The Control of Transpiration. Insights from Arabidopsis[1]

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Stomatal complexes in the epidermes of aerial plant parts are critical sites for the regulation of gas exchange between the plant and the atmosphere. Stomata consist of microscopic pores, each flanked by a pair of guard cells. Guard cells can increase or decrease the size of the pore via changes in their turgor status, hence regulating both CO_2 entry into the leaf and transpiration, or the loss of water from the leaf. This *Update* focuses on recent progress in our understanding of the regulation of transpiration and drought tolerance that has been garnered through the use of Arabidopsis (*Arabidopsis thaliana*) as a model experimental system.

The coordinated regulation of gas exchange is integral to land plant survival because CO_2 must be able to penetrate the leaf to allow photosynthesis, yet water loss (transpiration) must be minimized to prevent desiccation, drought stress, and plant death. Transpiration also provides the driving force for the transport of water and nutrients from the roots to the aerial tissues, and the evaporation of water from the substomatal cavity cools the plant (Lambers et al., 1998). While a number of morphological traits can contribute to the overall level of leaf gas exchange (e.g. the density and distribution of stomata, leaf epidermal structure and internal organization, cuticle thickness), the regulation of stomatal aperture size is unique in that it is a dynamic and reversible process by which water loss and CO_2 influx can be rapidly fine tuned in response to a number of environmental and intrinsic signals, such as light, CO_2, and the plant stress hormone abscisic acid (ABA; Schroeder et al., 2001). Because guard cells integrate and respond to a plethora of signals, they have become a model cell type in the field of plant cell signaling (Blatt, 2000; Schroeder et al., 2001; Roelfsema and Hedrich, 2005).

This *Update* highlights recent research reports on the guard cell physiology of Arabidopsis that include some quantitative measure of stomatal function. These measures include transpiration, stomatal conductance (stomatal conductance is defined as stomatal transpiration divided by the vapor pressure difference between the leaf and the air, and increases with increasing stomatal aperture), leaf water status, and water-use efficiency/transpiration efficiency (the ratio of photosynthetic assimilation to transpiration). By focusing the article in this manner, we hope to promote the synthesis of ideas and approaches between whole-plant physiologists and molecular biologists/geneticists. The former typically measure stomatal regulation of gas exchange and its impact on whole-plant physiology, and may treat the cellular and molecular biology of guard cells as a ''black box'' that receives and reacts to inputs. The latter typically use model plant species to investigate cell and molecular regulation of guard cell function, and may employ gene expression, stomatal aperture, or a specific guard cell parameter, such as ion fluxes, as a ''readout,'' without quantifying alterations in gas exchange and concomitant whole-plant impacts. Our premise is that Arabidopsis is an excellent reference plant in which these complementary approaches can be readily combined, and that such an integrated approach has great potential to yield new insights into the biology of transpiration in C_3 angiosperms.

GENETIC APPROACHES TOWARD THE CONTROL OF TRANSPIRATION

Arabidopsis is a powerful biological tool for the identification and characterization of the molecular regulators of transpiration because it has a small, sequenced genome and is easy to transform. These characteristics allow researchers to experimentally modulate the levels of candidate regulatory molecules via techniques such as RNA interference, insertional mutagenesis, or genetic overexpression, and many studies that employ such tools are discussed in the following sections. Additionally, the availability of collections of genetic mutants allows for large-scale screens for potential regulators of transpiration and for functional analyses of candidate regulators. For example, one such screen used infrared thermography to detect differences in leaf temperature, a correlate of transpiration, among a collection of Arabidopsis mutants (Merlot et al., 2002; Wang et al., 2004). The screen identified two novel mutations in stomatal regulation, *ost1* and *ost2*; *OST1* has been cloned and identified as encoding an Arabidopsis homolog of an ABA-activated protein kinase first identified in *Vicia faba* and is discussed further below (Li et al., 2000; Mustilli et al., 2002).

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Quantitative trait loci (QTL) analysis is an alternative to mutant analysis that harnesses naturally occurring variation within a species to identify putative genes and genomic regions involved in the regulation of quantitative traits such as transpiration (Alonso-Blanco and Koornneef, 2000). QTL mapping involves the generation of a segregating population for a particular trait, often either an F_2 population or a population of homozygous recombinant inbred lines. The population is then phenotyped for the traits of interest and genotyped using molecular markers. Statistical techniques are then employed to link specific genotypes to traits, which allows for the mapping of traits to particular chromosomal regions. Arabidopsis is a useful species for QTL analysis because of its small size and rapid life cycle; large mapping populations can be grown in a small space and recombinant inbred lines can be generated relatively quickly compared to other species (Alonso-Blanco and Koornneef, 2000). Additionally, once candidate genes of interest are identified, they can be further characterized using the molecular techniques mentioned above.

QTL analysis has led to the identification of a number of QTLs affecting transpiration efficiency in Arabidopsis (Juenger et al., 2005; Masle et al., 2005). It will be interesting to see to what extent these loci are found to encode known regulators of stomatal response, such as those discussed in subsequent sections, versus novel regulatory mechanisms. One example of the latter was provided by Masle and colleagues (Masle et al., 2005). Using QTL analysis, they identified one genetic locus, *ERECTA*, which encodes a Leu-rich repeat receptor-like kinase, as a genetic regulator of transpiration efficiency (Masle et al., 2005). Complementation of genotypes harboring mutations in *ERECTA* (including the common Arabidopsis ecotype Landsberg *erecta*) with the wild-type *ERECTA* allele results in increased transpiration efficiency and reduced stomatal conductance compared to *erecta* mutants.

HORMONAL REGULATION OF TRANSPIRATION

When plants are drought stressed, the plant hormone ABA accumulates in the shoot, where it both inhibits stomatal opening and promotes stomatal closure, resulting in reduced water loss from the plant. ABA is a key regulator of plant water status and stomatal function, and ABA and drought responses are the focus of the majority of the studies discussed in this *Update*. It is important to note that the terms drought stress and drought tolerance are used in this review just as they were reported in the original references. In these references, it is usually the case that a plant is deemed drought tolerant if it survives a restricted watering regime. However, if the effect of, e.g. a genetic manipulation, is to reduce transpiration, then, under identical watering regimes, the mutant plant is actually experiencing less drought stress than the wild-type control plant.

Research on the effects of altered levels of ABA on transpiration spans several decades, starting with the discovery of the wilty *flacca* mutant of tomato (*Lycopersicon esculentum*), which is deficient in ABA synthesis (Tal, 1966). Recent research on this topic has taken advantage of the molecular genetic tools available in the Arabidopsis model system. Production of xanthoxin from epoxycarotenoids is a key step in ABA synthesis (Nambara and Marion-Poll, 2005). A family of seven 9-cis-epoxycarotenoid dioxygenase (*NCED*) genes is implicated in this process in Arabidopsis, of which *NCED3* is most strongly induced by drought (Iuchi et al., 2001). Iuchi and co-authors demonstrated that overexpression of *NCED3* resulted in elevated ABA levels, strong induction of the *RD29B* ABA reporter gene following drought onset, reduced transpiration under well-watered conditions, and improved drought survival. Antisense and T-DNA knockout lines exhibited the opposite phenotypes.

ABA levels in the plant reflect a balance between ABA synthesis and ABA catabolism into inactive forms by conjugation or oxidation. ABA oxidation to 8′-hydroxyl ABA (from which spontaneous isomerization to phaseic acid occurs) is catalyzed by four cytochrome P450 monooxygenases in Arabidopsis: CYP707A1 to 4. Of these, *CYP707A3* is most strongly induced by ABA during dehydration and rehydration (Umezawa et al., 2006). In a recent study, Shinozaki and colleagues characterized T-DNA insertional mutants and constitutive overexpressing lines of CYP707A3 (Umezawa et al., 2006). The T-DNA mutants exhibited greater ABA content under all conditions, more rapid expression of ''classic'' markers of ABA-induced gene expression (such as *RD29A* and *RAB18*), reduced transpiration, and improved survival after drought treatment. Conversely, *CYP707A3*-overexpressing lines exhibited lowered ABA content coupled with higher levels of the ABA metabolites phaesic acid and dihydrophaseic acid; these lines exhibited increased transpiration. Interestingly, transgenic alterations in levels of two RING-finger proteins, the RING-H2 protein XERICO and the R2R3-type MYB transcription factor HOS10, strongly affect *NCED3* transcript levels, with correlated effects on ABA levels, drought tolerance, and water loss (Zhu et al., 2005; Ko et al., 2006).

Numerous genetic mutants in Arabidopsis with alterations in production, sensing, or response to all the major plant hormones provide a wealth of resources with which to investigate hormonal regulation of transpiration. Tanaka and colleagues have used such tools to investigate hormonal cross talk between ABA, ethylene, cytokinins, and auxins in the regulation of stomatal apertures (Tanaka et al., 2005, 2006). When ethylene levels were increased, either via provision of exogenous ethylene or through use of the ethylene-overproducing mutant *eto1-1*, ABA-induced stomatal closure in epidermal peels was retarded, and greater rates of fresh weight decrease in excised shoots were observed. The effects seem specific to the ABA response, as no alterations in dark-induced stomatal

closure were seen. Treatment of epidermal peels with cytokinins (6-benzyladenine or kinetin) or auxins (naphthaleneacetic acid or indole-3-acetic acid) similarly opposed ABA-induced stomatal closure. Tanaka et al. hypothesize that these hormones act indirectly, through enhancement of ethylene production, since the repressive effects of 6-benzyladenine and naphthaleneacetic acid on ABA-induced stomatal closure were negated by genetic (use of the *ein3-1* ethylene-insensitive mutant) or pharmacological (application of 1-methylcyclopropene, a competitive inhibitor of ethylene-receptor binding) abrogation of ethylene signaling. These studies illustrate the interconnectivity of hormone signaling in plant systems, an emerging theme in phytohormone research (Gazzarrini and McCourt, 2003; Ko et al., 2006).

REGULATION OF TRANSPIRATION BY ION CHANNELS AND TRANSPORTERS

Stomatal conductance is altered by the opening and closing of stomata, processes which in turn are mediated via changes in the turgor status of the adjacent guard cells. Changes in guard cell turgor result from water influx or efflux into the cell following changes in cell water potential, which arise from alterations in symplastic ion concentrations. Stomatal opening occurs when K^+, Cl^-, malate^{2-}, and Suc accumulate inside the cells, resulting in water entry into the guard cells and the outbowing and opening of the stomatal pore. Stomatal closure occurs following K^+ and anion efflux, resulting in loss of water from the cell, a reduction in cell turgor, and pore closure (Schroeder et al., 2001). Therefore, the channels and transporters responsible for ion transport across cell membranes are key regulators in the control of stomatal aperture and plant water loss.

Signals resulting in changes in stomatal aperture alter the activities of a number of ion channels and transporters. For example, ABA can promote stomatal closure and inhibit stomatal opening in part by stimulating an increase in cytosolic Ca^{2+} levels via activation of plasma membrane and endomembrane Ca^{2+}-permeable channels (Sanders et al., 2002; Fan et al., 2004; Hetherington and Brownlee, 2004). The increase in cytosolic Ca^{2+} is a signal that initiates anion efflux and consequent plasma membrane depolarization, which inhibits inward-rectifying K^+ channels and activates outward-rectifying K^+ channels (Schroeder et al., 2001). A net movement of ions out of the cell causes water efflux and closure of the stomatal pore.

The major outward-rectifying K^+ channel involved in guard cell closure in Arabidopsis is encoded by the *GORK* gene (Hosy et al., 2003). Functional analyses of *gork* mutants suggest that GORK plays an important role in the regulation of transpiration. *gork-1* T-DNA insertional mutants and *gork-dn1* dominant-negative mutants displayed reduced ABA- and dark-induced stomatal closure in isolated epidermal peels and increased water loss from excised rosettes compared to wild-type plants (Hosy et al., 2003). Whole-rosette gas-exchange analysis revealed that the *gork-1* mutants transpired more, especially under water-stressed conditions, and had slower reductions in transpiration when light-acclimated plants were placed in the dark (Hosy et al., 2003).

Because K^+ influx is critical for stomatal opening, inward-rectifying K^+ channels, such as KAT1, are also candidate transpiration regulators. Analysis of an Arabidopsis mutant harboring a transposon-induced mutation in *KAT1*, however, found no altered stomatal functioning or regulation of transpiration, suggesting genetic redundancy may exist for inward-rectifying K^+ channels in guard cells (Kwak et al., 2001; Szyroki et al., 2001). Indeed, a number of genes encoding inward-rectifying K^+ channels are expressed in guard cells of Arabidopsis, including *KAT1*, *KAT2*, *AKT1*, *AtKC1*, and *AKT2/3* (Szyroki et al., 2001). To avoid the confounding effects of likely functional redundancy among K^+ channels, Schroeder and colleagues used a dominant-negative approach to decrease the overall level of functional inward-rectifying K^+ channels in Arabidopsis (Kwak et al., 2001). Transgenic plants overexpressing a dominant-negative mutant form of KAT1 displayed reduced inward K^+ current and guard cell K^+ content (Kwak et al., 2001). These mutant *KAT1* lines also had reduced light-induced stomatal opening, reduced water loss from excised leaves, and increased water content in leaves following drought stress compared to empty-vector control lines, supporting a role for inward-rectifying K^+ channels in the regulation of transpiration.

In addition to functioning in cellular detoxification, two ATP-binding cassette transporters that are expressed in guard cells, AtMRP4 and AtMRP5, are also involved in the control of transpiration, possibly as regulators of ion channel activity (Leonhardt et al., 1997, 1999; Klein et al., 2003, 2004). *mrp5* mutants were insensitive to ABA promotion of stomatal closure but displayed reduced light-induced stomatal opening (Klein et al., 2003). Whole-plant and leaf gas-exchange measurements showed reduced transpiration in the *mrp5* mutant compared to control, concomitant with an approximately 20% increase in instantaneous water-use efficiency, and *mrp5* mutants had reduced water loss from excised leaves and were less wilty than wild-type plants under drought conditions (Klein et al., 2003). These data suggest that in the *mrp5* mutant, the reduction in light-induced stomatal opening and resultant decrease in transpiration are more important to maintaining whole-plant water status than any increase in water loss due to reduced ABA sensitivity of stomatal closure. Interestingly, *mrp4* mutants display phenotypes opposite to those of *mrp5*; *mrp4* mutants have larger stomatal apertures in both the light and the dark and exhibit increased water loss from excised leaves. Nevertheless, *mrp4* mutants retain ABA sensitivity of stomatal closure (Klein et al., 2004). Gas-exchange measurements reveal that *mrp4*

mutants have increased transpiration and reduced water-use efficiency, and wilt earlier than wild type when drought stressed (Klein et al., 2004).

To date, no genes encoding anion channels involved in stomatal movements have been definitively identified, although members of the ATP-binding cassette transporter family are being scrutinized as candidates. However, a guard cell-expressed NO_3^- transporter, AtNRT1.1/CHL1, has been shown to function in NO_3^--dependent stomatal opening and plant drought responses (Guo et al., 2003). *chl1* mutants show no altered sensitivity to ABA but show reduced NO_3^- uptake and light-stimulated stomatal opening when NO_3^- is the sole anion available, presumably because, under these conditions, NO_3^- is the only anion available to serve as a counter ion for K^+ uptake. Replacement of NO_3^- with Cl^- eliminates altered stomatal opening in the mutant (Guo et al., 2003). Additionally, when grown in substrates containing NO_3^-, *chl1* mutants are more drought tolerant and have reduced transpiration compared to wild type (Guo et al., 2003). Interestingly, wild-type plants lost more water from excised leaves when NO_3^- was present, suggesting that NO_3^- availability allowed for wider apertures (Guo et al., 2003). Taken together, these data suggest that the amount of NO_3^- in the soil can affect stomatal regulation and magnitude of transpiration, and this NO_3^- effect is in part mediated by NO_3^- uptake into guard cells via the NO_3^- transporter CHL1.

In Arabidopsis, 20 Glu receptor-like (*GLR*) genes have been identified, and evidence is accumulating that suggests that the GLR proteins may function as nonselective cation channels (Davenport, 2002). One putative plant Glu receptor, AtGLR1.1, has recently been implicated in functioning in ABA biosynthesis, ABA signaling, and control of transpiration (Kang et al., 2004). Antisense *AtGLR1.1* lines had smaller stomatal apertures, reduced transpiration rates, and were more drought resistant than wild-type plants (Kang et al., 2004). Consistent with these results, these lines also had higher transcript levels of ABA biosynthetic genes and higher levels of ABA, as well as reduced expression of *ABI1* and *ABI2* genes, which encode negative regulators of ABA response. The mechanism by which an alteration in cation flux would influence gene expression remains unknown.

CONTROL OF TRANSPIRATION BY CELLULAR SIGNALING MECHANISMS

The appropriate transduction of abiotic stress signals into cellular and developmental responses is of paramount importance in both natural and agroecosystems (J.Z. Zhang et al., 2004; Chaerle et al., 2005; Wang et al., 2005). Accordingly, the identification of intracellular second messengers for drought and ABA is a major area of research in plant biology (Rock, 2000). It is impossible to do justice to ABA signaling within the constraints of this article; for a more comprehensive discussion of this topic in the context of guard cell physiology, readers are pointed toward several excellent reviews (Blatt, 2000; Schroeder et al., 2001; Sheng, 2003; Roelfsema and Hedrich, 2005; Verslues and Zhu, 2005). Instead, in this section, we have chosen to exemplify the progress that is being made by focusing on just one second messenger of guard cell ABA signaling, ABI1, and the web of molecules with which it is being found to interact. ABI1 is chosen first because it is an important regulator of ABA responses and second because it is one of the best-studied second messengers in guard cells.

ABI1 is a type 2C protein phosphatase (PP2C). The first *ABI1* mutant to be characterized was the dominant-negative mutant *abi1-1* (Koornneef et al., 1989; Leung et al., 1994; Meyer et al., 1994). This mutant exhibits a strong ABA-insensitive, wilty phenotype (Koornneef et al., 1989), accompanied by elevated, ABA-insensitive stomatal conductance (Assmann et al., 2000). Subsequently, intragenic revertant recessive mutants and, more recently, T-DNA insertional mutants of *ABI1* were isolated (Gosti et al., 1999; Mishra et al., 2006; Saez et al., 2006). These mutants exhibit moderate ABA hypersensitivity in stomatal regulation, and this hypersensitivity is strongly enhanced when double mutants are created with the related PP2C genes *ABI2* (Merlot et al., 2001) or *HAB1* (Saez et al., 2004, 2006). Since loss of ABI1 results in ABA hypersensitivity, ABI1 is characterized as a negative regulator of ABA responses.

Some of the signaling components functioning upstream (Guo et al., 2002) and downstream of ABI1 have been identified. Downstream, production of reactive oxygen species (ROS) is impaired in the dominant-negative *abi1-1* mutant (Murata et al., 2001). Production of ROS is also impaired in the aforementioned *ost1* mutant (Mustilli et al., 2002); thus, OST1 likely functions upstream of the NADPH oxidases that produce ROS in guard cells (Kwak et al., 2003). However, the relative positions of OST1 and ABI1 in the signaling cascade are still unclear. ROS inhibit ABI1 activity (Meinhard and Grill, 2001), suggesting that that ROS and thus OST1 may function upstream of ABI1. On the other hand, OST1 was recently shown to physically interact with ABI1 in a yeast two-hybrid assay and the *abi1-1* mutant form of ABI1 inhibits ABA activation of OST1 (Yoshida et al., 2006), suggesting that OST1 might function downstream of ABI1. It is also important to note that these two possibilities are not mutually exclusive, e.g. OST1 activation of ROS production could be a regulatory, negative feedback mechanism on ABI1 and thus also feedback regulate OST1 activity. In the dominant *abi1-1* mutant, activation of ROS-activated, Ca^{2+}-permeable channels at the plasma membrane is also impaired (Murata et al., 2001), as is elevation of cytosolic Ca^{2+} (Allen et al., 1999). Activation of slow anion channels, which participate in the large anion efflux needed to drive stomatal closure, is likewise impaired in dominant-negative *abi1-1* plants because these channels are Ca^{2+} activated

(Pei et al., 1997). Because all of these responses are inhibited in dominant-negative *abi1-1*-insensitive mutant plants, it is reasonable to hypothesize that they may be strengthened in recessive, ABA-hypersensitive *abi1* mutants. ABI1 also physically interacts with the transcription factor ATHB6 (Himmelbach et al., 2002). As discussed in the next section, overexpression studies show that ATHB6 is a negative regulator of ABA-induced gene expression, and perhaps it is activated by ABI1.

Given that ABI1 is a negative regulator of ABA action, one would expect that the net result of ABA activation of components functioning upstream of ABI1 would be to inhibit the activity of this PP2C phosphatase. One of the enzymes activated by ABA in guard cells is phospholipase D (PLD; Jacob et al., 1999), which hydrolyzes phospholipids, producing a headgroup and phosphatidic acid (PA). Interestingly, the lipid metabolite PA binds to ABI1 and inhibits its activity (W. Zhang et al., 2004). Knockdown of PLDα1 in Arabidopsis by antisense methods increases stomatal conductance and impairs drought tolerance (Sang et al., 2001; W. Zhang et al., 2004), effects that would be consistent with loss of inhibition of ABI1 in the PLDα1 antisense guard cells. Indeed, a mutant version of ABI1 that is unable to bind PA but has normal phosphatase activity also results in hyposensitivity of ABA-induced stomatal closure (Mishra et al., 2006).

PLDα1 also has additional roles in modulation of ABA inhibition of inward K^+ channels and stomatal opening, through a pathway that involves the heterotrimeric G protein α-subunit GPA1(Jacob et al., 1999; Wang et al., 2001; Coursol et al., 2003; Zhao and Wang, 2004; Mishra et al., 2006). Since the GPA1-dependent pathway is proposed to be ABI1 independent (Mishra et al., 2006), readers are referred to the cited references for further details.

The above summary has focused only on ABI1, and literally dozens of ABA-regulated secondary messengers have been identified in guard cells. A figure that summarizes the current guard cell signaling network for ABA-induced stomatal closure, including the portion described above, has recently been published (Li et al., 2006). Ultimately, the power of computational and systems biology approaches will be needed to derive comprehensive and predictive models of ABA signaling, and the paper by Li et al. describes one such approach (Li et al., 2006).

CONTROL OF TRANSPIRATION VIA MODULATORS OF GENE EXPRESSION

Recent evidence suggests that, in addition to rapid cellular signaling events, gene expression changes also function in the regulation of stomatal aperture size and transpirational water loss in Arabidopsis. Table I summarizes names and functions of regulators of gene expression that have been implicated in the control of transpiration. Two R2R3-MYB domain transcription factors, *AtMYB60* and *AtMYB61*, both guard cell expressed, have been shown to play opposite roles in the regulation of diurnal stomatal movements (Cominelli et al., 2005; Liang et al., 2005). The *atmyb60-1* T-DNA insertional mutant displays reduced sensitivity toward light-induced stomatal opening, reduced water loss from excised leaves, and reduced transpirational water loss when drought stressed as measured by the relative water content of the rosette leaves (Cominelli et al., 2005). Conversely, *myb61* mutants display reduced dark-induced stomatal closure and increased stomatal conductance compared to wild type (Liang et al., 2005). The *atmyb60-1* and *atmyb61* mutants and overexpressing plants showed no altered sensitivities toward ABA (Cominelli et al., 2005; Liang et al., 2005). Therefore, it appears that AtMYB60 and AtMYB61 function specifically in the diurnal regulation of stomatal aperture and transpirational water loss.

Expression of a number of genes is controlled by ABA. Some of the ABA-induced genes serve protective functions in the plants, while others are regulatory in nature, such as protein kinases, protein phosphatases, and transcription factors (Rock, 2000). One method to identify potential regulators of ABA-modulated gene expression and thus of transpiration is to screen for proteins that bind to ABA-responsive cis-elements, such as ABREs, found in the promoters of a number of ABA up-regulated genes (Busk and Pages, 1998). ABF3 and ABF4 are basic Leu zipper (bZip) proteins that were identified via a yeast one-hybrid screen as ABRE-interacting proteins (Kang et al., 2002). Compared to wild type, transgenic lines overexpressing ABF3 or ABF4 exhibited drought tolerance and reduced water loss from excised rosette leaves (Kang et al., 2002). Conversely, *abf3* and *abf4* mutants are more susceptible to drought than wild type (Kim et al., 2004). Based on reporter gene analysis (Kang et al., 2002), both *ABF3* and *ABF4* are expressed in leaf tissues, including guard cells, suggesting that they may influence stomatal function in part through direct regulation of gene expression in guard cells. Consistent with this idea, transcripts of the *KAT1* and *KAT2* genes, which encode inward K^+ channels that mediate K^+ uptake during stomatal opening, are repressed in *ABF3*-overexpressing lines (Kang et al., 2002).

Another ABRE-binding protein, the bZip protein ABF2 (also known as AREB1), has been shown to confer drought tolerance when overexpressed (Kim et al., 2004). However, in this case, transgenics overexpressing a constitutively active form of ABF2 did not exhibit a reduction in water loss (Fujita et al., 2005; Furihata et al., 2006). Instead, drought tolerance may have been conferred because there was increased expression of a number of ABA-induced genes, including LATE EMBRYOGENESIS ABUNDANT class proteins, which are thought to serve protective functions. Thus, these experiments illustrate the fact that plants employ a diversity of mechanisms to achieve drought tolerance, only some of which involve alterations in stomatal regulation.

Table I. *Transcription factors, chromatin-remodeling factors, and RNA-processing proteins implicated in drought and ABA regulation of transpiration in Arabidopsis and discussed in this article*

In this table, "Mutant" refers to recessive underexpressing or null lines; "OEX" refers to overexpressing lines.

Locus	Gene Name	Function/Putative Function	Type of Line: Whole-Plant Phenotype	Putative Role in Transpiration Regulation	References
At1g08810	*AtMYB60*	R2R3-MYB transcription factor	Mutant: reduced water loss from excised and drought-stressed leaves	Function in diurnal regulation of transpiration	Cominelli et al. (2005)
At1g09540	*AtMYB61*	R2R3-MYB transcription factor	Mutant: increased stomatal conductance OEX: reduced stomatal conductance	Function in diurnal regulation of transpiration	Liang et al. (2005)
At1g35515	*HOS10*	R2R3-MYB transcription factor	Mutant: increased water loss from excised shoots	Positive regulator of ABA biosynthetic gene, *NCED3*, and ABA levels	Zhu et al. (2005)
At4g34000	*ABF3/AREB3*	ABRE-binding bZip transcription factor	Mutant: susceptible to drought stress OEX: reduced water loss from excised leaves, drought tolerant	Positive regulator of ABA response	Kang et al. (2002), Kim et al. (2004)
At3g19290	*ABF4/AREB2*	ABRE-binding bZip transcription factor	Mutant: susceptible to drought OEX: reduced water loss from excised leaves, drought tolerant	Positive regulator of ABA response	Kang et al. (2002), Kim et al. (2004)
At2g22430	*ATHB6*	HD-zip transcription factor	OEX: increased water loss from excised leaves	Negative regulator of ABA signaling	Himmelbach et al. (2002)
At3g20310	*AtERF7*	AP2/EREBP-type transcription factor	OEX: increased water loss from excised leaves, susceptible to drought	Negative regulator of ABA signaling	Song et al. (2005)
At5g03740	*AtHD2C/HDT3*	Histone deacetylase	OEX: reduced water loss from excised leaves	Tissue-specific regulator of ABA signaling	Sridha and Wu (2006)
At5g44200	*CBP20*	Nuclear mRNA cap-binding protein	Mutant: reduced stomatal conductance, drought tolerant	Negative regulator of ABA signaling	Papp et al. (2004)
At2g13540	*ABH1/CBP80*	Nuclear mRNA cap-binding protein	Mutant: reduced stomatal conductance, wilt tolerant	Negative regulator of ABA signaling	Hugouvieux et al. (2002)

Transcription factors serving as negative regulators of ABA signaling may also play a role in the regulation of transpiration. One such repressor is ATHB6, a HD-zip protein that interacts with ABI1, a PP2C and known negative regulator of ABA responses (Himmelbach et al., 2002). Transgenic plants overexpressing ATHB6 exhibit increased water loss from excised leaves and reduced stomatal closure following leaf detachment compared to control plants (Himmelbach et al., 2002). A second transcriptional repressor of ABA response is AtERF7, an AP2/EREBP-type transcription factor that binds to the GCC-box ABRE and can be phosphorylated by protein kinase PKS3, a negative regulator of ABA signaling (Guo et al., 2002; Song et al., 2005). In lines overexpressing AtERF7, ABA-induced up-regulation of two genes containing GCC boxes in their promoters was shown to be eliminated. These lines also displayed increased water loss from excised leaves, decreased drought tolerance compared to wild type, and hyposensitivity toward ABA-induced stomatal closure compared to wild type (Song et al., 2005), leading to the conclusion that AtERF7 suppresses positive regulators of ABA response. Conversely, RNA interference lines that had reduced levels of AtERF7 displayed ABA hypersensitivity (Song et al., 2005).

Interestingly, in transient expression assays, repression of ABA-induced genes by AtERF7 is enhanced by the histone deacetylase HDA19 (Song et al., 2005). In addition, AtERF7 interacts with a transcriptional corepressor, AtSin3, which may interact with HDA19 (Song et al., 2005). This suggests a role for histone deacetylation and chromatin remodeling in ABA regulation of gene expression (Song et al., 2005).

AtHD2C, one of four plant-specific HD2-type histone deacetylases, is also implicated in ABA regulation of gene expression (Sridha and Wu, 2006). AtHD2C-overexpressing plants display up-regulation of the ABA-responsive genes *RD29B* and *RAB18*, and reduced transcript levels of *ABI2*, a negative regulator of ABA response. Consistent with these results, AtHD2C-overexpressing plants also display drought tolerance

and reduced water loss from excised leaves (Sridha and Wu, 2006). However, overexpression of AtHD2C also confers reduced sensitivity toward ABA in ABA inhibition of germination and root growth, indicating that the role of AtHD2C in ABA response may exhibit tissue and cell specificity.

Proteins involved in the posttranscriptional modifications of mRNAs also play a role in the regulation of stomatal movements. Plants harboring mutations in genes encoding two subunits of the nuclear cap-binding complex, CBP20 and ABH1/CBP80, display marked ABA hypersensitivity (Hugouvieux et al., 2001; Papp et al., 2004). *abh1* mutants are hypersensitive to ABA induction of cytosolic Ca^{2+} elevation in guard cells and stomatal closure, and wilt less than wild type following drought stress (Hugouvieux et al., 2001). In the absence of exogenous ABA, *abh1* mutants exhibit reduced inward K^+ currents and enhanced anion efflux currents, responses that accord well with the reduced stomatal apertures and stomatal conductances seen under these conditions, and are consistent with hypersensitivity to endogenous ABA (Hugouvieux et al., 2002). *cbp20* mutants similarly display drought tolerance and have reduced stomatal conductance compared to wild type (Papp et al., 2004).

Although transcription factors have long been known to participate in ABA regulation of plant development, the studies cited above are providing new information on the roles of transcription factors in the dynamic regulation of stomatal movement (Rock, 2000). In addition, compelling new information on roles of chromatin-remodeling factors and RNA-processing proteins in ABA responses suggests that we have only scratched the surface with regard to the intricate mechanisms by which modulators of gene expression participate in the control of transpiration.

CONCLUSIONS AND PERSPECTIVES

This *Update* has illustrated some of the recent progress that is being made in understanding the control of transpiration at the whole-plant, cellular, and molecular levels, using Arabidopsis as a model system. We hope that this brief review will encourage increased collaboration among researchers studying this phenomenon at disparate levels of biological organization.

Drought and ABA are two environmental signals that were discussed in depth in this article. Yet, guard cells respond to a wide diversity of environmental cues (Hetherington and Woodward, 2003). Studies that assess impacts of light (Kinoshita et al., 2001; Sothern et al., 2002), CO_2 (Hashimoto et al., 2006; Teng et al., 2006; Young et al., 2006), and humidity (Assmann et al., 2000; Yoshida et al., 2002; Xie et al., 2006) on transpiration, while not discussed here, are equally important to our knowledge of transpirational control. Finally, while this article has focused on levels ranging from the molecular to the whole plant, it is important to note that Arabidopsis is found in natural ecosystems (Pigliucci, 2002; Mitchell-Olds and Schmitt, 2006). Thus, Arabidopsis is also proving to be a valuable tool for ecophysiological and ecological studies of how plant populations in situ respond to water availability and other environmental signals that impact the control of gas exchange (McKay et al., 2003; Engelmann and Schlichting, 2005), topics that were not covered in this brief *Update*.

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