tolerance in plants.

Networks describing responses in biological systems often reveal the extensive overlap of the many pathways of an organism with each other and the interaction of many pathways previously considered independent. The cold regulation pathway is no exception to this interconnectedness. The role of development and environmental conditions on the response of *Arabidopsis* to low temperature is only beginning to be analyzed, but there is a clear role for involvement of the circadian clock and light quality signaling (Fowler et al. 2005; Ramos et al. 2005; Franklin and Whitelam 2007; Bieniawska et al. 2008; Michael et al. 2008). In the course of this analysis additional evidence for the complex integration of environmental signals and development with the cold-response were identified. It was discovered that *Arabidopsis* has improved cold-acclimation ability when grown under short days, implicating a possible link between day-length signals and the cold response. Additionally, developmental phenotypes observed in the CBF2ΔC plants indicate that CBF may have roles in normal growth and development in addition to its role in cold acclimation.

Results

CAMTA3 is a Positive Regulator of *CBF* Expression in Response to Mechanical Agitation and Cycloheximide Treatment

CBF2 is induced in response to low-temperature, mechanical agitation, and cycloheximide treatment. One common link between these three stimuli is a rapid influx of cytosolic calcium (Knight et al. 1992; Knight et al. 1996).

CAMTA3 was identified as a positive regulator of low-temperature induction of *CBF2*. The binding of calmodulin by CAMTA proteins suggests a possible link to calcium signaling. It is therefore of interest to determine if CAMTAs are involved in the regulation of *CBF2* in response to these other stimuli. In support of this possibility, the CM2/CM1-Box:Gus reporter, which is regulated by CAMTA3 in response to low temperature, was shown by Van Buskirk to be induced in response to cycloheximide and mechanical treatment (Figure 4.1A, Unpublished Data).

To test the potential role for the CAMTAs in the regulation of *CBF2* in response to other environmental inputs, mRNA accumulation was measured in *camta* mutants after treatment with cycloheximide. As was seen in response to low temperature, loss of CAMTA3 resulted in lower induction of *CBF2* in response to cycloheximide treatment (Figure 4.1B). There also seems to be a role for CAMTA2 in the regulation of *CBF2* to cycloheximide as *CBF2* mRNA does not accumulate as high in the *camta2* mutant as in WT. In response to mechanical agitation, there is little effect on *CBF2* induction in the *camta3* mutant (Figure 4.1C). Indicating that induction of *CBF2* in response to mechanical agitation may occur through a distinct pathway than that of cold-induction. However, loss of CAMTA3, did have an effect on *CBF1* induction in respon to mechanical agitation (Figure 4.1D). This result suggests that CAMTAs may contribute to specificity in the response to various external signals.

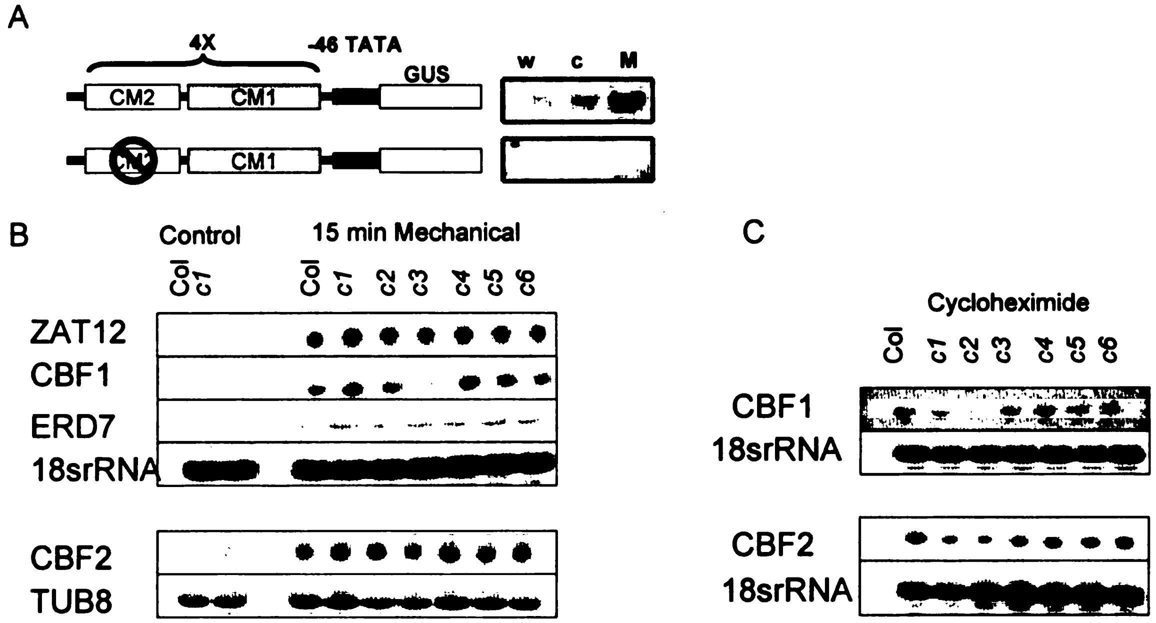


Figure 4.1 Role for CAMTAs in Induction of *CBFs* by Various Stimuli
(A)CM2/CM1-Box is sufficient for induction in response to mechanical agitation
(B)Northern blot analysis of plants treated for 15 min with mechanical agitation then harvested after 10 min. Control is untreated plants. mRNA levels of *ZAT12*, *CBF1*, and *ERD7* were analyzed in Columbia (Col), *camta1(c1)*, *camta2(c2)*, *camta3(c3)*, *camta4(c4)*, *camta5(c5)*, *camta6(c6)* plants and 18s rRNA was used as a loading control. *CBF2* mRNA levels were analyzed with *TUB8* as a loading control.
(C)Northern blots of plants treated with 10 µg ml^{-1} cycloheximide Columbia (Col), *camta1(c1)*, *camta2(c2)*, *camta3(c3)*, *camta6(c6)* analyzed for *CBF2* mRNA levels 18s rRNA was used as a loading control.

CAMTAs as Potential Hubs in Response to Stress in *Arabidopsis*

The ability for a similar calcium signature to precede a multitude of environmental responses indicates the importance of downstream signals in the calcium cascade for interpretation of the calcium signal and initiation of an appropriate response. The CAMTA proteins could be one potential method of interpreting this signal and deriving the specificity of the response. For example, loss of CAMTA3 showed a loss of GUS staining in the shoots of *Arabidopsis* expressing the CM2/CM1:Gus reporter, but not in the roots, indicating a possible role for CAMTA 3 in providing tissue specificity in response to low temperature signals (Figure 4.2) . The observation that CAMTAs are involved in the regulation of different stimuli to induce *CBF* expression indicates that CAMTA proteins are a potential hub for interaction of multiple signals and initiation of the desired response. If CAMTAs are a point of regulation for the calcium response they will be interesting targets for improving stress tolerance in plants.

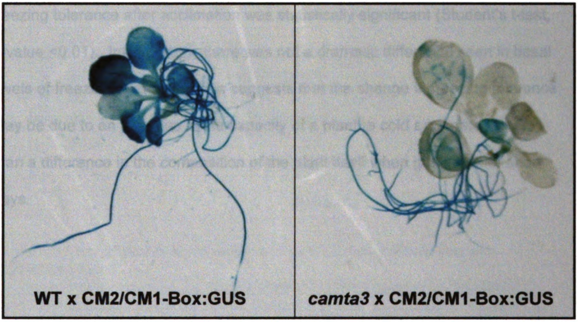


Figure 4.2 Histochemical staining of WT x CM2/CM1-Box:GUS and *camta3* x CM2/CM1-Box:GUS seedlings

***Arabidopsis* Has an Increased Capacity for Cold Acclimation When Grown in Short-Days**

There have been multiple environmental inputs demonstrated to affect the cold-acclimation pathway in *Arabidopsis*; induction of *CBFs* and other early cold-responsive genes is gated by the circadian clock, light-quality affects induction of downstream targets of CBFs, and pre-treatment with ABA or drought increases freezing tolerance (Mäntylä et al. 1995; Fowler et al. 2005; Franklin and Whitelam 2007). Results from this study indicated that there is a potential role for the integration of photoperiod signals into the cold acclimation ability in *Arabidopsis*.

Electrolyte leakage assays of plants grown in short-day photoperiods (8h light, 16h dark) showed a dramatic increase in the EL_{50} values of cold acclimated plants over plants grown in constant light (Figure 4.3, 4.4). The increased freezing tolerance after acclimation was statistically significant (Student's t-test, p-value <0.01). Interestingly, there was not a dramatic difference seen in basal levels of freezing tolerance. This suggests that the change in freezing tolerance may be due to an increase in the capacity of a plant to cold acclimate rather than a difference in the composition of the plant itself when grown under short days.

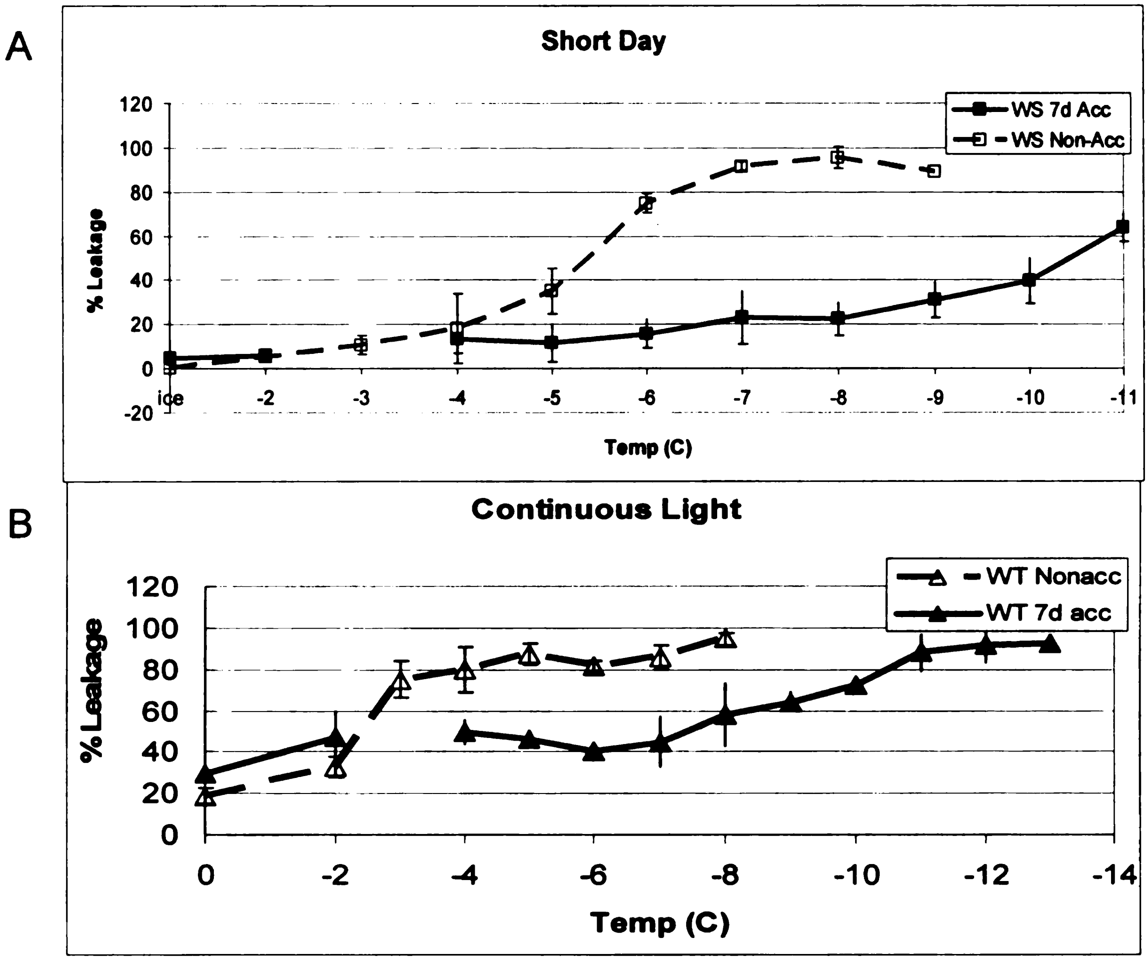


Figure4.3 Electrolyte Leakage Assay of Plants Grown in Short Day and Continuous Light

(A) Electrolyte leakage assay of non-acclimated WS (open squares, dashed lines) and WS acclimated for 7d4°C (closed squares, solid line) of plants grown in 8h light / 16h dark

(B) Electrolyte leakage assay of non-acclimated WS (open triangles, dashed lines) and WS acclimated for 7d4°C of plants grown in continuous light

A

Light Regimen	Non-Acclimated EL_{50} (°C)	Acclimated EL_{50}(°C)	Change in EL_{50} (°C)
Constant	-3	-7	4
Constant	-4.5	-6.5	2
Constant	-4	-5.5	1.5
8h	-6	-10	4
8h	-5.5	-10.5	5
8h	-4	-9.5	5.5

B

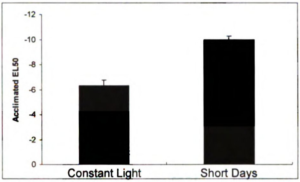


Figure 4.4 EL_{50} Values for Short Day and Constant Light Grown Plants
(A) Table of EL_{50}Values for plants grown in constant light and short days.
(B) Graph of acclimated EL_{50} values, (p-value <0.01)

The mechanisms for this increase in freezing tolerance are not clear. One possibility is that when grown in continuous light, plants make the switch from vegetative to reproductive state very early and that switch may be delayed in short day grown plants. In rye and wheat, after switching from vegetative to reproductive growth, there is a reduction in the ability of these plants to cold acclimate (Fowler et al. 1996). Another possibility is that the plant uses day-length clues to gate the response to low temperature. Gating of the response to low temperature to day-length would ensure that a full response to low temperature in preparation for the potential threat of freezing occurs only when days get shorter.

Potential Role for CBFs in Development

Many developmental changes occur in response to growth at low temperature. Exposure to short periods of low temperature causes a delay in flowering, while extended periods at low temperature are responsible for vernalization, which induces flowering (Sung and Amasino 2005; Franklin and Whitelam 2007). Inversion of thermocycles, warm nights and cold days, results in reduced growth (Thingnaes et al. 2003). Therefore, when constructing a network of cold-response signals, the integration of developmental and other environmental cues must be considered.

Interestingly, the loss of CBF results in a decrease in basal freezing tolerance of non-acclimated plants. This decrease in basal freezing tolerance suggests a potential role for CBF in normal growth and development in *Arabidopsis*. This role may simply be in maintaining a prepared state for cold acclimation. However, functional CBFs are present in many species of plants, including those which are chilling and freezing sensitive(Zhang et al. 2004; Ballou et al. 2007). The maintenance of a functional CBF in a diverse array of plant species, including those that are not chilling tolerant suggests that there is some pressure on preservation of a functional CBF, independent of its function in cold acclimation.

One would expect that if there were a role in development for CBF, CBF overexpressing plants and CBF2ΔC plants may show some developmental phenotypes. CBF overexpressing plants have a dramatic dwarf, late-flowering phenotype that appears to correlate with CBF function (Canella and Gilmour, unpublished data)(Gilmour et al. 2004). Comparison of plants under normal growth conditions revealed no obvious phenotype differences in adult plants between WT and CBF2ΔC. However, CBF2ΔC plants have a consistent developmental phenotype; approximately 10% of offspring have single, fused, or tri-cotyledons (Figure 4.5, 4.6). This result is consistent from generation to generation, regardless of the parent plant's cotyledon phenotype. NAM (non-apical meristem) transcription factors have a similar cotyledon phenotype to the one seen in CBF2ΔC plants. Comparison of the expression level of the NAM genes in *Arabidopsis* revealed that two NAM genes, AT5g22290 and AT5g39610 were two-fold or more higher in expression in CBF2ΔC than in WT plants with a p-value <0.05. Perhaps this indicates a role for CBF in the development of *Arabidopsis*, but that redundancy in the developmental network, like that of the cold network, provides the plant with plasticity to overcome the loss of CBF.

DISCUSSION

As would be expected from a living organism that has to survive in the environment where it landed as a seed, with only the information provided in its genome, there is a great deal of plasticity and redundancy in the response of *Arabidopsis* to environmental stresses. In this work, this plasticity is evident in the response of *Arabidopsis* to low temperature. Loss of the CBF pathway, which is sufficient for cold acclimation, does cause a reduction in freezing tolerance. However, in the absence of the CBF pathway *Arabidopsis* can still sense low temperature and make changes necessary to increase its ability to survive freezing, even without the ability to accumulate raffinose, an important cryoprotectant molecule. This redundancy implies a complicated network, complete with multiple back-ups, alternative routes, and fail-safe mechanisms to ensure that the plant has the best chance of survival no matter what environment it finds itself in.

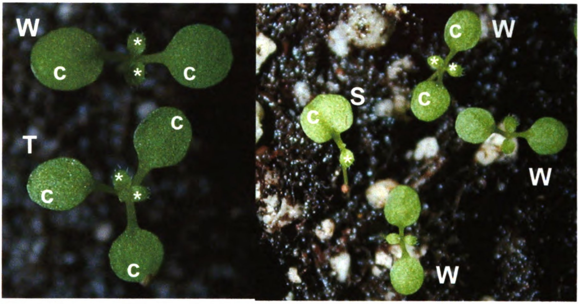


Figure 4.5 Cotyledon Phenotypes in CBF2ΔC Seedlings
CBF2ΔC seedlings with wild-type (W), tri-cotyledons (T), or single-cotyledon (S) phenotypes. Cotyledons are indicated by C and leaves are indicated by *.

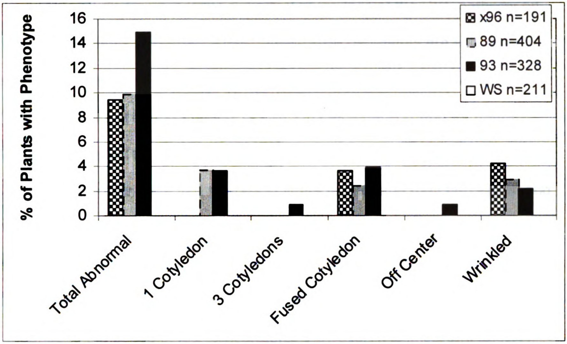


Figure 4.6 Quantification of Cotyledon Phenotypes in CBF2ΔC Seedlings
Percent of total plants with abnormal cotyledon phenotype from WS (white) and three lines of CBF2ΔC plants, 93 (solid black), 89 (grey), x96 (checkered). Total number of plants analyzed (n) is presented in the legend.

An important component in a preparedness system is to be able to rapidly and accurately predict changes in the environment and translate this into an appropriate response. It is easy to imagine the evolutionary pressure on plants to incorporate all possible environmental cues to anticipate changes in the environment and prepare for them when necessary, while not wasting resources preparing needlessly. In this study, the CAMTAs are identified as important regulators of the response to low temperature. Their possible role as integration points for processing and responding to multiple environmental signals makes them interesting targets for further study and potential targets for improving stress responses in crop plants.

This study also describes the effect of the loss of the CBF pathway on the ability of plants to cold acclimate. While CBF2ΔC plants are still able to cold acclimate, one level of redundancy in response to low temperature has been removed in these plants. The use of CBF2ΔC as a background for future studies will facilitate the identification of other pathways for cold acclimation.

While plants have had millions of years to fine tune their perception and response to environmental stimuli, the recent rapid climate changes may disrupt the ability of plants to accurately predict and prepare for fluctuations in the environment. It is encouraging to see the incredible plasticity of plants in their response to the stress of low temperature. However, it is important to remember that survival of *Arabidopsis* in the face of an impaired cold acclimation pathway does not directly translate into a sustained yield of crop plants in response to low temperature. Recognizing and investigating the ability of plants to incorporate these subtle signals into their response will allow us to describe more accurately the network of responses to low temperature and other stresses that plants encounter. A thorough understanding of the plant stress response network will allow us to target areas for improvement in plant species we depend on for survival.

MATERIALS AND METHODS

Plant Material and Growth Conditions

All plants were stratified for 3-5 days in the dark at 4°C then transferred to constant illumination at 24°C for 10 days prior to treatment. Mechanical treatment consisted of tapping the plates on the bench for 15 minutes prior to harvesting the tissue. Cycloheximide treatment consisted of floating seedlings grown on a filter paper in 10ug ml^{-1} cylcoheximide. Plants were grown on a filter placed on Gamborg's B5 nutrients (Caisson Laboratories, www.caissonlabs.com) and 0.8% phytagar (Caisson Laboratories, www.caissonlabs.com) without sucrose. All experiments were performed on tissue from whole seedlings.

RNA isolation and Analysis

Total RNA was extracted from plant material with the use of RNeasy Plant Mini kits (Qiagen, Valencia, CA) with modifications as described (Zarka et al. 2003). Northern transfers were prepared and hybridized as described (Hajela et al. 1990) probes prepared, and membranes washed with high-stringency conditions (Stockinger et al. 1997).

Electrolyte Leakage Freeze Test

Electrolyte leakage freeze tests were performed essentially as described (Gilmour et al. 2000) with minor modifications. The SAS system was not used to aid in randomization.

Staining for GUS Activity

Gus activity was analyzed as described by Zarka et al. (2003).

LITERATURE CITED

Ballou, S. M., K. Y. Yun, et al. (2007). "Cold Sensitivity Gradient in Tuber-Bearing Solanum Based on Physiological and Transcript Profiles." Crop Science **47**(5): 2027.

Bieniawska, Z., C. Espinoza, et al. (2008). "Disruption of the Arabidopsis Circadian Clock Is Responsible for Extensive Variation in the Cold-Responsive Transcriptome." Plant Physiol. **147**(1): 263-279.

Fowler, D. B., L. P. Chauvin, et al. (1996). "The regulatory role of vernalization in the expression of low-temperature. induced genes in wheat and rye." Theor Appl Genet **93**: 554-559.

Fowler, S. G., D. Cook, et al. (2005). "Low temperature induction of Arabidopsis CBF1, 2, and 3 is gated by the circadian clock." Plant Physiol **137**: 961 - 968.

Fowler, S. G., D. Cook, et al. (2005). "Low Temperature Induction of Arabidopsis CBF1, 2, and 3 Is Gated by the Circadian Clock." Plant Physiol **137**(3): 961 - 968.

Franklin, K. A. and G. C. Whitelam (2007). "Light-quality regulation of freezing tolerance in Arabidopsis thaliana." Nat Genet **39**(11): 1410-1413.

Gilmour, S. J., S. G. Fowler, et al. (2004). "Arabidopsis Transcriptional Activators CBF1, CBF2, and CBF3 have Matching Functional Activities." Plant Molecular Biology **54**(5): 767-781.

Gilmour, S. J., A. M. Sebolt, et al. (2000). "Overexpression of the Arabidopsis CBF3 Transcriptional Activator Mimics Multiple Biochemical Changes Associated with Cold Acclimation." Plant Physiol. **124**(4): 1854-1865.

Hajela, R. K., D. P. Horvath, et al. (1990). "Molecular Cloning and Expression of cor (Cold-Regulated) Genes in Arabidopsis thaliana." Plant Physiol. **93**(3): 1246-1252.

Knight, H., A. J. Trewavas, et al. (1996). "Cold Calcium Signaling in Arabidopsis Involves Two Cellular Pools and a Change in Calcium Signature after Acclimation." Plant Cell **8**(3): 489-503.

Knight, M. R., S. M. Smith, et al. (1992). "Wind-induced plant motion immediately increases cytosolic calcium." Proceedings of the National Academy of Sciences of the United States of America **89**(11): 4967-4971.

Mäntylä, E., V. Lång, et al. (1995). "Role of abscisic acid in drought-induced freezing tolerance, cold acclimation, and accumulation of LTI78 and RAB18 proteins in Arabidopsis thaliana." Plant Physiol **107**: 141-148.

Michael, T. P., T. C. Mockler, et al. (2008). "Network Discovery Pipeline Elucidates Conserved Time-of-Dayâ€"Specific cis-Regulatory Modules." PLoS Genetics **4**(2): e14.

Ramos, A., E. Perez-Solis, et al. (2005). "From the Cover: Winter disruption of the circadian clock in chestnut." Proceedings of the National Academy of Sciences **102**(19): 7037-7042.

Stockinger, Eric J., Sarah J. Gilmour, et al. (1997). "Arabidopsis thaliana CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit." Proceedings of the National Academy of Sciences **94**(3): 1035-1040.

Sung, S. and R. M. Amasino (2005). "REMEMBERING WINTER: Toward a Molecular Understanding of Vernalization." Annual Review of Plant Biology **56**(1): 491-508.

Thingnaes, E., S. Torre, et al. (2003). "Day and Night Temperature Responses in Arabidopsis: Effects on Gibberellin and Auxin Content, Cell Size, Morphology and Flowering Time." Ann Bot **92**(4): 601-612.

Zarka, D. G., J. T. Vogel, et al. (2003). "Cold Induction of Arabidopsis CBF Genes Involves Multiple ICE (Inducer of CBF Expression) Promoter Elements and a Cold-Regulatory Circuit That Is Desensitized by Low Temperature." Plant Physiol. **133**(2): 910-918.

Zhang, X., S. G. Fowler, et al. (2004). "Freezing-sensitive tomato has a functional CBF cold response pathway, but a CBF regulon that differs from that of freezing-tolerant Arabidopsis." The Plant Journal **39**(6): 905-919.

APPENDIX

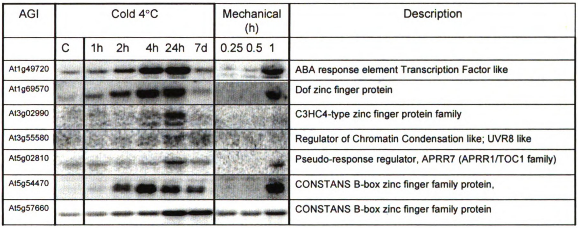
EAR Repression

Attempts were made to reduce CBF function using an EAR repressive domain (Hiratsu et al. 2003). CBF full length and two versions of the CBF DNA binding domain were fused to the EAR repressive domain. No viable colonies of the CBF full length/EAR fusion construct were obtained from cloning attempts by either Sarah Gilmour or me. Both versions of the CBF DNA binding domain fused to EAR were obtained and plants were transformed. Plants were tested for a reduction in COR15 and COR78 using northern blot analysis. lines were found with reduced COR15 and COR78 expression. However, the level of reduction was not as great as that seen in the CBF2ΔC lines.

ICICLES

In an interest to identify other factors that maybe involve in regulation of cold-responsive transcripts a reverse genetics approach was taken. Seven transcription factors that were induced in response to low temperature were studied for their role in cold acclimation. The group of transcription factors analyzed was classified as ICICLEs, Independent of CBF Influence and Cold Late Expressed, that is analysis of Affymetrix experiments by Jonathan Vogel indicated that they were induced after long-term exposure to low temperature and were not up-regulated by overexpression of CBF in the warm. The expression pattern of these transcripts was confirmed by northern blot analysis (Figure A1). Homozygous knockouts were obtained and tested for changes in freezing tolerance. No changes were seen in freezing tolerance in any of the homozygous knockout mutants either by electrolyte leakage assay or whole plant freeze tests.

In contrast to the transcription factors induced early in response to low temperature which were induced rapidly after mechanical agitation, these transcription factors either did not have a detectable response to mechanical agitation, At2g55580 and At3g02990, or were induced an hour after treatment (Figure A1).



AGI	Cold 4°C	Mechanical (h)	Description
	C | 1h 2h 4h 24h 7d	0.25 0.5 1	
At1g49720			ABA response element Transcription Factor like
At1g69570			Dof zinc finger protein
At3g02990			C3HC4-type zinc finger protein family
At3g55580			Regulator of Chromatin Condensation like; UVR8 like
At5g02810			Pseudo-response regulator, APRR7 (APRR1/TOC1 family)
At5g54470			CONSTANS B-box zinc finger family protein,
At5g57660			CONSTANS B-box zinc finger family protein

Figure A1 Induction of ICICLE Transcription Factors in Response to Low Temperature Treatment and Mechanical Agitation
Northern blot analysis for induction of each transcript for control (C), after treatment at 4°C for time specified, and after 15 min mechanical agitation and recovery for time specificed.

Silencing of CBF

A hairpin RNAi construct was designed that would target CBF1, 2, and 3 for silencing. This construct was based on CBF2 sequence homology. The construct was a 537bp sense construct fused to a reverse oriented 338bp antisense construct (Figure A2). The sense construct started 31bp upstream of the CBF2 translation start site. Both the sense and the anti-sense construct ended at bp 509. Thus the overlapping region targeted the 3' end of the DNA binding domain and the CBF specific sequence, PKKPAGER. Initial results from the T2 generation of plants transformed with this construct showed a reduced binding to CBF1 full-length probe indicating a reduction in *CBF 1, 2*, and *3* levels (Figure A3). However, when homozygous offspring from the lines which showed the lowest levels were analyzed for *CBF* mRNA levels there was a distribution of reduction similar to that seen in the original lines indicating that this reduction was not stable.

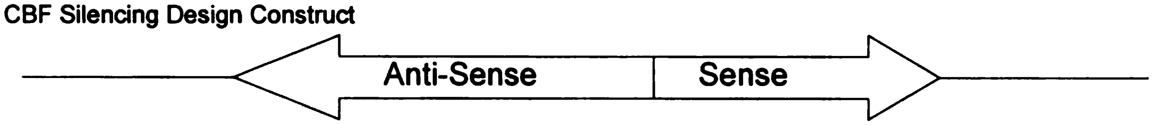


Figure A2 Design of CBF Silencing Contruct.

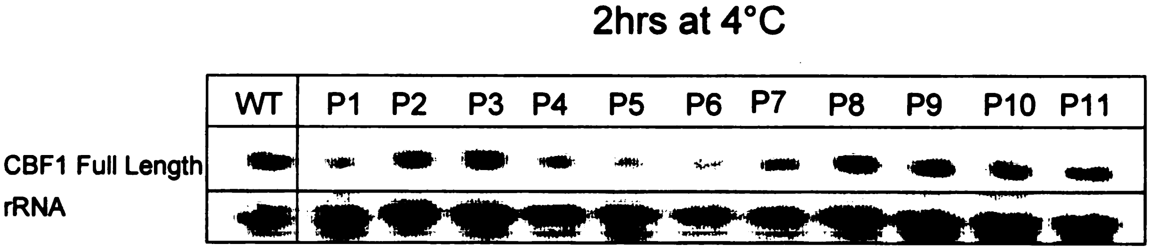


Figure A3 CBF-Targeting RNAi Silencing Construct Produced Lines with Reduced CBF Levels.
Northern blot analysis of CBF levels after treatment for 2h at 4°C in T2 plants containing the CBF silencing construct. Wild-type (WT) plants were used as a control for comparisons of lines P1 through P11. rRNA was probed as a loading control.

TRP Channels

Members of the Transient Receptor Potential (TRP) family of calcium ion channel proteins have been shown to be involved in sensing cold and heat in mammals ((McKemy et al. 2002). There are four homologs of these channels in Arabidopsis, TC1-4. Dr. Richard Amasino provided homozygous T-DNA insertion lines for each of these TC channels for analysis of CBF induction in these channels in response to low temperature treatment. There was no significant difference in the early cold responsive transcripts levels of *CBF* or *ZAT12* in these lines in response to low temperature compared with wild type plants. However, there was a reduction in two downstream cold-responsive genes, both *COR15* and *COR78* mRNA did not reach the cold-induced level of wild type plants in the *–tc1* and *–tc2* T-DNA insertion lines (Figure A4). Not all late-cold-induced transcript levels were affected in *–tc1* and *–tc2* plants, however. Phenyl ammonia lyase (PAL2) was present at levels comparable to wild type in both *-tc1* and *–tc2* plants (Figure A4). The analysis on CBF mRNA levels was performed using a full length CBF probe which measures levels of CBF 1, 2 and 3. Analysis with probes specific for each CBF might provide additional information since we now know that there are differences in regulation of each of these three cold-responsive CBFs.

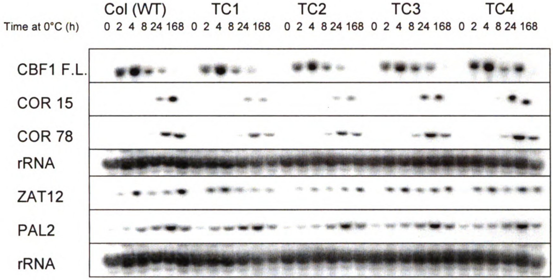


Figure A4 Cold-Induced Transcript Levels in –*tc* T-DNA Insertion Lines
Northern blot analysis of mRNA levels for cold-responsive transcripts in Columbia (Col), -*tc1* (TC1), -*tc2* (TC2), -*tc3* (TC3), -*tc4* (TC4) plants. Probes were for *CBF1* full length (CBF1 F.L.), *COR15, COR78, ZAT12, PAL2*. *rRNA* was probed as a loading control.

Phenotypes of CBF Overexpressing Plant Crossed to CBF2ΔC

CBF2 overexpressing plants crossed to CBF2ΔC plants produced T3 offspring that were near WT size however showed a thickening of the leaves and stem compared to WT. Plants that contained both constructs showed an earlier flowering time and recovery of WT size compared to CBF2 overexpressing plants. However, since both constructs were in the same vector and the sequences only differed by one base pair, it was difficult to identify offspring homozygous for both CBF2 and CBF2ΔC constructs.

Phenotypes of CBF Overexpressing Plants Crossed to ZAT12 Overexpressing Plants

CBF overexpressing plants crossed to ZAT12 overexpressing plants showed an interesting phenotype. While both CBF overexpressors and ZAT12 overexpressors show a dwarf phenotype plants containing at least one copy of each construct showed a recovery of WT size. The plants also had a phenotype in flower development with downturned buds (Figure A5). This phenotype was consistent across crosses with several different ZAT12 overexpressing lines.

Figure A5 Flower Phenotype of CBF Overexpressing Plants Crossed to ZAT12 Overexpressing Plants.
Plants containing at least one copy of both the CBF overexpression construct (35s:CBF2) and one copy of the ZAT12 overexpression construct (35s:ZAT12) showed a downturned flower phenotype indicated by arrow. Siliques remain downturned as indicated by dashed arrow.

Other CAMTA Phenotypes

CAMTA3 overexpressing plants are early flowering.

camta1/3 double mutants are chlorotic, have lower FV/FM in both warm and low temperature and show early senescence of leaves.

COR Protein Levels in CBF2ΔC Plants

The protein levels of two CBF target genes COR15 and COR78 were analyzed in CBF2ΔC expressing plants. These plants showed a dramatic decrease in both COR15 levels (Figure A6) and COR78 levels.

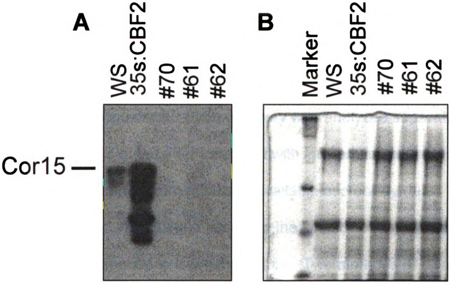


Figure A6 COR15 Protein Levels in CBF2ΔC Plants.
(A) Western blot analysis of COR15 in plants treated for 7d at 4°C. Multiple bands in 35s:CBF2 are possibly due to cross-reactivity with other COR genes or breakdown products of COR15.
(B) Comassie stain of samples in (A) for loading comparison

Analysis of CBF2ΔC Transcript Categories for Novel Cis-Elements

The categories of transcripts based on CBF dependence identified in the analysis of CBF2ΔC plants (Chapter 3) were tested for the overrepresentation of novel cis-elements in the promoters of each group of transcripts. The analysis was performed with Motif Sampler and Weeder. Overrepresentation of the known elements, CRT, CG-1, TBX, and ABRE was identified by both programs. However, no novel element was consistently picked in any category of transcripts by both programs.

Kinetic Analysis of Known Cis-Elements Identified in CBF2ΔC Plants

The known cis-elements identified as being overrepresented in each category of transcripts based on CBF dependence (Chapter 3) were analyzed for their potential role in the different kinetic responses to low temperature of the cold-regulated transcripts. Each category was analyzed separately. The expression levels of the after treatment with low temperature for different periods of time were obtained from publically available microarray data. Each category was then divided into clusters based on the kinetics of the induction of its transcripts in response to low temperature. Two methods of clustering were used, K-means and the pattern-based method of Haystack. These sub-clusters were then analyzed for the overrepresentation of known motifs. For the motifs analyzed, ABRE-like, CG-1, CRT, and TBX only CG-1 showed significant enrichment in any kinetic category. As discussed in Chapter 2, CG-1 was enriched in those transcripts with an early response to low temperature.

LC-MS Analysis of CBF2ΔC Plants

Initial attempts at characterizing the affects of the CBF2ΔC construct on the metabolite profile in response to low temperature involved using LC-MS analysis of metabolites. This analysis identified some metabolites that were significantly difference between CBF2ΔC and wild-type (WT) samples after treatment at low temperature. However, the majority of metabolites did not show a significant difference between WT and CBF2ΔC. There was also no detectable difference in the majority of metabolites between CBF2 overexpressing plants and WT plants in the warm as was previously seen by Cook et al. (Cook et al. 2004). This discrepancy is most likely due to the fact that many of the metabolite differences detected were in polar metabolites, detectable by the GC-MS method used by Cook et al. These polar metabolites are possibly excluded by the LC/MS method employed. Therefore, I switched to GC/MS analysis and identification of these metabolites was not pursued.

Identification of CBF2 T-DNA Insertion Lines with Reduced Expression of *CBF2* mRNA

Isolating knock-out plants for each of the three cold-inducible CBFs would provide a tool for understanding the function of each CBF and the possible redundancy and specificity of the three proteins. In collaboration with Ryan Sartor, we attempted to identify T-DNA insertion lines affecting the CBF 1, 2, or 3 genes. However, the position of the T-DNA insertion in all publically available lines in the CBF locus is limited to the promoter regions of the three CBFS.

Therefore, to determine if plants that were homozygous for the T-DNA insertion had significantly reduced mRNA levels we extracted RNA and performed semi-quantitative RT PCR with primers specific for each CBF. The two T-DNA insertion lines in the promoters of CBF2, CBF2c P7 and CBF2a P23, showed almost no accumulation of *CBF2* mRNA in response to low temperature. These were the only two lines with any affect on the expression levels of *CBF1, 2* or *3.* The two CBF2 knock-down lines were analyzed for their affect on the other CBF transcripts and downstream target genes. No dramatic differences, as detectable by semi-quantitative RT PCR, were seen. However, quantitative differences could not be determined by this method.

CM2/CM1 Mutant Screen

In collaboration with Megan Sargent a population of plants containing the CM2/CM1:GUS reporter construct were mutagenized with ethyl methane-sulphonate (EMS). The plants were screened for mutants that no longer stained for GUS after exposure to low temperature. Megan identified two lines with reduced staining, L-3 and S-14. Line S-14 still showed GUS staining in the warm although at a lower level than the parental plants and showed almost no GUS staining after treatment at low temperature. L-3 had normal staining in the warm, and after low temperature treatment had a blotchy staining pattern.

Table A.1 1279 Transcripts Differentially Regulated in Response to Low Temperature at 24h in Both WS and Col.

Probe Set	AGI	Description
261572_at	AT1G01170	ozone-responsive stress-related protein
261048_at	AT1G01420	UDP-glucoronosyl/UDP-glucosyl transferase family protein
261049_at	AT1G01430	similar to unknown protein
259426_at	AT1G01470	Encodes late-embryogenesis abundant protein
259436_at	AT1G01500	similar to unknown protein
259431_at	AT1G01620	a member of the plasma membrane intrinsic protein subfamily PIP1.
261558_at	AT1G01770	similar to Protein of unknown function DUF1446
261655_at	AT1G01940	peptidyl-prolyl cis-trans isomerase cyclophilin-type family protein
264123_at	AT1G02270	endonuclease/exonuclease/phosphatase family protein
259442_at	AT1G02310	glycosyl hydrolase family protein 5 / cellulase family protein
259444_at	AT1G02370	pentatricopeptide (PPR) repeat-containing protein
260903_at	AT1G02460	glycoside hydrolase family 28 protein / polygalacturonase (pectinase) family protein
260914_at	AT1G02640	encodes a protein similar to a beta-xylosidase located in the extracellular matrix.
262113_at	AT1G02820	late embryogenesis abundant 3 family protein / LEA3 family protein
262112_at	AT1G02870	similar to hypothetical protein MtrDRAFT_AC161032g9v1
263118_at	AT1G03090	MCCA is the biotinylated subunit of the dimer MCCase
263114_at	AT1G03130	Encodes a protein predicted by sequence similarity with spinach PsaD
264357_at	AT1G03360	ATRRP4; exonuclease; similar to Os04g0520000
264843_at	AT1G03400	A single copy gene that encodes a protein with sequence similarity to tomato E8
264818_at	AT1G03530	similar to unknown protein
265066_at	AT1G03870	fasciclin-like arabinogalactan-protein 9 (Fla9)
265093_at	AT1G03905	ABC transporter family protein; Identical to Non-intrinsic ABC protein 4
263656_at	AT1G04240	SHY2/IAA3 regulates multiple auxin responses in roots.
263668_at	AT1G04350	encodes a protein whose sequence is similar to 2-oxoglutarate-dependent dioxygenase
261130_at	AT1G04870	protein arginine N-methyltransferase family protein
261168_at	AT1G04945	similar to Os01g0962500
264583_at	AT1G05170	similar to galactosyltransferase family protein
263231_at	AT1G05680	UDP-glucoronosyl/UDP-glucosyl transferase family protein
263179_at	AT1G05710	ethylene-responsive protein
260955_at	AT1G06000	encodes a flavonol-7-O-rhamnosyltransferase
259392_at	AT1G06380	ribosomal protein-related
260824_at	AT1G06720	similar to unknown protein
260832_at	AT1G06780	Encodes a protein with putative galacturonosyltransferase activity.
256065_at	AT1G07070	60S ribosomal protein L35a (RPL35aA); Identical to 60S ribosomal protein L35a-1
261081_at	AT1G07350	transformer serine/arginine-rich ribonucleoprotein
261077_at	AT1G07430	protein phosphatase 2C
261084_at	AT1G07440	tropinone reductase
264803_at	AT1G08580	similar to Pm52
264806_at	AT1G08610	pentatricopeptide (PPR) repeat-containing protein
264652_at	AT1G08920	sugar transporter

264656_at	AT1G09010	glycoside hydrolase family 2 protein
264261_at	AT1G09240	nicotianamine synthase
264511_at	AT1G09350	ATGOLS3 (ARABIDOPSIS THALIANA GALACTINOL SYNTHASE 3)
264501_at	AT1G09390	GDSL-motif lipase/hydrolase family protein
264672_at	AT1G09750	chloroplast nucleoid DNA-binding protein-related
264668_at	AT1G09780	2,3-biphosphoglycerate-independent phosphoglycerate mutase, putative
264515_at	AT1G10090	similar to RXW8
264452_at	AT1G10270	GRP23 (GLUTAMINE-RICH PROTEIN23)
264435_at	AT1G10360	Encodes glutathione transferase belonging to the tau class of GSTs.
264436_at	AT1G10370	ATGSTU17/ERD9/GST30/GST30B (EARLY-RESPONSIVE TO DEHYDRATION 9
264458_at	AT1G10410	similar to CW14
262784_at	AT1G10760	SEX1
260481_at	AT1G10960	ATFD1 (FERREDOXIN 1); electron carrier/ iron ion binding
262452_at	AT1G11210	similar to unknown protein
261818_at	AT1G11390	ABC1 family protein; similar to ABC1 family protein
260969_at	AT1G12240	ATBETAFRUCT4/VAC-INV (VACUOLAR INVERTASE)
259537_at	AT1G12370	significant homology to the recently characterized type II photolyases
261211_at	AT1G12780	Encodes a UDP-glucose epimerase
262777_at	AT1G13030	sphere organelles protein-related; similar to unknown protein
262766_at	AT1G13160	SDA1 family protein; similar to SDA1 family protein
259364_at	AT1G13260	Encodes an AP2/B3 domain transcription factor
256096_at	AT1G13650	similar to 18S pre-ribosomal assembly protein gar2-related
256098_at	AT1G13700	glucosamine/galactosamine-6-phosphate isomerase family protein
262611_at	AT1G14060	similar to unknown protein
261485_at	AT1G14360	ATUTR3/UTR3 (UDP-GALACTOSE TRANSPORTER 3)
262830_at	AT1G14700	ATPAP3/PAP3 (purple acid phosphatase 3)
262840_at	AT1G14900	Encodes a protein belonging to the subgroup of HMGA (high mobility group A) proteins
262544_at	AT1G15420	similar to transducin family protein / WD-40 repeat family protein
262584_at	AT1G15440	transducin family protein / WD-40 repeat family protein
259500_at	AT1G15740	leucine-rich repeat family protein; similar to leucine-rich repeat family protein
261845_at	AT1G15960	member of Nramp2 family
262706_at	AT1G16280	DEAD/DEAH box helicase
262703_at	AT1G16510	auxin-responsive family protein
255764_at	AT1G16720	oxidoreductase/ transcriptional repressor; similar to transcriptional repressor
256114_at	AT1G16850	similar to unknown protein
262518_at	AT1G17170	Encodes glutathione transferase belonging to the tau class of GSTs.
262517_at	AT1G17180	Encodes glutathione transferase belonging to the tau class of GSTs.
262539_at	AT1G17200	integral membrane family protein; similar to integral membrane family protein
261060_at	AT1G17340	phosphoinositide phosphatase family protein
259398_at	AT1G17700	prenylated rab acceptor (PRA1) family protein
261661_at	AT1G18360	hydrolase

255774_at	AT1G18620	similar to unknown protein
255779_at	AT1G18650	glycosyl hydrolase family protein 17; similar to glycosyl hydrolase family protein 17
261377_at	AT1G18850	similar to Os01g0112100
261428_at	AT1G18870	Encodes a protein with isochorismate synthase activity
259461_at	AT1G18900	pentatricopeptide (PPR) repeat-containing protein
259476_at	AT1G19000	myb family transcription factor; similar to myb family transcription factor
256015_at	AT1G19150	PSI type II chlorophyll a/b-binding protein (Lhca2*1) mRNA
256017_at	AT1G19180	similar to unknown protein
260674_at	AT1G19370	similar to unknown protein
260668_at	AT1G19530	unknown protein
255786_at	AT1G19670	Chlorophyllase is the first enzyme involved in chlorophyll degradation.
261143_at	AT1G19770	Member of a family of proteins related to PUP1
261248_at	AT1G20030	pathogenesis-related thaumatin family protein
259570_at	AT1G20440	Belongs to the dehydrin protein family
259516_at	AT1G20450	Encodes a gene induced by low temperature and dehydration.
256091_at	AT1G20693	Encodes a protein belonging to the subgroup of HMGB (high mobility group B) proteins
259560_at	AT1G21270	cytoplasmic serine/threonine protein kinase
260876_at	AT1G21460	nodulin MtN3 family protein; similar to nodulin MtN3 family protein
260877_at	AT1G21500	similar to conserved hypothetical protein
260921_at	AT1G21540	AMP-binding protein
262503_at	AT1G21670	similar to unknown protein
262496_at	AT1G21790	similar to Os01g0869600
255968_at	AT1G22270	Identical to TRM112-like protein At1g22270
255962_at	AT1G22335	similar to RNA recognition motif (RRM)-containing protein
264211_at	AT1G22770	GI
264774_at	AT1G22890	similar to unknown protein
264751_at	AT1G23020	Encodes a ferric chelate reductase whose transcription is regulated by FIT1.
264895_at	AT1G23100	10 kDa chaperonin
264893_at	AT1G23140	C2 domain-containing protein; similar to C2 domain-containing protein
262986_at	AT1G23390	kelch repeat-containing F-box family protein; similar to F-box family protein
263014_at	AT1G23400	ATCAF2/CAF2 (ARABIDOPSIS THALIANA HOMOLOG OF MAIZE CAF2)
263016_at	AT1G23410	ubiquitin extension protein
265175_at	AT1G23480	encodes a gene similar to cellulose synthase
265188_at	AT1G23800	Encodes a mitochondrial aldehyde dehydrogenase
263035_at	AT1G23860	Encodes a 9G8-like serine-arginine rich (SR) protein that interacts in vivo with U1-70K
263019_at	AT1G23870	Encodes an enzyme putatively involved in trehalose biosynthesis.
264864_at	AT1G24310	similar to transporter
245637_at	AT1G25230	purple acid phosphatase family protein
245639_at	AT1G25260	acidic ribosomal protein P0-related; similar to 60S acidic ribosomal protein P0 (RPP0C)
245845_at	AT1G26150	protein kinase; similar to protein kinase family protein
261011_at	AT1G26340	member of Cytochromes b5

261276_at	AT1G26670	member of VTI1 Gene Family. les.
261263_at	AT1G26790	Dof-type zinc finger domain-containing protein
264989_at	AT1G27200	similar to zinc finger (C3HC4-type RING finger) family protein
264990_at	AT1G27210	binding; similar to binding [Arabidopsis thaliana] (TAIR:AT1G59850.1)
264445_at	AT1G27290	similar to conserved hypothetical protein
262296_at	AT1G27630	cyclin family protein; similar to cyclin family protein
261648_at	AT1G27730	Related to Cys2/His2-type zinc-finger proteins found in higher plants.
261651_at	AT1G27760	interferon-related developmental regulator family protein
259588_at	AT1G27930	similar to unknown protein
259582_at	AT1G28060	small nuclear ribonucleoprotein family protein / snRNP family protein
245668_at	AT1G28330	dormancy-associated protein (DRM1)
261500_at	AT1G28400	similar to unknown protein
259789_at	AT1G29395	encodes a protein similar to the cold acclimation protein WCOR413 in wheat.
259773_at	AT1G29500	auxin-responsive protein
260049_at	AT1G29940	Encodes a subunit of RNA polymerase 1 (aka RNA polymerase A).
245770_at	AT1G30240	binding; similar to unnamed protein product
261801_at	AT1G30520	acyl-activating enzyme 14 (AAE14)
263221_at	AT1G30620	encodes a type-II membrane protein that catalyzes 4-epimerization of UDP-D-Xylose to UDP-L-Arabinose in vitro
256497_at	AT1G31580	Encodes cell wall protein. ECS1 is not a Xcc750 resistance gene
246575_at	AT1G31660	similar to Os09g0352400
260653_at	AT1G32440	pyruvate kinase
261711_at	AT1G32700	zinc-binding family protein; similar to zinc-binding family protein
261187_at	AT1G32860	glycosyl hydrolase family 17 protein; Identical to Putative glucan endo-1
261192_at	AT1G32870	ANAC013 (Arabidopsis NAC domain containing protein 13)
261566_at	AT1G33230	similar to unknown protein
261594_at	AT1G33240	GTL1
261981_at	AT1G33811	GDSL-motif lipase/hydrolase family protein
259577_at	AT1G35340	ATP-dependent protease La (LON) domain-containing protein
260869_at	AT1G43800	acyl-(acyl-carrier-protein) desaturase
260868_at	AT1G43860	transcription factor; similar to Shwachman-Bodian-Diamond syndrome
260870_at	AT1G43890	ras-related small GTPase
245803_at	AT1G47128	cysteine proteinase (RD21A) / thiol protease
262440_at	AT1G47710	serpin
260727_at	AT1G48100	glycoside hydrolase family 28 protein / polygalacturonase (pectinase) family protein
262248_at	AT1G48370	Arabidopsis thaliana metal-nicotianamine transporter YSL4
261294_at	AT1G48430	dihydroxyacetone kinase family protein; similar to dihydroxyacetone kinase family protein
261308_at	AT1G48480	Arabidopsis thaliana receptor-like protein kinase (RKL1) gene
261301_at	AT1G48570	zinc finger (Ran-binding) family protein; similar to zinc finger (Ran-binding) family protein
246623_at	AT1G48920	The predominant form of the two nucleolin genes in Arabidopsis.
260769_at	AT1G49010	myb family transcription factor

260770_at	AT1G49200	zinc finger (C3HC4-type RING finger) family protein; Identical to ATL1I (ATL1I)
262448_at	AT1G49450	transducin family protein / WD-40 repeat family protein
262400_at	AT1G49480	RTV1 (RELATED TO VERNALIZATION1 1)
261610_at	AT1G49560	myb family transcription factor; similar to myb family transcription factor
261613_at	AT1G49720	Identified as a protein that binds to abscisic acid response elements.
261611_at	AT1G49730	protein kinase family protein; similar to protein kinase family protein
261638_at	AT1G49975	similar to expressed protein
245749_at	AT1G51090	heavy-metal-associated domain-containing protein; similar to metal ion binding
265142_at	AT1G51360	similar to unknown protein
265147_at	AT1G51380	eukaryotic translation initiation factor 4A
265149_at	AT1G51400	photosystem II 5 kD protein
260489_at	AT1G51610	cation efflux family protein / metal tolerance protein
259839_at	AT1G52190	proton-dependent oligopeptide transport (POT) family protein
262159_at	AT1G52720	similar to unknown protein
260203_at	AT1G52890	encodes a NAC transcription factor whose expression is induced by drought
260157_at	AT1G52930	brix domain-containing protein; similar to brix domain-containing protein
261318_at	AT1G53035	similar to unknown protein
260614_at	AT1G53390	ATPase
260983_at	AT1G53560	similar to unknown protein
260984_at	AT1G53645	hydroxyproline-rich glycoprotein family protein; similar to actin binding
262956_at	AT1G54270	member of eIF4A - eukaryotic initiation factor 4A
262964_at	AT1G54380	spliceosome protein-related; similar to spliceosome protein-related
264186_at	AT1G54570	esterase/lipase/thioesterase family protein
264191_at	AT1G54730	sugar transporter
264238_at	AT1G54740	similar to unknown protein
264240_at	AT1G54820	protein kinase family protein; similar to protein kinase family protein
256353_at	AT1G55000	peptidoglycan-binding LysM domain-containing protein
259659_at	AT1G55170	similar to unknown protein
259666_at	AT1G55310	Encodes a SR spliceosome protein that is localized to nuclear specks
259664_at	AT1G55330	Encodes a putative arabinogalactan-protein (AGP21).
257510_at	AT1G55360	similar to unknown protein
265078_at	AT1G55500	similar to ECT2
264535_at	AT1G55690	SEC14 cytosolic factor family protein / phosphoglyceride transfer family protein
260592_at	AT1G55850	encodes a protein similar to cellulose synthase
260596_at	AT1G55900	component of a translocase in the mitochondrial inner membrane
260603_at	AT1G55960	similar to unknown protein
262094_at	AT1G56110	NOP56-like protein
262098_at	AT1G56170	Encodes a protein with similarity to a subunit of the CCAAT promoter motif binding complex
256225_at	AT1G56220	dormancy/auxin associated family protein

256221_at	AT1G56300	DNAJ heat shock N-terminal domain-containing protein
245628_at	AT1G56650	Encodes a putative MYB domain containing transcription factor
245868_at	AT1G58030	Arabidopsis thaliana amino acid permease family protein (At1g58030)
246396_at	AT1G58180	carbonic anhydrase family protein / carbonate dehydratase family protein
256022_at	AT1G58360	neutral amino acid transporter expressed in seeds
262076_at	AT1G59580	encodes a mitogen-activated kinase involved in innate immunity
263679_at	AT1G59990	DEAD/DEAH box helicase
264217_at	AT1G60190	armadillo/beta-catenin repeat family protein / U-box domain-containing protein
264940_at	AT1G60470	ATGOLS4 (ARABIDOPSIS THALIANA GALACTINOL SYNTHASE 4)
264920_at	AT1G60550	naphthoate synthase
264963_at	AT1G60600	Encodes a protein similar to 1
259908_at	AT1G60850	ATRPAC42 (Arabidopsis thaliana RNA polymerase I subunit 42)
259721_at	AT1G60890	phosphatidylinositol-4-phosphate 5-kinase family protein
265061_at	AT1G61640	ABC1 family protein
264398_at	AT1G61730	DNA-binding storekeeper protein-related; similar to transcription regulator
264400_at	AT1G61800	glucose6-Phosphate/phosphate transporter 2
264289_at	AT1G61890	MATE efflux family protein; similar to MATE efflux family protein
264736_at	AT1G62200	proton-dependent oligopeptide transport (POT) family protein
265119_at	AT1G62570	flavin-containing monooxygenase family protein / FMO family protein
262644_at	AT1G62710	Encodes a vacuolar processing enzyme
262691_at	AT1G62740	stress-inducible protein
259690_at	AT1G63160	replication factor C 40 kDa
260109_at	AT1G63260	Member of TETRASPANIN family
260323_at	AT1G63780	Small nucleolar ribonucleoprotein protein involved in ribosomal RNA processing.
260317_at	AT1G63800	UBC5 (ubiquitin-conjugating enzyme 37); ubiquitin-protein ligase
260316_at	AT1G63810	similar to Nrap protein
262354_at	AT1G64200	VHA-E3 (VACUOLAR H+-ATPASE SUBUNIT E ISOFORM 3)
261956_at	AT1G64590	short-chain dehydrogenase/reductase (SDR) family protein
261972_at	AT1G64600	similar to unknown protein
261949_at	AT1G64670	BDG1
262884_at	AT1G64720	membrane related protein CP5
262881_at	AT1G64890	integral membrane transporter family protein;
263142_at	AT1G65230	similar to hypothetical protein MtrDRAFT_AC148171g2v1
262930_at	AT1G65690	NDR1/HIN1-LIKE 25
260140_at	AT1G66390	production of anthocyanin pigment 2 protein (PAP2)
255852_at	AT1G66970	glycerophosphoryl diester phosphodiesterase family protein
255857_at	AT1G67080	Involved in the photoprotection of PSII.
264471_at	AT1G67120	midasin-related; similar to unknown protein
264992_at	AT1G67300	hexose transporter
264968_at	AT1G67360	rubber elongation factor (REF) family protein
264229_at	AT1G67480	kelch repeat-containing F-box family protein
245196_at	AT1G67750	pectate lyase family protein; Identical to Probable pectate lyase 5 precursor
245200_at	AT1G67850	similar to unknown protein

259996_at	AT1G67910	similar to unknown protein
260014_at	AT1G68010	Encodes hydroxypyruvate reductase.
260431_at	AT1G68190	zinc finger (B-box type) family protein; Identical to Putative zinc finger protein At1g68190
260262_at	AT1G68470	exostosin family protein; similar to MUR3 (MURUS 3)
260264_at	AT1G68500	unknown protein
260266_at	AT1G68520	zinc finger (B-box type) family protein; Identical to CONSTANS-LIKE 6 (COL6)
262281_at	AT1G68570	proton-dependent oligopeptide transport (POT) family protein
262286_at	AT1G68585	metal ion binding; contains InterPro domain Heavy metal transport/detoxification protein
260037_at	AT1G68840	RAV2
259672_at	AT1G68990	DNA-directed RNA polymerase
260338_at	AT1G69250	nuclear transport factor 2 (NTF2) family protein
260357_at	AT1G69260	similar to unknown protein
260360_at	AT1G69370	Encodes chorismate mutase 3 (CM3).
256299_at	AT1G69530	Member of Alpha-Expansin Gene Family.
260410_at	AT1G69870	proton-dependent oligopeptide transport (POT) family protein
264700_at	AT1G70100	similar to unknown protein
264697_at	AT1G70210	Encodes a D-type cyclin that physically interacts with CDC2A.
264339_at	AT1G70290	Encodes an enzyme putatively involved in trehalose biosynthesis.
262288_at	AT1G70760	inorganic carbon transport protein-related; similar to NADH dehydrogenase subunit
262313_at	AT1G70900	similar to unknown protein
261516_at	AT1G71750	phosphoribosyltransferase family protein
257487_at	AT1G71850	similar to unknown protein
260167_at	AT1G71970	similar to unknown protein
259803_at	AT1G72150	novel cell-plate-associated protein
260398_at	AT1G72320	APUM23 (ARABIDOPSIS PUMILIO 23)
260427_at	AT1G72430	auxin-responsive protein-related
260425_at	AT1G72440	EDA25 (embryo sac development arrest 25); similar to unknown protein
259891_at	AT1G72730	eukaryotic translation initiation factor 4A
245734_at	AT1G73480	hydrolase
260076_at	AT1G73630	calcium-binding protein
260075_at	AT1G73700	MATE efflux family protein; similar to MATE efflux family protein
260070_at	AT1G73830	BEE3 (BR ENHANCED EXPRESSION 3)
260388_at	AT1G74070	peptidyl-prolyl cis-trans isomerase cyclophilin-type family protein
260221_at	AT1G74670	gibberellin-responsive protein
259953_at	AT1G74810	anion exchange family protein; Identical to Putative boron transporter-like protein 3
259925_at	AT1G75040	Thaumatin-like protein involved in response to pathogens.
259927_at	AT1G75100	Contains a J-domain at the C-terminus which is similar to the J-domain of auxilin
259954_at	AT1G75130	member of CYP721A
256458_at	AT1G75220	integral membrane protein
261116_at	AT1G75370	SEC14 cytosolic factor
261114_at	AT1G75390	bZIP transcription factor family protein
261118_at	AT1G75460	ATP-dependent protease La (LON) domain-containing protein
262953_at	AT1G75670	similar to RNA polymerase Rpa43 subunit-like

262677_at	AT1G75860	similar to unknown protein
262682_at	AT1G75900	family II extracellular lipase 3 (EXL3); similar to family II extracellular lipase 1 (EXL1)
261751_at	AT1G76080	ATCDSP32/CDSP32
261769_at	AT1G76100	plastocyanin
261782_at	AT1G76110	high mobility group (HMG1/2) family protein
261726_at	AT1G76270	similar to unknown protein
259971_at	AT1G76580	transcription factor; Identical to (SPL1-related protein 3) (SPL16)
259977_at	AT1G76590	zinc-binding family protein; similar to zinc-binding family protein
256332_at	AT1G76890	encodes a plant trihelix DNA-binding protein
264953_at	AT1G77120	Catalyzes the reduction of acetaldehyde using NADH as reductant.
264482_at	AT1G77210	sugar transporter
259708_at	AT1G77420	hydrolase
259711_at	AT1G77570	DNA binding / transcription factor; similar to AT-HSFA5
259713_at	AT1G77610	glucose-6-phosphate/phosphate translocator-related
262136_at	AT1G77850	Posttranscriptionally regulated by miR160 and is essential for proper development.
262164_at	AT1G78070	WD-40 repeat family protein; similar to WD-40 repeat family protein
263127_at	AT1G78610	mechanosensitive ion channel domain-containing protein
264118_at	AT1G79150	binding; similar to unknown protein
264131_at	AT1G79150	binding; similar to unknown protein
262892_at	AT1G79440	Encodes a mitochondrial succinic semialdehyde dehydrogenase (SSADH).
262943_at	AT1G79470	inosine-5'-monophosphate dehydrogenase
262941_at	AT1G79490	EMB2217 (EMBRYO DEFECTIVE 2217)
262039_at	AT1G80050	Encodes an adenosine phosphoribosyl transferase(E.C:2.4.2.7)
262061_at	AT1G80110	ATPP2-B11
262050_at	AT1G80130	binding; similar to unknown protein
262049_at	AT1G80180	similar to unknown protein
260331_at	AT1G80270	DNA-binding protein
260287_at	AT1G80440	kelch repeat-containing F-box family protein
261911_at	AT1G80750	60S ribosomal protein L7 (RPL7A); Identical to 60S ribosomal protein L7-1 (RPL7A)
261887_at	AT1G80780	CCR4-NOT transcription complex protein
257474_at	AT1G80850	methyladenine glycosylase family protein
261901_at	AT1G80920	J8 mRNA
266300_at	AT2G01420	Encodes a putative auxin efflux carrier
266348_at	AT2G01450	member of MAP Kinase
266119_at	AT2G02100	LCR69/PDF2.2 (Low-molecular-weight cysteine-rich 69); protease inhibitor;+C494
266141_at	AT2G02120	LCR70/PDF2.1 (Low-molecular-weight cysteine-rich 70); protease inhibitor
266707_at	AT2G03310	unknown protein
265699_at	AT2G03550	similar to unknown protein
264027_at	AT2G03670	CDC48 - like protein AAA-type ATPase
264037_at	AT2G03750	sulfotransferase family protein; similar to sulfotransferase family protein
264042_at	AT2G03760	High similarity to flavonol sulfotransferases (FSTs).
263334_at	AT2G03820	nonsense-mediated mRNA decay NMD3 family protein

263811_at	AT2G04350	long-chain-fatty-acid--CoA ligase family protein
263647_at	AT2G04690	cellular repressor of E1A-stimulated genes (CREG) family
263674_at	AT2G04790	similar to conserved hypothetical protein
265511_at	AT2G05540	glycine-rich protein; similar to glycine-rich protein
263391_at	AT2G11810	MGDG synthase type C
257444_at	AT2G12550	ubiquitin-associated (UBA)/TS-N domain-containing protein; similar to H0413E07.5
266614_at	AT2G14910	similar to unknown protein
265481_at	AT2G15960	unknown protein
265480_at	AT2G15970	encodes an alpha form of a protein similar to WCOR413 in wheat.
263239_at	AT2G16570	Amidophosphoribosyltransferase (ATase: EC 2.4.2.14)
265354_at	AT2G16700	Encodes actin depolymerizing factor 5 (ADF5).
266532_at	AT2G16890	UDP-glucoronosyl/UDP-glucosyl transferase family protein; similar to transferase
263574_at	AT2G16990	tetracycline transporter; similar to unknown protein
263415_at	AT2G17250	similar to Os04g0585300
264906_at	AT2G17270	mitochondrial substrate carrier family protein; similar to mitochondrial phosphate transporter
264907_at	AT2G17280	phosphoglycerate/bisphosphoglycerate mutase family protein
264591_at	AT2G17670	pentatricopeptide (PPR) repeat-containing protein
264590_at	AT2G17710	similar to Os04g0560700
264787_at	AT2G17840	Identified as drought-inducible gene by differential hybridization.
265326_at	AT2G18220	Identical to Nucleolar complex protein 2 homolog (Protein NOC2 homolog)
265342_at	AT2G18300	basic helix-loop-helix (bHLH) family protein
265931_at	AT2G18520	pentatricopeptide (PPR) repeat-containing protein
266072_at	AT2G18700	Encodes an enzyme putatively involved in trehalose biosynthesis.
266934_at	AT2G18900	transducin family protein / WD-40 repeat family protein
267436_at	AT2G19190	Receptor-like protein kinase. Involved in early defense signaling.
267336_at	AT2G19310	similar to HSP18.2 (HEAT SHOCK PROTEIN 18.2)
267309_at	AT2G19385	nucleic acid binding / zinc ion binding
267280_at	AT2G19450	Encodes Acyl-CoA:diacylglycerol acyltransferase (DGAT)
265947_at	AT2G19540	transducin family protein / WD-40 repeat family protein
265935_at	AT2G19580	Member of TETRASPANIN family
266693_at	AT2G19800	Encodes a myo-inositol oxygenase family gene.
266695_at	AT2G19810	zinc finger (CCCH-type) family protein; similar to zinc finger (CCCH-type) family protein
265584_at	AT2G20180	Member of the basic helix-loop-helix (bHLH) family of transcription factors.
263712_at	AT2G20585	NFD6 (NUCLEAR FUSION DEFECTIVE 6); similar to unknown protein
265394_at	AT2G20725	CAAX amino terminal protease family protein
264019_at	AT2G21130	peptidyl-prolyl cis-trans isomerase / cyclophilin (CYP2) / rotamase
264024_at	AT2G21180	similar to unknown protein
264014_at	AT2G21210	Putative auxin-regulated protein whose expression is downregulated in response to chitin oligomers.
263739_at	AT2G21320	zinc finger (B-box type) family protein; similar to zinc finger (B-box type) family protein
263544_at	AT2G21590	Encodes the large subunit of ADP-glucose pyrophosphorylase

263517_at	AT2G21620	Encodes gene that is induced in response to dessication
263875_at	AT2G21970	stress enhanced protein 2 (SEP2) chlorophyll a/b-binding protein
263352_at	AT2G22080	similar to zinc finger protein-related
263452_at	AT2G22190	trehalose-phosphatase; similar to trehalose-6-phosphate phosphatase
264052_at	AT2G22330	Encodes a cytochrome P450.
264001_at	AT2G22420	peroxidase 17 (PER17) (P17)
264000_at	AT2G22500	mitochondrial substrate carrier family protein
265290_at	AT2G22590	glycosyltransferase family protein
267265_at	AT2G22980	SCPL13; serine carboxypeptidase; similar to SCPL7
267262_at	AT2G22990	sinapoylglucose:malate sinapoyltransferase.
267261_at	AT2G23120	similar to unknown protein
267266_at	AT2G23150	Encodes a member of the Nramp2 metal transporter family
245078_at	AT2G23340	encodes a member of the DREB subfamily A-5 of ERF/AP2 transcription factor family.
266578_at	AT2G23910	cinnamoyl-CoA reductase-related; similar to cinnamoyl-CoA reductase-related
266567_at	AT2G24050	MIF4G domain-containing protein / MA3 domain-containing protein
265999_at	AT2G24100	similar to unknown protein
266001_at	AT2G24150	heptahelical transmembrane protein HHP3
265990_at	AT2G24280	serine carboxypeptidase S28 family protein
265662_at	AT2G24500	Encodes a C2H2 zinc finger protein FZF.
263799_at	AT2G24550	similar to unknown protein
257435_at	AT2G24590	splicing factor
264379_at	AT2G25200	similar to unknown protein
265614_at	AT2G25355	exonuclease-related; similar to unknown protein
265634_at	AT2G25530	AFG1-like ATPase family protein; similar to ATPase
265886_at	AT2G25620	protein phosphatase 2C
265913_at	AT2G25625	similar to Os05g0575000
266656_at	AT2G25900	putative Cys3His zinc finger protein (ATCTH) mRNA
267377_at	AT2G26250	epidermis-specific
263082_at	AT2G27200	GTP-binding family protein
266209_at	AT2G27550	encodes a protein similar to TFL1
266253_at	AT2G27840	Belongs to the plant specific HD2 type proteins
263435_at	AT2G28600	ATP binding / ATP-dependent helicase/ nucleic acid binding
263443_at	AT2G28630	beta-ketoacyl-CoA synthase family protein
263412_at	AT2G28720	histone H2B
266225_at	AT2G28900	Encodes AtOEP16
266790_at	AT2G28950	Encodes an expansin. Naming convention from the Expansin Working Group
266778_at	AT2G29090	Encodes a protein with ABA 8'-hydroxylase activity
266279_at	AT2G29290	tropinone reductase
266277_at	AT2G29310	tropinone reductase
266265_at	AT2G29340	short-chain dehydrogenase/reductase (SDR) family protein
266237_at	AT2G29540	RNA polymerase I(A) and III(C) 14 kDa subunit
266617_at	AT2G29670	binding; similar to binding [Arabidopsis thaliana] (TAIR:AT2G29670.1)
266668_at	AT2G29760	pentatricopeptide (PPR) repeat-containing protein
255866_at	AT2G30350	endo/excinuclease amino terminal domain-containing protein; similar to Os07g0230500

267516_at	AT2G30520	light inducible root phototropism 2
267517_at	AT2G30520	light inducible root phototropism 2
267576_at	AT2G30640	Mutator-like transposase family
266471_at	AT2G31060	elongation factor family protein; similar to elongation factor family protein
266474_at	AT2G31110	similar to unknown protein
263249_at	AT2G31360	homologous to delta 9 acyl-lipid desaturases of cyanobacteria
263252_at	AT2G31380	a B-box zinc finger protein that interacts with COP1.
263474_at	AT2G31725	similar to unknown protein
263467_at	AT2G31730	ethylene-responsive protein
265680_at	AT2G32150	haloacid dehalogenase-like hydrolase family protein
265730_at	AT2G32220	60S ribosomal protein L27 (RPL27A); Identical to 60S ribosomal protein L27-1 (RPL27A)
267116_at	AT2G32560	F-box family protein; similar to F-box family protein
245164_at	AT2G33210	chaperonin
255793_at	AT2G33250	similar to unknown protein
255817_at	AT2G33330	33 kDa secretory protein-related; similar to 33 kDa secretory protein-related
255795_at	AT2G33380	Encodes a calcium binding protein whose mRNA is induced upon treatment with NaCl
267454_at	AT2G33730	DEAD box RNA helicase
267004_at	AT2G34260	transducin family protein / WD-40 repeat family protein
266996_at	AT2G34490	Encodes a protein with C22-sterol desaturase activity.
266903_at	AT2G34570	MEE21 (maternal effect embryo arrest 21); similar to Protein of unknown function DUF652
266899_at	AT2G34620	mitochondrial transcription termination factor-related / mTERF-related
267406_at	AT2G34780	EMB1611/MEE22 (EMBRYO DEFECTIVE 1611
267429_at	AT2G34850	MEE25 (maternal effect embryo arrest 25); catalytic
267432_at	AT2G35020	UTP--glucose-1-phosphate uridylyltransferase family protein
265795_at	AT2G35780	SCPL26 (serine carboxypeptidase-like 26)
263951_at	AT2G35960	Encodes a protein whose sequence is similar to tobacco hairpin-induced gene (HIN1)
263946_at	AT2G36000	mitochondrial transcription termination factor-related / mTERF-related
263919_at	AT2G36470	similar to unknown protein
265197_at	AT2G36750	UGT72C1 (UDP-glucosyl transferase 72C1)
263867_at	AT2G36830	encodes a tonoplast intrinsic protein
263866_at	AT2G36950	heavy-metal-associated domain-containing protein
265471_at	AT2G37130	peroxidase 21 (PER21) (P21) (PRXR5)
265953_at	AT2G37480	similar to unknown protein
265952_at	AT2G37480	similar to unknown protein
267163_at	AT2G37520	PHD finger family protein; similar to PHD finger transcription factor
267177_at	AT2G37580	zinc finger (C3HC4-type RING finger) family protein
267168_at	AT2G37770	aldo/keto reductase family protein; similar to aldo/keto reductase family protein
266093_at	AT2G37990	ribosome biogenesis regulatory protein (RRS1) family protein
267034_at	AT2G38310	similar to unknown protein
267036_at	AT2G38465	unknown protein
266411_at	AT2G38730	peptidyl-prolyl cis-trans isomerase
266979_at	AT2G39470	photosystem II reaction center PsbP family protein

266965_at	AT2G39510	nodulin MtN21 family protein; similar to nodulin MtN21 family protein
266984_at	AT2G39570	ACT domain-containing protein; similar to ACT domain-containing protein
267592_at	AT2G39710	Encodes a Cysteine-rich peptide (CRP) family protein
245063_at	AT2G39795	mitochondrial glycoprotein family protein / MAM33 family protein
267356_at	AT2G39930	Encodes an isoamylase-type debranching enzyme.
267359_at	AT2G40020	similar to unknown protein
263824_at	AT2G40360	transducin family protein / WD-40 repeat family protein
263802_at	AT2G40430	Identical to Protein At2g40430
255877_at	AT2G40460	proton-dependent oligopeptide transport (POT) family protein
255822_at	AT2G40610	member of Alpha-Expansin Gene Family
266076_at	AT2G40700	DEAD/DEAH box helicase
266049_at	AT2G40780	RNA binding / translation initiation factor
267080_at	AT2G41190	amino acid transporter family protein; similar to amino acid transporter family protein
263495_at	AT2G42530	cold-responsive protein / cold-regulated protein (cor15b)
263497_at	AT2G42540	A cold-regulated gene whose product is targeted to the chloroplast
263987_at	AT2G42690	lipase
265265_at	AT2G42900	similar to Os05g0582000
260540_at	AT2G43500	RWP-RK domain-containing protein
260556_at	AT2G43620	chitinase
260585_at	AT2G43650	Sas10/U3 ribonucleoprotein (Utp) family protein; similar to unknown protein
267367_at	AT2G44210	similar to unknown protein
267344_at	AT2G44230	similar to unknown protein
267371_at	AT2G44510	p21Cip1-binding protein-related; Identical to Protein BCCIP homolog
266873_at	AT2G44740	CYCP4;1 (cyclin p4;1); cyclin-dependent protein kinase; similar to CYCP4
266876_at	AT2G44820	similar to Os01g0224500
266822_at	AT2G44860	60S ribosomal protein L24
266820_at	AT2G44940	encodes a member of the DREB subfamily A-4 of ERF/AP2 transcription factor family.
266106_at	AT2G45170	Involved in autophagy. Under nutrient starvation the protein localizes to autophagosomes.
267509_at	AT2G45660	AGL20
266925_at	AT2G45740	member of the peroxin11 (PEX11) gene family
266927_at	AT2G45960	a member of the plasma membrane intrinsic protein subfamily PIP1.
266604_at	AT2G46030	Ubiquitin conjugating enzyme E2
266583_at	AT2G46220	similar to unknown protein
266591_at	AT2G46225	Encodes a subunit of the WAVE complex.
266552_at	AT2G46330	Encodes arabinogalactan protein (AGP16).
266327_at	AT2G46680	encodes a putative transcription factor
266324_at	AT2G46710	rac GTPase activating protein
266719_at	AT2G46830	CCA1
263320_at	AT2G47180	ATGOLS1 (ARABIDOPSIS THALIANA GALACTINOL SYNTHASE 1)
266503_at	AT2G47780	rubber elongation factor (REF) protein-related
266514_at	AT2G47890	zinc finger (B-box type) family protein; Identical to CONSTANS-LIKE 13 (COL13)

266483_at	AT2G47910	CRR6 (CHLORORESPIRATORY REDUCTION 6); similar to conserved hypothetical protein
266460_at	AT2G47930	AGP26/ATAGP26 (ARABINOGALACTAN PROTEINS 26)
266510_at	AT2G47990	Encodes a transducin family nucleolar protein with six WD40
259278_at	AT3G01160	similar to unknown protein
258949_at	AT3G01370	similar to unknown protein
259181_at	AT3G01690	similar to unknown protein
258996_at	AT3G01800	ribosome recycling factor family protein / ribosome releasing factor family protein
258998_at	AT3G01820	adenylate kinase family protein; similar to ADK/ATPADK1 (ADENOSINE KINASE)
258859_at	AT3G02120	hydroxyproline-rich glycoprotein family protein
259104_at	AT3G02170	Encodes LONGIFOLIA2 (LNG2).
259131_at	AT3G02180	SPIRAL1-LIKE3 belongs to a six-member gene family in Arabidopsis+C949
259132_at	AT3G02250	similar to unknown protein
259075_at	AT3G02320	RNA binding / tRNA (guanine-N2-)-methyltransferase
258502_at	AT3G02490	pentatricopeptide (PPR) repeat-containing protein
258487_at	AT3G02550	LOB domain protein 41 / lateral organ boundaries domain protein 41 (LBD41)
258474_at	AT3G02650	pentatricopeptide (PPR) repeat-containing protein; similar to binding
258618_at	AT3G02885	GASA5 (GAST1 PROTEIN HOMOLOG 5); similar to gibberellin-regulated family protein
258603_at	AT3G02990	member of Heat Stress Transcription Factor (Hsf) family
258871_at	AT3G03060	ATPase; similar to AAA-type ATPase family protein
258849_at	AT3G03250	Is thought to encode a cytosolic UDP-glucose pyrophosphorylase
259173_at	AT3G03640	Encodes beta-glucosidase (GLUC).
259347_at	AT3G03920	Gar1 RNA-binding region family protein
258805_at	AT3G04010	glycosyl hydrolase family 17 protein; similar to glycosyl hydrolase family 17 protein
258537_at	AT3G04210	disease resistance protein (TIR-NBS class)
258800_at	AT3G04550	similar to unknown protein
259311_at	AT3G05060	SAR DNA-binding protein
259105_at	AT3G05500	rubber elongation factor (REF) family protein
258893_at	AT3G05660	kinase/ protein binding; similar to disease resistance family protein
258742_at	AT3G05800	transcription factor; similar to transcription factor
258562_at	AT3G05980	similar to unknown protein
258468_at	AT3G06070	similar to unknown protein
258472_at	AT3G06080	similar to unknown protein
256401_at	AT3G06200	guanylate kinase
258882_at	AT3G06330	zinc finger (C3HC4-type RING finger) family protein
258505_at	AT3G06530	BAP28-related; Identical to Hypothetical protein At3g06530
258535_at	AT3G06750	hydroxyproline-rich glycoprotein family protein
258527_at	AT3G06850	dihydrolipoamide branched chain acyltransferase
258552_at	AT3G07010	pectate lyase family protein; Identical to Probable pectate lyase 8 precursor
258545_at	AT3G07050	GTP-binding family protein; similar to GTP-binding family protein
258834_at	AT3G07270	GTP cyclohydrolase I; similar to GTP cyclohydrolase I
259017_at	AT3G07310	similar to unknown protein

259018_at	AT3G07390	isolated from differential screening of a cDNA library from auxin-treated root culture.
259068_at	AT3G07560	Encodes peroxin 13 (PEX13) involved in protein transport into peroxisomes.
259227_at	AT3G07750	3' exoribonuclease family domain 1-containing protein
258692_at	AT3G08640	alphavirus core protein family; similar to unknown protein
258993_at	AT3G08940	Lhcb4.2 protein (Lhcb4.2
258987_at	AT3G08950	electron transport SCO1/SenC family protein
259207_at	AT3G09050	similar to Os01g0853000
259037_at	AT3G09350	armadillo/beta-catenin repeat family protein
258979_at	AT3G09440	heat shock cognate 70 kDa protein 3 (HSC70-3) (HSP70-3)
258719_at	AT3G09540	pectate lyase family protein; similar to pectate lyase family protein
258708_at	AT3G09580	amine oxidase family protein; similar to HEMG2/MEE61 (maternal effect embryo arrest 61)
258723_at	AT3G09600	myb family transcription factor; similar to DNA binding / transcription factor
258928_at	AT3G10070	putative TBP-associated 58 kDa subunit protein (TAFII58)
258965_at	AT3G10530	transducin family protein / WD-40 repeat family protein
258774_at	AT3G10740	Encodes a bifunctional alpha-l-arabinofuranosidase/beta-d-xylosidase
256427_at	AT3G11090	LOB domain family protein / lateral organ boundaries domain family protein (LBD21)
258727_at	AT3G11930	universal stress protein (USP) family protein
256661_at	AT3G11964	S1 RNA-binding domain-containing protein
256262_at	AT3G12150	similar to unnamed protein product
256288_at	AT3G12270	methyltransferase; similar to protein arginine N-methyltransferase
256266_at	AT3G12320	similar to unknown protein
256230_at	AT3G12340	FK506 binding / peptidyl-prolyl cis-trans isomerase; similar to immunophilin-related
256235_at	AT3G12490	cysteine protease inhibitor
256245_at	AT3G12580	HSP70 (heat shock protein 70); ATP binding
256237_at	AT3G12610	Plays role in DNA-damage repair/toleration.
257702_at	AT3G12670	EMB2742 (EMBRYO DEFECTIVE 2742); CTP synthase; similar to CTP synthase
257694_at	AT3G12860	nucleolar protein Nop56
257860_at	AT3G13062	similar to unknown protein
257188_at	AT3G13150	pentatricopeptide (PPR) repeat-containing protein
257193_at	AT3G13160	pentatricopeptide (PPR) repeat-containing protein
257658_at	AT3G13230	nucleic acid binding; similar to Isopenicillin N synthetase; KH
257708_at	AT3G13330	binding; similar to proteasome activator subunit 4-like
256649_at	AT3G13570	encodes an SC35-like splicing factor of 30 kD that is localized to the nuclear specks.
256772_at	AT3G13750	beta-galactosidase
256779_at	AT3G13784	ATCWINV5 (ARABIDOPSIS THALIANA CELL WALL INVERTASE 5)
258202_at	AT3G13940	DNA binding / DNA-directed RNA polymerase; similar to Os11g0615100
257280_at	AT3G14440	Encodes 9-<i>cis</i>-epoxycarotenoid dioxygenase
258094_at	AT3G14690	putative cytochrome P450
257237_at	AT3G14890	phosphoesterase; similar to poly (ADP-ribose) polymerase
257211_at	AT3G15080	exonuclease family protein; similar to exonuclease

257058_at	AT3G15352	Encodes protein similar to yeast COX17
258397_at	AT3G15357	similar to zinc finger protein-related
258402_at	AT3G15450	similar to unknown protein
258396_at	AT3G15460	brix domain-containing protein; similar to brix domain-containing protein
258225_at	AT3G15630	similar to unknown protein
258270_at	AT3G15650	phospholipase/carboxylesterase family protein
258054_at	AT3G16240	Delta tonoplast intrinsic protein
258055_at	AT3G16250	ferredoxin-related; similar to ferredoxin-related
259329_at	AT3G16360	Encodes AHP4
259375_at	AT3G16370	GDSL-motif lipase/hydrolase family protein; similar to GDSL-motif lipase
257206_at	AT3G16530	Lectin like protein whose expression is induced upon treatment with chitin oligomers.
258434_at	AT3G16770	RAP2.3
257650_at	AT3G16800	protein phosphatase 2C
257652_at	AT3G16810	APUM24 (ARABIDOPSIS PUMILIO 24)
257648_at	AT3G16840	ATP-dependent helicase
256790_at	AT3G16857	Encodes an Arabidopsis response regulator (ARR) protein
257880_at	AT3G16910	Encodes a peroxisomal protein with acetyl-CoA synthetase activity
257876_at	AT3G17130	invertase/pectin methylesterase inhibitor family protein
258375_at	AT3G17470	RelA/SpoT domain-containing protein / calcium-binding EF-hand family protein
258214_at	AT3G17970	chloroplast outer membrane translocon subunit
258215_at	AT3G17970	chloroplast outer membrane translocon subunit
258218_at	AT3G18000	Arabidopsis thaliana N-methyltransferase-like protein mRNA.
258156_at	AT3G18050	similar to unknown protein
258155_at	AT3G18130	RACK1
256797_at	AT3G18600	DEAD/DEAH box helicase
257801_at	AT3G18750	Encodes a member of the WNK family (9 members in all) of protein kinases
257803_at	AT3G18790	similar to 2 coiled coil domains of eukaryotic ori (GB:BAD19345.1)
257035_at	AT3G19270	Encodes a protein with ABA 8'-hydroxylase activity
258015_at	AT3G19340	sodium:dicarboxylate symporter; similar to unknown protein
258024_at	AT3G19360	zinc finger (CCCH-type) family protein; similar to zinc finger (CCCH-type) family protein
258006_at	AT3G19400	cysteine proteinase
258009_at	AT3G19440	pseudouridine synthase family protein
257966_at	AT3G19800	similar to conserved hypothetical protein
257964_at	AT3G19850	phototropic-responsive NPH3 family protein
257131_at	AT3G20240	mitochondrial substrate carrier family protein
258034_at	AT3G21300	RNA methyltransferase family protein; similar to zinc finger (CCCH-type) family protein
258166_at	AT3G21540	transducin family protein / WD-40 repeat family protein
258167_at	AT3G21560	Encodes a protein with sinapic acid:UDP-glucose glucosyltransferase activity.
257262_at	AT3G21890	zinc finger (B-box type) family protein; similar to DNA binding / zinc ion binding
256796_at	AT3G22210	similar to fortune-1
258316_at	AT3G22660	rRNA processing protein-related

258317_at	AT3G22670	pentatricopeptide (PPR) repeat-containing protein; similar to binding
258321_at	AT3G22840	Encodes an early light-inducible protein.
258101_at	AT3G23590	similar to structural constituent of ribosome
258104_at	AT3G23620	brix domain-containing protein; Identical to Brix domain-containing protein 1 homolog
257203_at	AT3G23730	xyloglucan:xyloglucosyl transferase
257171_at	AT3G23760	similar to transferase
257173_at	AT3G23810	SAHH2 (S-ADENOSYL-L-HOMOCYSTEINE (SAH) HYDROLASE 2)
256890_at	AT3G23830	encodes a glycine-rich RNA binding protein.
256914_at	AT3G23880	F-box family protein; similar to F-box family protein
256905_at	AT3G23990	mitochondrial chaperonin HSP
257253_at	AT3G24190	ABC1 family protein; similar to ABC1 family protein
257868_at	AT3G25070	RIN4
256754_at	AT3G25690	actin binding protein required for normal chloroplast positioning
257643_at	AT3G25730	AP2 domain-containing transcription factor
258078_at	AT3G25870	similar to unknown protein
258079_at	AT3G25940	transcription factor S-II (TFIIS) domain-containing protein
256881_at	AT3G26410	methyltransferase/ nucleic acid binding; similar to RNA methylase-like protein
256880_at	AT3G26450	major latex protein-related / MLP-related; similar to major latex protein-related / MLP-related
257615_at	AT3G26510	octicosapeptide/Phox/Bem1p (PB1) domain-containing protein
257313_at	AT3G26520	gamma tonoplast intrinsic protein 2 (TIP2)
257611_at	AT3G26580	binding; similar to hypothetical protein
257832_at	AT3G26740	CCL
258254_at	AT3G26782	binding; similar to pentatricopeptide (PPR) repeat-containing protein
258256_at	AT3G26890	similar to unknown protein
256980_at	AT3G26932	DRB3 (DSRNA-BINDING PROTEIN 3)
257789_at	AT3G27020	Arabidopsis thaliana metal-nicotianamine transporter YSL6
257154_at	AT3G27210	similar to unknown protein
257149_at	AT3G27280	ATPHB4 (PROHIBITIN 4)
257970_at	AT3G27570	similar to unknown protein
257226_at	AT3G27880	similar to unknown protein
257271_at	AT3G28007	nodulin MtN3 family protein
257299_at	AT3G28050	nodulin MtN21 family protein
257300_at	AT3G28080	nodulin MtN21 family protein
257071_at	AT3G28180	encodes a gene similar to cellulose synthase
256577_at	AT3G28220	meprin and TRAF homology domain-containing protein
257745_at	AT3G29240	similar to unknown protein
256924_at	AT3G29590	At3g29590 (At5MAT) encodes a malonyl-CoA
245228_at	AT3G29810	phytochelatin synthetase family protein / COBRA cell expansion protein COBL2
252661_at	AT3G44450	similar to unknown protein
252639_at	AT3G44550	oxidoreductase
252625_at	AT3G44750	Encodes a histone deacetylase.
252615_at	AT3G45230	hydroxyproline-rich glycoprotein family protein; similar to unknown protein
252591_at	AT3G45600	Member of TETRASPANIN family
252548_at	AT3G45850	kinesin motor protein-related

252534_at	AT3G46130	Encodes a putative transcription factor (MYB48).
252508_at	AT3G46210	3' exoribonuclease family domain 1-containing protein
252529_at	AT3G46490	oxidoreductase
252467_at	AT3G47080	binding; similar to binding [Arabidopsis thaliana] (TAIR:AT3G47080.1)
252429_at	AT3G47500	Dof-type zinc finger domain-containing protein
252433_at	AT3G47560	esterase/lipase/thioesterase family protein
252374_at	AT3G48100	Encodes a transcription repressor
252353_at	AT3G48200	similar to Os05g0594500
252355_at	AT3G48250	pentatricopeptide (PPR) repeat-containing protein
252367_at	AT3G48360	BT2
252321_at	AT3G48510	similar to unknown protein
252316_at	AT3G48700	similar to unknown protein
252317_at	AT3G48720	transferase family protein
252252_at	AT3G49180	transducin family protein / WD-40 repeat family protein
252305_at	AT3G49240	EMB1796 (EMBRYO DEFECTIVE 1796)
252281_at	AT3G49320	similar to unknown protein
252250_at	AT3G49790	similar to ATPP2-A10 (Phloem protein 2-A10)
252239_at	AT3G49990	similar to Os03g0372700
252214_at	AT3G50260	encodes a member of the DREB subfamily A-5 of ERF/AP2 transcription factor family.
252199_at	AT3G50270	transferase family protein
252168_at	AT3G50440	hydrolase; similar to esterase
252167_at	AT3G50560	short-chain dehydrogenase/reductase (SDR) family protein
252178_at	AT3G50750	brassinosteroid signalling positive regulator-related; Identical to BEH1
252179_at	AT3G50760	Encodes a protein with putative galacturonosyltransferase activity.
252154_at	AT3G50880	HhH-GPD base excision DNA repair family protein
252127_at	AT3G50960	similar to unknown protein
252102_at	AT3G50970	Belongs to the dehydrin protein family
252123_at	AT3G51240	Encodes flavanone 3-hydroxylase
252092_at	AT3G51420	strictosidine synthase family protein; similar to YLS2 (yellow-leaf-specific gene 2)
252117_at	AT3G51430	strictosidine synthase-like protein
252076_at	AT3G51660	macrophage migration inhibitory factor family protein / MIF family protein
246307_at	AT3G51800	putative nuclear DNA-binding protein G2p (AtG2) mRNA
246304_at	AT3G51840	Encodes a short-chain acyl-CoA oxidase
246310_at	AT3G51895	Encodes a sulfate transporter.
252034_at	AT3G52040	similar to unknown
252040_at	AT3G52060	similar to unknown protein
256676_at	AT3G52180	Encodes a plant-specific protein phosphatase
256677_at	AT3G52190	Encodes a plant specific protein structurally related to the SEC12
256671_at	AT3G52290	IQD3 (IQ-domain 3); calmodulin binding; similar to IQD2 (IQ-domain 2)
256674_at	AT3G52360	similar to unknown protein
252011_at	AT3G52720	carbonic anhydrase family protein; similar to carbonic anhydrase family protein
251973_at	AT3G53180	glutamate-ammonia ligase; similar to glutamine synthetase
251975_at	AT3G53230	cell division cycle protein 48

251984_at	AT3G53260	Encodes phenylalanine lyase.
251987_at	AT3G53280	cytochrome P450 monooxygenase
251941_at	AT3G53470	similar to Os03g0285100
251931_at	AT3G53950	glyoxal oxidase-related; similar to glyoxal oxidase-related
251927_at	AT3G53990	universal stress protein (USP) family protein
251886_at	AT3G54260	similar to unknown protein
251899_at	AT3G54400	aspartyl protease family protein; similar to pepsin A
251827_at	AT3G55120	Catalyzes the conversion of chalcones into flavanones.
251800_at	AT3G55510	similar to unknown protein
251793_at	AT3G55580	regulator of chromosome condensation (RCC1) family protein
251759_at	AT3G55630	ATDFD (A. THALIANA DHFS-FPGS HOMOLOG D)
251753_at	AT3G55760	similar to unknown protein
251768_at	AT3G55940	phosphoinositide-specific phospholipase C
251740_at	AT3G56070	rotamase cyclophilin 2 (ROC2)
251725_at	AT3G56260	similar to unknown protein
251657_at	AT3G57000	nucleolar essential protein-related; similar to Os02g0290400
251668_at	AT3G57010	strictosidine synthase family protein; similar to strictosidine synthase family protein
251667_at	AT3G57150	Encodes a putative pseudouridine synthase (NAP57).
251629_at	AT3G57410	Encodes a protein with high homology to animal villin.
251641_at	AT3G57470	peptidase M16 family protein / insulinase family protein
251638_at	AT3G57490	40S ribosomal protein S2 (RPS2D); Identical to 40S ribosomal protein S2-4 (RPS2D)
251593_at	AT3G57660	Encodes a subunit of RNA polymerase I (aka RNA polymerase A).
251620_at	AT3G58060	cation efflux family protein / metal tolerance protein
251575_at	AT3G58120	bZIP transcription factor family protein
251529_at	AT3G58570	DEAD box RNA helicase
251538_at	AT3G58660	60S ribosomal protein-related
251506_at	AT3G59090	similar to TOM1 (TOBAMOVIRUS MULTIPLICATION 1)
251476_at	AT3G59670	similar to unknown protein
251432_at	AT3G59820	calcium-binding mitochondrial protein-related
251427_at	AT3G60130	glycosyl hydrolase family 1 protein / beta-glucosidase
251371_at	AT3G60360	EDA14/UTP11 (U3 SMALL NUCLEOLAR RNA-ASSOCIATED PROTEIN 11
251372_at	AT3G60520	zinc ion binding; similar to unknown protein
251391_at	AT3G60910	catalytic; similar to catalytic [Arabidopsis thaliana] (TAIR:AT3G17365.1)
251346_at	AT3G60980	pentatricopeptide (PPR) repeat-containing protein+C612
251356_at	AT3G61060	ATPP2-A13; similar to ATPP2-A12 (Phloem protein 2-A12)
251336_at	AT3G61190	Encodes a protein with a C2 domain that binds to BON1 in yeast two hybrid analyses.
251360_at	AT3G61210	embryo-abundant protein-related; similar to embryo-abundant protein-related
251324_at	AT3G61430	a member of the plasma membrane intrinsic protein subfamily PIP1.
251323_at	AT3G61580	delta-8 sphingolipid desaturase (SLD1); similar to delta-8 sphingolipid desaturase
251296_at	AT3G62010	metal ion binding / oxidoreductase; similar to Os03g0586700
251265_at	AT3G62310	RNA helicase
251267_at	AT3G62330	zinc knuckle (CCHC-type) family protein; similar to unknown protein

251218_at	AT3G62410	CP12-2 encodes a small peptide found in the chloroplast stroma.
251227_at	AT3G62700	member of MRP subfamily
251229_at	AT3G62740	glycosyl hydrolase family 1 protein; similar to glycosyl hydrolase family 1 protein
251235_at	AT3G62860	esterase/lipase/thioesterase family protein
251200_at	AT3G63010	Encodes a gibberellin (GA) receptor ortholog of the rice GA receptor gene (OsGID1).
251205_at	AT3G63080	Encodes glutathione peroxidase.
251169_at	AT3G63210	encodes a novel zinc-finger protein with a proline-rich N-terminus
255700_at	AT4G00200	DNA binding; similar to DNA-binding family protein
255637_at	AT4G00750	dehydration-responsive family protein; similar to dehydration-responsive family protein
255626_at	AT4G00780	meprin and TRAF homology domain-containing protein / MATH domain-containing protein
255645_at	AT4G00880	auxin-responsive family protein
255617_at	AT4G01330	protein kinase family protein; similar to protein kinase family protein
255579_at	AT4G01460	basic helix-loop-helix (bHLH) family protein
255543_at	AT4G01870	tolB protein-related; similar to unknown protein
255561_at	AT4G02050	sugar transporter
255517_at	AT4G02290	glycosyl hydrolase family 9 protein; Identical to Endoglucanase 17 precursor (EC 3.2.1.4)
255524_at	AT4G02330	pectinesterase family protein; similar to pectinesterase family protein
255501_at	AT4G02400	similar to U3 ribonucleoprotein (Utp) family protein
255452_at	AT4G02880	similar to unknown protein
255411_at	AT4G03110	RNA-binding protein
255436_at	AT4G03150	similar to Os12g0534100
255434_at	AT4G03180	similar to hypothetical protein DDBDRAFT_0185878
255365_at	AT4G04040	MEE51 (maternal effect embryo arrest 51)
255278_at	AT4G04940	transducin family protein / WD-40 repeat family protein
255310_at	AT4G04955	Encodes an allantoinase which is involved in allantoin degradation and assimilation.
255259_at	AT4G05020	NDB2 (NAD(P)H DEHYDROGENASE B2); disulfide oxidoreductase
255225_at	AT4G05410	transducin family protein / WD-40 repeat family protein
255070_at	AT4G09020	Encodes an isoamylase-like protein.
255032_at	AT4G09500	glycosyltransferase family protein
255011_at	AT4G10040	Encodes cytochrome c
255008_at	AT4G10060	similar to unknown protein
255807_at	AT4G10270	wound-responsive family protein
254991_at	AT4G10620	similar to GTP binding
254938_at	AT4G10770	oligopeptide transporter
254919_at	AT4G11360	Encodes a putative RING-H2 finger protein RHA1b.
254931_at	AT4G11460	protein kinase family protein; similar to protein kinase family protein
254890_at	AT4G11600	Encodes glutathione peroxidase.
254848_at	AT4G11960	similar to unknown protein
254850_at	AT4G12000	similar to unknown protein
254815_at	AT4G12420	encodes a protein of unknown function involved in directed root tip growth.

254806_at	AT4G12430	trehalose-6-phosphate phosphatase
254818_at	AT4G12470	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein
254805_at	AT4G12480	a putative lipid transfer protein
254832_at	AT4G12490	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein
254819_at	AT4G12500	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein
254747_at	AT4G13020	Encodes a member of the cdc2+ family of protein kinases MHK.
254688_at	AT4G13830	DnaJ-like protein (J20); nuclear gene
245325_at	AT4G14130	xyloglucan endotransglycosylase-related protein (XTR7)
245605_at	AT4G14300	heterogeneous nuclear ribonucleoprotein
245265_at	AT4G14400	encodes a novel protein with putative ankyrin and transmembrane regions.
245592_at	AT4G14540	CCAAT-box binding transcription factor subunit B (NF-YB) (HAP3) (AHAP3) family
245563_at	AT4G14580	CBL-interacting protein kinase
245306_at	AT4G14690	Encodes an early light-induced protein.
245533_at	AT4G15130	cholinephosphate cytidylyltransferase
245352_at	AT4G15490	Encodes a protein that might have sinapic acid:UDP-glucose glucosyltransferase activity.
245321_at	AT4G15545	similar to unknown protein
245281_at	AT4G15560	Encodes a protein with 1-deoxyxylulose 5-phosphate synthase activity
245512_at	AT4G15770	60S ribosome subunit biogenesis protein
245523_at	AT4G15910	DI21
245319_at	AT4G16146	similar to unknown protein
245391_at	AT4G16520	ATG8F (AUTOPHAGY 8F); microtubule binding; similar to AtATG8e (AUTOPHAGY 8E)
245318_at	AT4G16980	arabinogalactan-protein family
245266_at	AT4G17070	similar to Os03g0100300
245346_at	AT4G17090	Encodes a beta-amylase targeted to the chloroplast.
245264_at	AT4G17245	zinc finger (C3HC4-type RING finger) family protein
245399_at	AT4G17340	DELTA-TIP2/TIP2;2 (tonoplast intrinsic protein 2;2)
245362_at	AT4G17460	Encodes homeobox protein HAT1.
245308_at	AT4G17486	Identical to UPF0326 protein At4g17486
245426_at	AT4G17540	similar to Os10g0563400
245427_at	AT4G17550	transporter-related; similar to glycerol-3-phosphate transporter
245401_at	AT4G17670	senescence-associated protein-related
254656_at	AT4G18070	similar to unknown protein
254662_at	AT4G18270	Encodes protein similar to similar to bacterial translocase I (mra Y).
254667_at	AT4G18280	glycine-rich cell wall protein-related
254636_at	AT4G18700	Encodes CBL-interacting protein kinase 12 (CIPK12).
254609_at	AT4G18970	GDSL-motif lipase/hydrolase family protein
254563_at	AT4G19120	ERD3 (EARLY-RESPONSIVE TO DEHYDRATION 3)
254561_at	AT4G19160	binding; similar to similar to Uncharacterized conserved protein
254564_at	AT4G19170	similar to nine-cis-epoxycarotenoid dioxygenase
254562_at	AT4G19230	Encodes a protein with ABA 8'-hydroxylase activity
254580_at	AT4G19390	similar to structural constituent of ribosome
254573_at	AT4G19420	pectinacetylesterase family protein; similar to pectinacetylesterase

254574_at	AT4G19430	unknown protein
254553_at	AT4G19530	disease resistance protein (TIR-NBS-LRR class)
254543_at	AT4G19810	glycosyl hydrolase family 18 protein; similar to glycosyl hydrolase family 18 protein
254547_at	AT4G19860	lecithin:cholesterol acyltransferase family protein / LACT family protein
254505_at	AT4G19985	GCN5-related N-acetyltransferase (GNAT) family protein
254446_at	AT4G20890	tubulin 9
254452_at	AT4G21100	DDB1
254455_at	AT4G21140	similar to unknown protein
254378_at	AT4G21810	Der1-like family protein / degradation in the ER-like family protein
254387_at	AT4G21850	methionine sulfoxide reductase domain-containing protein / SelR domain-containing protein
254384_at	AT4G21870	26.5 kDa class P-related heat shock protein (HSP26.5-P)
254305_at	AT4G22200	Encodes a photosynthate- and light-dependent inward rectifying potassium channel
254304_at	AT4G22270	similar to unknown protein
254355_at	AT4G22380	ribosomal protein L7Ae/L30e/S12e/Gadd45 family protein
254314_at	AT4G22470	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein
254321_at	AT4G22590	trehalose-6-phosphate phosphatase
254333_at	AT4G22753	Encodes a member of the SMO1 family of sterol 4alpha-methyl oxidases.
254256_at	AT4G23180	Encodes a receptor-like protein kinase.
254239_at	AT4G23400	PIP1
254210_at	AT4G23450	zinc finger (C3HC4-type RING finger) family protein
254232_at	AT4G23600	Encodes cystine lyase which is expected to be involved in amino acid metabolism
254227_at	AT4G23630	BTI1 (VIRB2-INTERACTING PROTEIN 1)
254188_at	AT4G23920	Encodes a protein with UDP-D-glucose 4-epimerase activity.
254185_at	AT4G23990	encodes a protein similar to cellulose synthase
254157_at	AT4G24220	encodes a novel protein containing mammalian death domain
254145_at	AT4G24700	similar to Os02g0595200
254119_at	AT4G24780	pectate lyase family protein
254133_at	AT4G24810	similar to ABC1 family protein
254085_at	AT4G24960	Homologous to a eukaryote specific ABA- and stress-inducible gene
254105_at	AT4G25080	Encodes a protein with methyltransferase
254110_at	AT4G25260	invertase/pectin methylesterase inhibitor family protein
254054_at	AT4G25320	DNA-binding protein-related; similar to DNA-binding protein-related
254076_at	AT4G25340	immunophilin-related / FKBP-type peptidyl-prolyl cis-trans isomerase-related
254075_at	AT4G25470	CBF2
254066_at	AT4G25480	CBF3
254073_at	AT4G25500	encodes an arginine/serine-rich splicing factor.
254080_at	AT4G25630	encodes a fibrillarin
254077_at	AT4G25640	MATE efflux family protein; similar to MATE efflux family protein
254079_at	AT4G25730	FtsJ-like methyltransferase family protein
254032_at	AT4G25940	epsin N-terminal homology (ENTH) domain-containing protein
254043_at	AT4G25990	chloroplast import apparatus CIA2-like.

253994_at	AT4G26080	Involved in abscisic acid (ABA) signal transduction.
253971_at	AT4G26530	fructose-bisphosphate aldolase
253973_at	AT4G26555	immunophilin / FKBP-type peptidyl-prolyl cis-trans isomerase family protein
253975_at	AT4G26600	nucleolar protein
253949_at	AT4G26780	unknown function
253917_at	AT4G27380	similar to hypothetical protein MtrDRAFT_AC126013g5v1
253872_at	AT4G27410	Encodes a NAC transcription factor induced in response to dessication.
253874_at	AT4G27450	similar to unknown protein
253875_at	AT4G27520	plastocyanin-like domain-containing protein
253886_at	AT4G27710	member of CYP709B
253835_at	AT4G27820	glycosyl hydrolase family 1 protein; similar to hydrolase
253841_at	AT4G27830	glycosyl hydrolase family 1 protein; similar to glycosyl hydrolase family 1 protein
253824_at	AT4G27940	mitochondrial substrate carrier family protein
253828_at	AT4G27970	C4-dicarboxylate transporter/malic acid transport family protein
253849_at	AT4G28080	binding; similar to binding [Arabidopsis thaliana] (TAIR:AT4G28080.1)
253803_at	AT4G28200	similar to Ribosomal protein L29
253815_at	AT4G28250	putative beta-expansin/allergen protein.
253806_at	AT4G28270	zinc finger (C3HC4-type RING finger) family protein; similar to RMA1
253777_at	AT4G28450	transducin family protein / WD-40 repeat family protein
253722_at	AT4G29190	zinc finger (CCCH-type) family protein; similar to zinc finger (CCCH-type) family protein
253709_at	AT4G29220	phosphofructokinase family protein
253695_at	AT4G29510	protein arginine N-methyltransferase
253684_at	AT4G29690	type I phosphodiesterase/nucleotide pyrophosphatase family protein
253608_at	AT4G30290	putative xyloglucan endotransglycosylase/hydrolase
253627_at	AT4G30650	hydrophobic protein
253597_at	AT4G30690	translation initiation factor 3 (IF-3) family protein
253595_at	AT4G30830	similar to unknown protein
253548_at	AT4G30993	similar to unknown protein
253559_at	AT4G31140	glycosyl hydrolase family 17 protein; Identical to Putative glucan endo-1
253534_at	AT4G31500	Encodes an oxime-metabolizing enzyme in the biosynthetic pathway of glucosinolates.
253490_at	AT4G31790	diphthine synthase
253425_at	AT4G32190	centromeric protein-related; similar to protein transport protein-related
253440_at	AT4G32570	contains InterPro domain ZIM; (InterPro:IPR010399)
253411_at	AT4G32980	Encodes transcription factor involved in photomorphogenesis.
253412_at	AT4G33000	Encodes a member of the calcineurin B-like calcium sensor gene family.
253416_at	AT4G33070	pyruvate decarboxylase
253302_at	AT4G33660	similar to proline-rich family protein
253305_at	AT4G33666	unknown protein
253293_at	AT4G33905	peroxisomal membrane protein 22 kDa
253322_at	AT4G33980	similar to unknown protein
253282_at	AT4G34120	LEJ1 (LOSS OF THE TIMING OF ET AND JA BIOSYNTHESIS 1)

253277_at	AT4G34230	Encodes a catalytically active cinnamyl alcohol dehydrogenase
253254_at	AT4G34650	squalene synthase
253203_at	AT4G34710	encodes a arginine decarboxylase (ADC)
253252_at	AT4G34740	Encodes glutamine 5-phosphoribosylpyrophosphate amidotransferase.
253255_at	AT4G34760	auxin-responsive family protein
253219_at	AT4G34990	Member of the R2R3 factor gene family.
253141_at	AT4G35440	CLC-e chloride channel protein
253130_at	AT4G35510	similar to unknown protein
253163_at	AT4G35750	Rho-GTPase-activating protein-related; similar to unknown protein
253161_at	AT4G35770	Senescence-associated gene that is strongly induced by phosphate starvation.
253104_at	AT4G36010	pathogenesis-related thaumatin family protein;
253129_at	AT4G36020	CSDP1 (COLD SHOCK DOMAIN PROTEIN 1)
246275_at	AT4G36540	BEE2 (BR ENHANCED EXPRESSION 2); DNA binding / transcription factor
246282_at	AT4G36580	AAA-type ATPase family protein; similar to AAA-type ATPase family protein
246249_at	AT4G36680	pentatricopeptide (PPR) repeat-containing protein
246196_at	AT4G37090	similar to Os02g0186700
253049_at	AT4G37300	MEE59 (maternal effect embryo arrest 59); similar to Os05g0451300
253097_at	AT4G37320	member of CYP81D
253061_at	AT4G37610	BT5 (BTB and TAZ domain protein 5); protein binding / transcription regulator
253040_at	AT4G37800	xyloglucan:xyloglucosyl transferase
253021_at	AT4G38050	permease; Identical to Nucleobase-ascorbate transporter 11 (AtNAT11)
253027_at	AT4G38150	pentatricopeptide (PPR) repeat-containing protein
252986_at	AT4G38380	MATE efflux protein-related; similar to MATE efflux family protein
252997_at	AT4G38400	member of EXPANSIN-LIKE. Naming convention from the Expansin Working Group
252990_at	AT4G38440	similar to Os06g0574400
252956_at	AT4G38580	putative farnesylated protein (At4g38580) mRNA
252950_at	AT4G38690	1-phosphatidylinositol phosphodiesterase-related
252972_at	AT4G38840	auxin-responsive protein
252965_at	AT4G38860	auxin-responsive protein
252968_at	AT4G38890	dihydrouridine synthase family protein; similar to FAD binding / oxidoreductase
252888_at	AT4G39210	Encodes the large subunit of ADP-Glucose Pyrophosphorylase which catalyzes the first
252882_at	AT4G39675	unknown protein
252880_at	AT4G39730	lipid-associated family protein; similar to lipid-associated family protein
252863_at	AT4G39800	Columbia myo-inositol-1-phosphate synthase mRNA
252871_at	AT4G40000	NOL1/NOP2/sun family protein; similar to NOL1/NOP2/sun family protein
251142_at	AT5G01015	similar to unknown protein
251136_at	AT5G01290	mRNA guanylyltransferase; similar to mRNA capping enzyme family protein
251090_at	AT5G01340	mitochondrial substrate carrier family protein

251084_at	AT5G01520	zinc finger (C3HC4-type RING finger) family protein
251063_at	AT5G01850	protein kinase
251066_at	AT5G01880	zinc finger (C3HC4-type RING finger) family protein; Identical to ATL5A (ATL5A)
251068_at	AT5G01920	STN8
251029_at	AT5G02050	mitochondrial glycoprotein family protein / MAM33 family protein
250994_at	AT5G02490	heat shock cognate 70 kDa protein 2 (HSC70-2) (HSP70-2)
251005_at	AT5G02590	chloroplast lumen common family protein
251017_at	AT5G02760	protein phosphatase 2C family protein / PP2C family protein
250974_at	AT5G02820	Involved in the patterning and shape of leaf trichomes.
250987_at	AT5G02860	pentatricopeptide (PPR) repeat-containing protein
245701_at	AT5G04140	Encodes a gene whose sequence is similar to Fd-GOGAT
245699_at	AT5G04250	OTU-like cysteine protease family protein
245714_at	AT5G04280	glycine-rich RNA-binding protein; similar to glycine-rich RNA-binding protein
245711_at	AT5G04340	putative c2h2 zinc finger transcription factor mRNA
250842_at	AT5G04490	Encodes a protein with phytol kinase activity involved in tocopherol biosynthesis.
250891_at	AT5G04530	beta-ketoacyl-CoA synthase family protein
250856_at	AT5G04810	pentatricopeptide (PPR) repeat-containing protein
250817_at	AT5G04940	Encodes a SU(VAR)3-9 homolog
250825_at	AT5G05210	nucleolar matrix protein-related; similar to nucleolar matrix protein-related
250777_at	AT5G05440	similar to unknown protein
250779_at	AT5G05470	protein synthesis initiation factor eIF2 alpha
250752_at	AT5G05690	Encodes a member of the CP90A family
250742_at	AT5G05800	similar to unknown protein
250758_at	AT5G06000	One of the 2 genes that code for the G subunit of eukaryotic initiation factor 3 (EIF3).
250711_at	AT5G06110	DNAJ heat shock N-terminal domain-containing protein / cell division protein-related
250729_at	AT5G06460	Encodes a ubiquitin-activating enzyme (E1)
250679_at	AT5G06550	similar to transcription factor jumonji (jmjC) domain-containing protein
250648_at	AT5G06760	late embryogenesis abundant group 1 domain-containing protein
250669_at	AT5G06870	polygalacturonase inhibiting protein 2 (PGIP2) mRNA
250665_at	AT5G06980	similar to unknown protein
250582_at	AT5G07580	encodes a member of the ERF subfamily B-3 of ERF/AP2 transcription factor family.
250558_at	AT5G07990	Required for flavonoid 3' hydroxylase activity.
250546_at	AT5G08180	ribosomal protein L7Ae/L30e/S12e/Gadd45 family protein
246011_at	AT5G08330	TCP family transcription factor
250529_at	AT5G08610	DEAD box RNA helicase (RH26)
250538_at	AT5G08620	DEAD box RNA helicase (RH25)
250533_at	AT5G08640	Encodes a flavonol synthase that catalyzes formation of flavonols from dihydroflavonols.
245879_at	AT5G09420	chloroplast outer membrane translocon subunit
250502_at	AT5G09590	heat shock protein 70 (Hsc70-5); nuclear
250504_at	AT5G09840	similar to unknown protein
250452_at	AT5G10630	elongation factor 1-alpha
245906_at	AT5G11070	similar to unknown protein

245904_at	AT5G11110	Encodes a protein with putative sucrose-phosphate synthase activity.
250418_at	AT5G11240	transducin family protein / WD-40 repeat family protein
250318_at	AT5G12200	dihydropyrimidinase / DHPase / dihydropyrimidine amidohydrolase / hydantoinase (PYD2)
250309_at	AT5G12220	las1-like family protein; similar to Las1-like
245984_at	AT5G13090	similar to unknown protein
250279_at	AT5G13200	GRAM domain-containing protein / ABA-responsive protein-related
250252_at	AT5G13750	ZIFL1 (ZINC INDUCED FACILITATOR-LIKE 1)
250203_at	AT5G13980	glycosyl hydrolase family 38 protein
250222_at	AT5G14050	transducin family protein / WD-40 repeat family protein
250217_at	AT5G14120	nodulin family protein; similar to nodulin family protein
250180_at	AT5G14450	GDSL-motif lipase/hydrolase family protein
250192_at	AT5G14520	pescadillo-related; similar to BRCT; Pescadillo
250194_at	AT5G14550	similar to unknown protein
250196_at	AT5G14580	polyribonucleotide nucleotidyltransferase
246596_at	AT5G14740	Encodes a beta carbonic anhydrase likely to be localized in the cytoplasm.
246597_at	AT5G14760	At5g14760 encodes for L-aspartate oxidase involved in the early steps of NAD biosynthesis
246566_at	AT5G14940	proton-dependent oligopeptide transport (POT) family protein
250152_at	AT5G15120	similar to unknown protein
250158_at	AT5G15190	unknown protein
250110_at	AT5G15350	plastocyanin-like domain-containing protein
246559_at	AT5G15550	transducin family protein / WD-40 repeat family protein
246528_at	AT5G15640	mitochondrial substrate carrier family protein
246527_at	AT5G15750	RNA-binding S4 domain-containing protein; similar to hypothetical protein
246487_at	AT5G16030	similar to unknown protein
246484_at	AT5G16040	regulator of chromosome condensation (RCC1) family protein
246457_at	AT5G16750	mutant has Female gametophyte; WD-40 Repeat Protein
246461_at	AT5G16930	AAA-type ATPase family protein; similar to AAA-type ATPase family protein
246419_at	AT5G17030	UDP-glucoronosyl/UDP-glucosyl transferase family protein
246467_at	AT5G17040	UDP-glucoronosyl/UDP-glucosyl transferase family protein
246468_at	AT5G17050	The At5g17050 encodes a anthocyanidin 3-O-glucosyltransferase
250072_at	AT5G17210	similar to unknown protein
250083_at	AT5G17220	Encodes glutathione transferase belonging to the phi class of GSTs.
246435_at	AT5G17460	similar to conserved hypothetical protein
250062_at	AT5G17760	AAA-type ATPase family protein; similar to AAA-type ATPase family protein
250053_at	AT5G17850	cation exchanger
250066_at	AT5G17930	RNA binding; similar to MIF4G domain-containing protein / MA3 domain-containing protein
250012_x_at	AT5G18060	auxin-responsive protein
250016_at	AT5G18100	A putative peroxisomal CuZnSOD inducible by a high-light pulse.
250017_at	AT5G18140	DNAJ heat shock N-terminal domain-containing protein
249984_at	AT5G18400	similar to Protein of unknown function DUF689
250009_at	AT5G18440	similar to Os01g0814000

249996_at	AT5G18600	glutaredoxin family protein; Identical to Monothiol glutaredoxin-S2 (AtGrxS2) (GRXS2)
250008_at	AT5G18630	lipase class 3 family protein; similar to lipase class 3 family protein
250000_at	AT5G18650	zinc finger (C3HC4-type RING finger) family protein
250007_at	AT5G18670	putative beta-amylase BMY3 (BMY3)
249977_at	AT5G18820	EMB3007 (EMBRYO DEFECTIVE 3007); ATP binding / protein binding
249923_at	AT5G19120	pepsin A; similar to extracellular dermal glycoprotein
245957_at	AT5G19590	similar to unknown protein
245913_at	AT5G19860	similar to unknown protein
246125_at	AT5G19875	similar to oxidoreductase/ transition metal ion binding
246149_at	AT5G19890	peroxidase
246070_at	AT5G20160	ribosomal protein L7Ae/L30e/S12e/Gadd45 family protein
246073_at	AT5G20180	ribosomal protein L36 family protein; similar to Os03g0811800
246099_at	AT5G20230	Al-stress-induced gene
246114_at	AT5G20250	encodes a member of glycosyl hydrolase family 36.
246088_at	AT5G20600	similar to unknown
246001_at	AT5G20790	similar to unknown protein
245998_at	AT5G20830	Encodes a protein with sucrose synthase activity (SUS1).
246189_at	AT5G20910	zinc finger (C3HC4-type RING finger) family protein
246021_at	AT5G21100	L-ascorbate oxidase
246028_at	AT5G21170	5'-AMP-activated protein kinase beta-2 subunit
249941_at	AT5G22270	similar to unknown protein
249886_at	AT5G22320	leucine-rich repeat family protein; similar to leucine-rich repeat family protein
249938_at	AT5G22330	ATTIP49A/RIN1
249862_at	AT5G22920	zinc finger (C3HC4-type RING finger) family protein
249848_at	AT5G23220	isochorismatase hydrolase family protein; similar to isochorismatase hydrolase family protein
249830_at	AT5G23300	dihydroorotate dehydrogenase
249806_at	AT5G23850	similar to unknown protein
249818_at	AT5G23860	beta-tubulin
249774_at	AT5G24150	squalene monooxygenase gene homolog
249775_at	AT5G24160	squalene monooxygenase 1
249777_at	AT5G24210	lipase class 3 family protein; similar to triacylglycerol lipase
249785_at	AT5G24300	SSI
249732_at	AT5G24420	glucosamine/galactosamine-6-phosphate isomerase-related
249741_at	AT5G24470	APRR5
249742_at	AT5G24490	30S ribosomal protein
246965_at	AT5G24840	methyltransferase; Identical to Probable tRNA (guanine-N(7)-)-methyltransferase
246922_at	AT5G25110	member of AtCIPKs
246932_at	AT5G25190	encodes a member of the ERF subfamily B-6 of ERF/AP2 transcription factor family.
246917_at	AT5G25280	serine-rich protein-related
246901_at	AT5G25630	pentatricopeptide (PPR) repeat-containing protein
246909_at	AT5G25770	similar to Os06g0163200
246831_at	AT5G26340	Encodes a protein with high affinity
246796_at	AT5G26770	similar to unknown protein
246781_at	AT5G27350	Encodes a sugar-porter family protein that is induced during leaf senescence.

246783_at	AT5G27360	Encodes a sugar-porter family protein that unlike the closely related gene
246759_at	AT5G27950	kinesin motor protein-related; similar to kinesin motor protein-related
246701_at	AT5G28020	Encodes cysteine synthase AtcysD2.
246708_at	AT5G28150	similar to unknown protein
245925_at	AT5G28770	bZIP protein BZO2H3 mRNA
246651_at	AT5G35170	adenylate kinase family protein; similar to adenylate kinase
249694_at	AT5G35790	Encodes a plastidic glucose-6-phosphate dehydrogenase
249645_at	AT5G36910	Encodes a thionin
249622_at	AT5G37550	similar to unknown protein
249542_at	AT5G38140	histone-like transcription factor (CBF/NF-Y) family protein
249528_at	AT5G38720	similar to unknown protein
249475_at	AT5G38890	exoribonuclease-related; similar to exoribonuclease-like
249494_at	AT5G39050	transferase family protein
249493_at	AT5G39080	transferase family protein
249426_at	AT5G39840	ATP-dependent RNA helicase
249410_at	AT5G40380	protein kinase family protein; similar to protein kinase family protein
249411_at	AT5G40390	Encodes a protein which might be involved in the formation of verbascose.
249378_at	AT5G40450	similar to unknown protein
249355_at	AT5G40500	similar to Os04g0482900
249327_at	AT5G40890	Encodes a member of the voltage-dependent chloride channel.
249337_at	AT5G41080	glycerophosphoryl diester phosphodiesterase family protein
249315_at	AT5G41190	similar to unnamed protein product
249303_at	AT5G41460	fringe-related protein; similar to fringe-related protein
249266_at	AT5G41670	6-phosphogluconate dehydrogenase family protein
249265_at	AT5G41700	One of the polypeptides that constitute the ubiquitin-conjugating enzyme E2
249233_at	AT5G42150	electron carrier/ protein disulfide oxidoreductase
249204_at	AT5G42570	similar to unknown protein
249190_at	AT5G42750	Encodes a plasma-membrane associated phosphoprotein
249174_at	AT5G42900	similar to unknown protein
249138_at	AT5G43070	WPP1 (WPP domain protein 1)
249134_at	AT5G43150	similar to hypothetical protein MtrDRAFT_AC141109g4v1
249148_at	AT5G43260	chaperone protein dnaJ-related; similar to drought-induced protein 1
249091_at	AT5G43860	Encodes a chlorophyllase
249073_at	AT5G44020	acid phosphatase class B family protein; similar to acid phosphatase class B family protein
249011_at	AT5G44670	similar to unknown protein
249008_at	AT5G44680	methyladenine glycosylase family protein
248912_at	AT5G45670	GDSL-motif lipase/hydrolase family protein
248879_at	AT5G46180	ornithine delta-aminotransferase
248839_at	AT5G46690	BHLH071 (BETA HLH PROTEIN 71)
248870_at	AT5G46710	zinc-binding family protein; similar to zinc-binding family protein
248868_at	AT5G46780	VQ motif-containing protein
248820_at	AT5G47060	senescence-associated protein-related
248795_at	AT5G47390	myb family transcription factor; similar to myb family transcription factor
248786_at	AT5G47410	similar to hypothetical protein 25.t00068

248790_at	AT5G47450	Tonoplast intrinsic protein
248762_at	AT5G47455	similar to unknown protein
248753_at	AT5G47630	acyl carrier family protein / ACP family protein; similar to mtACP-1
248749_at	AT5G47880	Encodes a eukaryotic release factor 1 homolog.
248732_at	AT5G48070	putative xyloglucan endotransglycosylase/hydrolase
248744_at	AT5G48250	zinc finger (B-box type) family protein; Identical to CONSTANS-LIKE 10 (COL10)
248683_at	AT5G48490	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein
248686_at	AT5G48540	33 kDa secretory protein-related; similar to receptor protein kinase-related
248625_at	AT5G48880	Encodes a peroxisomal 3-keto-acyl-CoA thiolase 2 precursor.
248596_at	AT5G49330	Member of the R2R3 factor gene family.
248622_at	AT5G49360	encodes a beta-xylosidase located in the extracellular matrix.
248607_at	AT5G49480	AtCP1 encodes a novel Ca2+-binding protein
248614_at	AT5G49560	similar to unknown protein
248510_at	AT5G50315	Mutator-like transposase family
248466_at	AT5G50720	Encodes one of five HVA22 homologs in Arabidopsis.
248467_at	AT5G50800	nodulin MtN3 family protein; similar to nodulin MtN3 family protein
248471_at	AT5G50840	similar to nuclear matrix constituent protein-related
248463_at	AT5G51130	similar to Os08g0540500
248451_at	AT5G51180	similar to unknown protein
248410_at	AT5G51570	band 7 family protein
248375_at	AT5G51710	member of Putative potassium proton antiporter family
248381_at	AT5G51830	pfkB-type carbohydrate kinase family protein
248398_at	AT5G51970	sorbitol dehydrogenase
248337_at	AT5G52310	cold regulated gene
248357_at	AT5G52380	zinc knuckle (CCHC-type) family protein; similar to zinc knuckle (CCHC-type) family protein
248329_at	AT5G52780	similar to unknown protein
248326_at	AT5G52820	WD-40 repeat family protein / notchless protein
248303_at	AT5G53170	encodes an FtsH protease that is localized to the chloroplast and the mitochondrion
248252_at	AT5G53250	AGP22/ATAGP22 (ARABINOGALACTAN PROTEINS 22)
248243_at	AT5G53590	auxin-responsive family protein
248236_at	AT5G53870	plastocyanin-like domain-containing protein
248186_at	AT5G53880	unknown protein
248207_at	AT5G53970	encodes tyrosine aminotransferase
248185_at	AT5G54060	UF3GT (UDP-GLUCOSE:FLAVONOID 3-O-GLUCOSYLTRANSFERASE)
248160_at	AT5G54470	zinc finger (B-box type) family protein; similar to zinc finger (B-box type) family protein
248136_at	AT5G54910	DEAD/DEAH box helicase
248140_at	AT5G54980	integral membrane family protein; similar to integral membrane family protein
248100_at	AT5G55180	glycosyl hydrolase family 17 protein; similar to hydrolase
248101_at	AT5G55200	co-chaperone grpE protein
248082_at	AT5G55400	fimbrin-like protein
248036_at	AT5G55920	nucleolar protein
248045_at	AT5G56030	a member of heat shock protein 90 (HSP90) gene family.

248007_at	AT5G56260	Regulator of ribonuclease-like protein 3
247989_at	AT5G56350	pyruvate kinase
247983_at	AT5G56630	phosphofructokinase family protein
247977_at	AT5G56850	similar to unknown protein
247954_at	AT5G56870	beta-galactosidase
247957_at	AT5G57050	Encodes a protein phosphatase 2C
247937_at	AT5G57110	Arabidopsis-autoinhibited Ca2+ -ATPase
247942_at	AT5G57120	similar to unknown protein
247926_at	AT5G57280	similar to SAM (and some other nucleotide) binding motif
247914_at	AT5G57540	xyloglucan:xyloglucosyl transferase
247921_at	AT5G57660	zinc finger (B-box type) family protein; Identical to CONSTANS-LIKE 5 (COL5)
247880_at	AT5G57780	similar to transcription factor
247882_at	AT5G57785	contains InterPro domain Helix-loop-helix DNA-binding; (InterPro:IPR011598)
247838_at	AT5G57990	Encodes a ubiquitin-specific protease.
247851_at	AT5G58070	lipocalin
247816_at	AT5G58260	Encodes subunit NDH-N of NAD(P)H:plastoquinone dehydrogenase complex (Ndh complex)
247819_at	AT5G58350	Encodes a member of the WNK family (9 members in all) of protein kinases
247786_at	AT5G58600	Belongs to a large family of plant-specific genes of unknown function.
247775_at	AT5G58690	phosphoinositide-specific phospholipase C family protein
247776_at	AT5G58700	phosphoinositide-specific phospholipase C family protein
247754_at	AT5G59080	similar to unknown protein
247763_at	AT5G59180	DNA-directed RNA polymerase II; Identical to DNA-directed RNA polymerase II
247739_at	AT5G59240	40S ribosomal protein S8 (RPS8B); Identical to 40S ribosomal protein S8-2 (RPS8B)
247655_at	AT5G59820	ZAT12
247650_at	AT5G59960	similar to Os01g0151600
247649_at	AT5G60030	similar to unknown protein
247638_at	AT5G60490	FLA12 (fasciclin-like arabinogalactan-protein 12)
247593_at	AT5G60790	member of GCN subfamily
247608_at	AT5G60990	DEAD/DEAH box helicase
247575_at	AT5G61030	encodes a glycine-rich RNA binding protein.
247550_at	AT5G61370	pentatricopeptide (PPR) repeat-containing protein
247524_at	AT5G61440	thioredoxin family protein; Identical to Thioredoxin-like 3
247540_at	AT5G61590	encodes a member of the ERF subfamily B-3 of ERF/AP2 transcription factor family.
247497_at	AT5G61770	brix domain-containing protein; Identical to Peter Pan-like protein (PPAN)
247498_at	AT5G61810	mitochondrial substrate carrier family protein
247488_at	AT5G61820	similar to hypothetical protein
247487_at	AT5G62150	peptidoglycan-binding LysM domain-containing protein
247463_at	AT5G62210	embryo-specific protein-related; similar to ATS3 (ARABIDOPSIS THALIANA SEED GENE 3)
247478_at	AT5G62360	invertase/pectin methylesterase inhibitor family protein
247454_at	AT5G62440	similar to defective chloroplasts and leaves protein-related / DCL protein-related
247453_at	AT5G62440	similar to defective chloroplasts and leaves protein-related / DCL protein-related

247396_at	AT5G62930	GDSL-motif lipase/hydrolase family protein; similar to carboxylic ester hydrolase
247378_at	AT5G63120	ethylene-responsive DEAD box RNA helicase
247377_at	AT5G63180	pectate lyase family protein; Identical to Probable pectate lyase 22 precursor
247374_at	AT5G63190	MA3 domain-containing protein
247361_at	AT5G63480	similar to OSJNBa0053B21.8
247356_at	AT5G63800	member of Glycoside Hydrolase Family 35
247318_at	AT5G63990	3'(2')
247268_at	AT5G64080	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein
247323_at	AT5G64170	dentin sialophosphoprotein-related; similar to unknown protein
247287_at	AT5G64230	similar to unknown protein
247282_at	AT5G64240	latex-abundant family protein (AMC3) / caspase family protein
247278_at	AT5G64380	fructose-1
247284_at	AT5G64410	oligopeptide transporter
247277_at	AT5G64420	DNA polymerase V family; similar to zinc finger protein-related
247241_at	AT5G64680	similar to Os01g0259900
247175_at	AT5G65280	lanthionine synthetase C-like family protein; similar to catalytic
247162_at	AT5G65730	xyloglucan:xyloglucosyl transferase
247168_at	AT5G65860	ankyrin repeat family protein
247119_at	AT5G65900	DEAD/DEAH box helicase
247072_at	AT5G66490	similar to unknown protein
247043_at	AT5G66880	encodes a member of SNF1-related protein kinases (SnRK2)
246985_at	AT5G67290	FAD-dependent oxidoreductase family protein; similar to Os07g0155100
246996_at	AT5G67420	LOB domain protein 37 / lateral organ boundaries domain protein 37 (LBD37)
247013_at	AT5G67480	BT4 (BTB AND TAZ DOMAIN PROTEIN 4)
264270_at	AT1G60260	Unknown Protein
251230_at	AT3G62750	Unknown Protein
265572_at	AT2G28210	Unknown Protein
248743_at	AT5G48240	Unknown Protein
254963_at	AT4G11060	Unknown Protein
267315_at	AT2G34720	Unknown Protein
245866_s_at	multiple	purine permease-related; similar to ATPUP18 (Arabidopsis thaliana purine permease 18)
254331_s_at	multiple	member of CYP706A
248566_s_at	multiple	Encodes a putative ferric chelate reductase.
249658_s_at	multiple	phosphoglycolate phosphatase
258977_s_at	multiple	encodes a monofunctional aspartate kinase
265444_s_at	multiple	a member of the plasma membrane intrinsic protein subfamily PIP2.
263048_s_at	multiple	similar to unknown protein
245877_at	multiple	Potential natural antisense gene
255908_s_at	multiple	similar to unknown protein
247388_s_at	multiple	Encodes a protein with similarity to a subunit of the CCAAT promoter motif binding complex
257175_s_at	multiple	cyclopropane-fatty-acyl-phospholipid synthase
245783_s_at	multiple	similar to unknown protein
247266_at	multiple	Encodes a beta-d-xylosidase that belongs to family 3 of glycoside hydrolases.

261144_s_at	multiple	wound-responsive family protein; similar to wound-responsive protein-related
257634_s_at	multiple	putative cytochrome P450
255895_at	multiple	12-oxophytodienoate reductase
256337_at	multiple	serine-type endopeptidase inhibitor; similar to Os03g0734300
264022_at	multiple	unknown protein
267126_s_at	multiple	hydrolase
262733_s_at	multiple	lipase
250670_at	multiple	polygalacturonase inhibiting protein 1 (PGIP1) mRNA
264654_s_at	multiple	sugar transporter family protein
253879_s_at	multiple	glycosyltransferase family protein
266932_s_at	multiple	DEAD box RNA helicase
250151_at	multiple	Encodes a microRNA.
261664_s_at	multiple	mitochondrial import inner membrane translocase subunit Tim17/Tim22/Tim23 family protein
265941_s_at	multiple	recA family protein
266720_s_at	multiple	pseudo-response regulator
258449_s_at	multiple	DEAD box RNA helicase
267162_s_at	multiple	phosphoribosylaminoimidazole carboxylase family protein / AIR carboxylase family protein
251775_s_at	multiple	encodes a delta1-pyrroline-5-carboxylate synthase
266184_s_at	multiple	phosphate transporter (AtPT2)
255685_s_at	multiple	tetrahydrofolate dehydrogenase/cyclohydrolase
249888_s_at	multiple	zinc finger (ZPR1-type) family protein
263823_s_at	multiple	encodes a member of the DREB subfamily A-2 of ERF/AP2 transcription factor family
246173_s_at	multiple	pentatricopeptide (PPR) repeat-containing protein
265670_s_at	multiple	similar to unknown protein
259077_s_at	multiple	reversibly glycosylated polypeptide possibly involved in plant cell wall synthesis
254740_s_at	multiple	SHM5 (SERINE HYDROXYMETHYLTRANSFERASE 5)
256376_s_at	multiple	S-adenosyl-L-methionine:carboxyl methyltransferase family protein
262054_s_at	multiple	heat shock protein 70
262306_s_at	multiple	SYNC3
267335_s_at	multiple	glycosyl hydrolase family 17 protein
256595_x_at	multiple	gypsy-like retrotransposon family (Athila)
254952_at	multiple	lipase class 3 family protein
246481_s_at	multiple	cold and ABA inducible protein kin1

Literature Cited

Cook, D., S. Fowler, et al. (2004). "From The Cover: A prominent role for the CBF cold response pathway in configuring the low-temperature metabolome of Arabidopsis." Proceedings of the National Academy of Sciences 101(42): 15243.

Hiratsu, K., K. Matsui, et al. (2003). "Dominant repression of target genes by chimeric repressors that include the EAR motif, a repression domain, in Arabidopsis." The Plant Journal **34**(5): 733-739.

McKemy, D. D., W. M. Neuhausser, et al. (2002). "Identification of a cold receptor reveals a general role for TRP channels in thermosensation." Nature **416**(6876): 52-58.