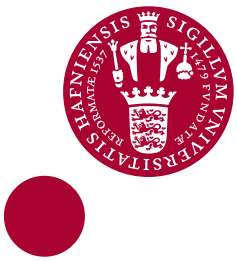

ATAF1 transcription factor directly regulates abscisic acid biosynthetic gene NCED3 in Arabidopsis thaliana

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ATAF1 transcription factor directly regulates abscisic acid biosynthetic gene *NCED3* in *Arabidopsis thaliana*☆

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ABSTRACT

ATAF1, an *Arabidopsis thaliana* NAC transcription factor, plays important roles in plant adaptation to environmental stress and development. To search for ATAF1 target genes, we used protein binding microarrays and chromatin-immunoprecipitation (ChIP). This identified T[A,C,G]CGT[A,G] and TT[A,C,G]CGT as ATAF1 consensus binding sequences. Co-expression analysis across publicly available microarray experiments identified 25 genes co-expressed with *ATAF1*. The promoter regions of *ATAF1* co-expressors were significantly enriched for ATAF1 binding sites, and TTGCGTA was identified in the promoter of the key abscisic acid (ABA) phytohormone biosynthetic gene *NCED3*. ChIP-qPCR and expression analysis showed that ATAF1 binding to the *NCED3* promoter correlated with increased *NCED3* expression and ABA hormone levels. These results indicate that ATAF1 regulates ABA biosynthesis.

Introduction

Abscisic acid (ABA) controls numerous physiological processes in plants and is best known for its regulatory role in abiotic stress responses [1,2]. Upon drought and high salinity, ABA promotes desiccation tolerance by stomatal closure, enabling plants to adapt to water stress. ABA also regulates developmental processes such as seed germination, vegetative growth and bud dormancy [3–5]. More recent studies have shown that ABA also impacts plant biotic stress signaling [6,7].

Consequently, although the pathways of ABA biosynthesis and catabolism are largely defined (reviewed by Nambara and Marion-Poll [8]), understanding the regulation of these pathways is important. In particular, the cleavage of 9-cis-epoxycarotenoids to xanthoxin catalyzed by 9-cis-epoxycarotenoid dioxygenases (NCEDs) during ABA biosynthesis, which is believed to be the key regulatory step of ABA biosynthesis [9], deserves elucidation. In rice, Yaish et al. reported that over-expression of the APETALA-2-like transcription factor (TF) *OsAP2–39* is associated with the up-regulation of the ABA biosynthetic gene *OsNCED-I* leading to an increase in endogenous ABA levels [10]. Among the five *NCED* genes in *Arabidopsis*, *NCED3* plays a key role in ABA biosynthesis during water deficit [11,12], and *nced3* mutants exhibit increased water loss and reduced ABA levels in vegetative tissues [12]. Recently, Jiang et al. reported the identification of a gain-of-function *acquired drought tolerance* (*adt*) mutant to be a WRKY TF conferring increased drought tolerance, ABA levels and direct *NCED3* promoter binding [13]. Apart from APETALA-2-like OsAP2–39 and *adt*, no other direct transcriptional regulators of *NCED* genes have been reported.

We previously highlighted NAC (petunia NAM and Arabidopsis ATAF1, ATAF2, and CUC2) TFs as components related to ABA and biotic stress signaling [6,14]. Overall, NAC genes encode a large, plant-specific family of TFs with roles in many aspects of growth, development and environmental stresses [15,16]. The N-terminal region of NAC proteins contains the highly conserved NAC domain encompassing a homo- and heterodimerization region indispensable for DNA-binding [17]. Moreover, a number of reports have identified core NAC

Abbreviations: ABA, abscisic acid; ATAF1, *Arabidopsis thaliana* activating factor 1; DBD, DNA-binding domain; ChIP, chromatin-immunoprecipitation; NAC, NAM, ATAF1/2, CUC2; NCED3, 9-cis-epoxycarotenoid dioxygenase-3; PBM, protein-binding microarrays; PWM, position weight matrix; SnRK, Sucrose nonfermenting 1(SNF1)-related serine/threonine-protein kinase; TF, transcription factor.

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DNA-binding sites [18–20], and the consensus binding site (BS) [T,A][T,G][T,A,C,G]CGT[G,A] has been proposed from studies of *Arabidopsis* ANAC019 [17]. Indeed, phylogenetically distant members of the NAC TF family bind this NAC-BS *in vitro*, albeit with various affinities [21]. This suggests that there may be non-canonical *k*-mers relevant for various NAC TF family members that are not yet defined.

NAC members of the ATAF clade of the NAC family exhibit strong and transient expression patterns to ABA treatment and environmental stress [22–24]. Additionally, *ATAF1* is ubiquitously expressed in various organs, including stomatal guard cells [23]. We previously reported that *ataf1* mutants are ABA-hyposensitive during seedling development and germination [14]. In line with this, plants over-expressing *ATAF1* were shown to be ABA hypersensitive [24]. Also, ATAF1 was identified in a yeast two-hybrid screen to interact with Sucrose nonfermenting 1(SNF1)-related serine/threonine-protein kinase 1 (SnRK1) subunits AKIN10 and AKIN11 [25], positive regulators of ABA metabolism and key integrators of transcription networks in response to stress and energy signaling [26,27].

To improve our understanding of the regulatory potential of ATAF1, we here delineate the DNA-binding specificity of ATAF1 using protein binding microarrays (PBM), co-expression analyses and chromatin-immunoprecipitation (ChIP). This identifies abscisic acid biosynthetic *NCED3* as an ATAF1 regulatory target gene. In plants over-expressing *ATAF1*, this correlates with increased *NCED3* transcript abundance and, most importantly, increased ABA phytohormone levels. Taken together, our data indicate that ATAF1 is a regulator of ABA biosynthesis in *Arabidopsis*.

Results

ATAF1 consensus-binding site

We used protein-binding microarrays (PBM) as an unbiased strategy to search for ATAF1 consensus-binding motifs. PBM permits the identification of TF DNA binding specificities at single base resolution [28,29]. Since *Arabidopsis* NACs only bind DNA as dimers [17], we first established whether ATAF1 can homodimerize. Yeast-2-hybrid experiments showed that ATAF1 homodimerizes independently from the ATAF1 C-terminal transcriptional regulatory domain (Fig. 1A). We then heterologously expressed and purified a GST-tagged version of the ATAF1 DNA-binding NAC domain (residues 1–165; Fig. 1B) and incubated PBMs using this protein. Subsequent PBM analysis identified T[A,C,G]CGT[A,G] and TT[A,C,G]CGT 6-mers as the most significant descriptors for ATAF1 binding. The ATAF1 binding specificity position weight matrix (PWM), derived from all relevant 8-mers bound by ATAF1, is graphically illustrated in Fig. 1C (see also Supplementary Fig. S1).

The ATAF1 co-expression cluster is enriched for ATAF1 consensus binding sites

Co-expression occurs among TFs and target genes, and co-expression clusters can be enriched for common TF binding-sites [30,31].

To complement our PBM data to search for direct target genes of ATAF1, we data-mined >8.500 ATH1 microarray samples from the Genevestigator [32] data repository. Using a stringent (>2-fold regulation, $P < 0.05$) selection criterion for *ATAF1* transcript level perturbations, we found 403 microarray slides from 87 perturbations. Using this data set we identified 25 top-ranking genes co-expressed with *ATAF1* ($r \geq 0.66$) (Table 1). Hierarchal clustering of *ATAF1* and its co-expressors identified strong induction by ABA application, drought, osmotic and salt stresses (Fig. 2A), confirming earlier ATAF1 studies [23,24]. Also, the *ATAF1* cluster exhibited distinct expression patterns during biotic stress (Fig. 2A). Using the PBM-derived ATAF1 binding oligomers and the POBO program [33], we analyzed whether the promoters of the *ATAF1* gene cluster have an over-representation of ATAF1 binding sites. Bootstrapping analysis using 1000 promoter sets of the background *Arabidopsis* genome and the *ATAF1* co-expression cluster, respectively, identified a significant ($P < 0.001$) enrichment of both T[A,C,G]CGT[A,G] and TT[A,C,G]CGT in the *ATAF1* cluster compared to background genomic distribution (Fig. 2B).

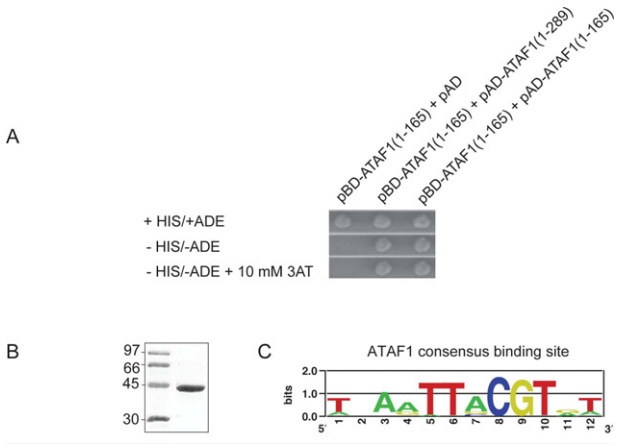

Fig. 1. ATAF1 homodimerization and consensus binding site. (A) Fusion proteins of Gal4-(DBD)–ATAF1(1–165), Gal4-(AD)–ATAF1(1–289), Gal4-(AD)–ATAF1(1–165), and empty Gal4-(DBD) were co-transformed, expressed in yeast, and screened after 7 days for transactivation activity of *HIS3* and *ADE2* reporter genes. (B) SDS–PAGE and Coomassie Blue staining of gel molecular-mass-markers (lane 1; molecular masses in kDa at left) and approx. 5 μg of affinity-purified recombinant GST–ATAF1(1–165). (C) Consensus binding site of ATAF1 from duplicate PBM experiments.

ATAF1 directly regulates the abscisic acid biosynthetic gene NCED3

In addition to ABA-inducible expression, the list of *ATAF1* co-expressed genes also includes the key regulatory ABA biosynthetic gene *NCED3*. We performed two experiments to investigate whether ATAF1 directly regulates ABA biosynthesis. First, to investigate the correlation between ATAF1 and ABA hormone biosynthesis *in planta*, we produced plants over-expressing *ATAF1* (*35S:ATAF1-HA*). In agreement with earlier studies [24,25,34], these plants showed stunted growth and delayed flowering (Fig. 3A–C, and Supplementary Fig. S2). The observed phenotype of these plants correlated with increased *ATAF1* transcript levels and were not associated with transgene silencing of *ATAF1* and other *ATAF* subclade members, as reported by Kleinow et al. ([25], Figs. 3B and 4A). Using these plants, we observed *ATAF1* over-expressing plants to have significant (approx. 6- and 7.5-fold) increased ABA levels ($P < 0.01$) compared to wild-type and *ataf1* mutants, respectively (Fig. 3D).

Second, to determine whether ATAF1 binds the promoter of *NCED3 in vivo*, we performed ChIP on wild-type and *35S:ATAF1-HA* plants (Fig. 3A). Subsequent qPCR identified a region (position −1134 to −1265 bp) including a TTGCGTA ATAF1 binding motif to be enriched in ChIPs from *ATAF1* over-expressing plants (Fig. 3E). ATAF1 did not bind a region between −120 and −218 bp (non-binding; NB), confirming ATAF1 binding specificity. As a technical control, the *FT* locus targeted by the HA-tagged TFL2 TF was included [35] (Fig. 3E). Finally, to assess the regulatory potential of ATAF1 we determined the transcript levels of *NCED3* and found ~10-fold increase in *ATAF1* over-expressing plants compared to wild-type. In contrast, mean *NCED3* levels were significantly lower in *ataf1* mutants (Fig. 3F), substantiating the strong positive correlation between *ATAF1* and *NCED3* transcript levels (Fig. 2A). In addition to *NCED3*, transcript levels of several other top-ranking *ATAF1* co-expressed genes displayed *ATAF1*-dependent expression perturbations, though with a narrower dynamic range compared to *NCED3* (Fig. 4B).

Table 1
Top 25 *ATAF1* co-expressed genes.[a]

AGI	Pear. corr. coeff	Description	TTVCGT[b]	TVCGTR[b]
AT5G61820	0.7855	Unknown protein		+
AT5G05410	0.7705	DRE-binding protein 2A (DREB2A)	+ + + + +	+ + + + + + + + + +
AT1G77450	0.7477	NAC domain containing protein 32	+ +	+ + +
AT3G19580	0.7384	Zinc-finger protein 2	+ +	+ +
AT3G62260	0.7287	Protein phosphatase 2C family protein	+	+ +
AT5G59220	0.7167	Highly ABA-induced PP2C gene 1 (HAI1)	+ + +	+ + +
AT5G63790	0.7147	NAC domain containing protein 102	+ +	+ +
AT3G17770	0.7132	Dihydroxyacetone kinase	+	+ +
AT1G61340	0.7052	F-box family protein		
AT1G21410	0.7037	F-box/RNI-like superfamily protein	+	+ + +
AT5G02020	0.6946	Encodes a protein involved in salt tolerance, names SIS		
AT5G04080	0.6932	Unknown protein	+ +	+ + +
AT2G33700	0.6925	Protein phosphatase 2C family protein	+	+ +
AT4G27410	0.6901	NAC (No Apical Meristem) domain protein	+	+ + +
AT1G05100	0.6893	Mitogen-activated protein kinase kinase kinase 18	+ +	+ + + +
AT5G62020	0.6889	Heat shock transcription factor B2A	+	+
AT5G04250	0.6865	Cysteine proteinases superfamily protein	+ + + +	+ + + + + + + +
AT4G37180	0.6788	Homeodomain-like superfamily protein	+	
AT3G14440	0.6773	Nine-cis-epoxycarotenoid dioxygenase 3 (NCED3)	+	+ + +
AT5G13810	0.6713	Glutaredoxin family protein		
AT4G34000	0.6675	Abscisic acid responsive elements-binding factor 3	+	+
AT5G57050	0.6659	Protein phosphatase 2C family protein (ABI2)	+	+
AT5G04340	0.661	Zinc finger of *Arabidopsis thaliana* 6	+ +	+
AT1G60190	0.659	ARM repeat superfamily protein	+	+
AT4G23050	0.6556	PAS domain-containing tyrosine kinase protein		

[a] Using 403 microarray samples from 87 different conditions where *ATAF1* was >2-fold regulated in treated samples compared to control samples.
[b] V = [A,C,G] and R = [A,G], + indicates number of ATAF1 binding 6-mers in 1 kb promoters.

Collectively, our data indicate that ATAF1 activates ABA hormone biosynthesis in plants, through transcriptional activation of *NCED3*.

Discussion

Here we identify ATAF1 as a positive regulator of ABA biosynthesis. We show that *ATAF1* and *NCED3* are co-expressed, and that ATAF1 binds the *NCED3* promoter *in vivo*. Most importantly, ATAF1-mediated induction of *NCED3* in plants over-expressing *ATAF1* correlates with increased ABA levels. In support of our observations, *ATAF1* exhibits strong expression in stomatal guard cells and in the vasculature [23], and plants over-expressing *ATAF1* have reduced stomatal aperture [24]. Interestingly, *NCED3* has a constrained spatial expression in vascular tissues [36], yet transient expression of *NCED3* in guard cells causes a decrease in stomatal aperture [37]. Hence, both at the transcript level and at the physiological level, the overlap between ATAF1 and NCED3 abundances and functionalities is evident. Hypersensitivity to ABA and increased endogenous ABA levels are often associated with increased tolerance to salt and drought stresses [38,39]. Thus, reduced ABA levels in plants may be associated with drought sensitivity [40]. We have previously reported that *ataf1* mutants are ABA-hyposensitive during seedling development and germination [14], and plants over-expressing *ATAF1* have been shown to be hypersensitive to ABA and drought tolerant [24]. Moreover, plants over-expressing *ATAF1* display stunted growth and delayed flowering, alike ABA-hypersensitive plants over-expressing the ATAF1 interaction partner SnRK1.1/AKIN10 [25,26,41]. This agrees with reports on ABA-deficient mutants displaying early flowering [42], and exogenous ABA application to delay flowering [43].

In addition to *NCED3*, several other *ATAF1* co-expressors showed displayed *ATAF1*-dependent expression perturbations. Specifically, significant changes in expression levels of both *ABI2* and *DREB2A* were observed in *aaf1–2* and *ATAF1*-overexpressing plants, respectively, compared to wild-type plants (Fig. 4B). *DREB2A* encodes a transcription factor regulating drought and osmotic-inducible genes [44,45], and ABI2, and other clade A type 2C protein phosphatases (PP2Cs), are known to be negative regulators of ABA signaling by dephosphorylation of ABA-activated Sucrose nonfermenting 1(SNF1)-related serine/threonine-protein kinases (SnRKs) [46–48]. We speculate that the positive correlation between *ATAF1* and transcript levels of genes encoding PP2Cs (Fig. 2, Table 1, and Fig. 4A) may reflect a negative feed-back loop needed to dampen the increased endogenous ABA

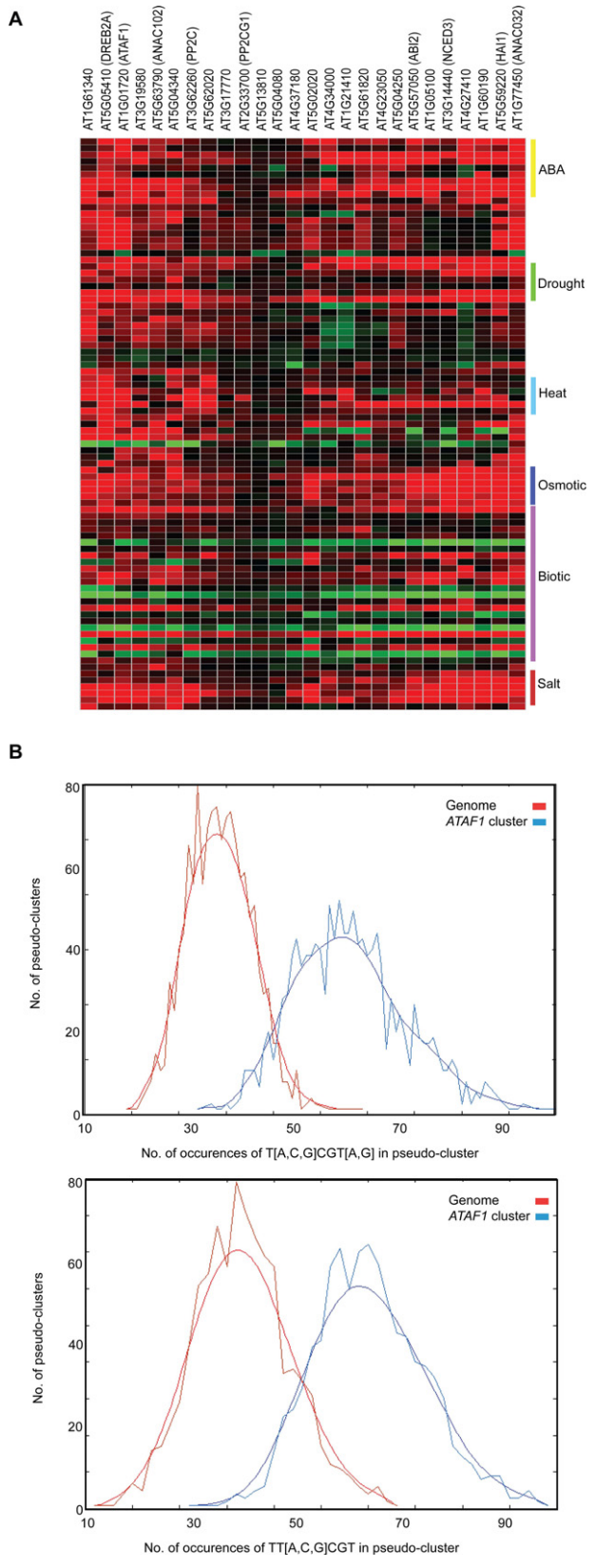

Fig. 2. *ATAF1* co-expression gene cluster is enriched for ATAF1 binding sites. (A) Using 403 microarray samples from 87 conditions either significantly inducing or repressing ATAF1 expression, identifies 25 co-expressed genes with a Pearson correlation coefficient >0.66. (B) Bootstrapping analyses using POBO [33] show that promoters of ATAF1 co-expressed genes are significantly ($P < 0.001$) enriched for ATAF1 consensus binding sites T[A,C,G]CGT[A,G] and TT[A,C,G]CGT.

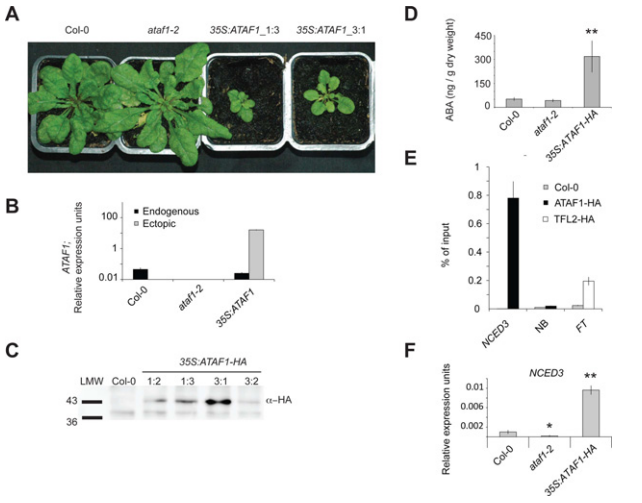

Fig. 3. Ectopic expression of *ATAF1* arrests plant development and activates ABA biosynthesis. (A) Eight week-old short-day grown Col-0 wild-type and *ataf1–2*, compared to plants ectopically expressing HA-tagged ATAF1. Two independent *35S:ATAF1-HA* lines (1:3 and 3:1) display growth reduction. (B) Quantitative expression profiles of endogenous and ectopic *ATAF1* in Col-0 wild-type, *ataf1–2* and *35S:ATAF1-HA* plants. Mean (±sem) relative expression units (log 10 scale) are displayed using *ACT2* as reference. Bars represent the mean of three biological replicates. (C) Expression of HA-tagged ATAF1 using SDS–PAGE and Western blot. The blot was probed with an anti-HA antibody. Representative result from three replicates is shown. The unspecific band at 37 kDA serves as a loading control. (D) Endogenous levels of ABA were measured. Bars represent the mean of three biological replicates. Error bars represent ±1 standard deviation. Statistical analyses were performed using Student's *t*-test of the differences between individual means compared to Col-0 ($^{**}P < 0.01$). (E) Direct binding of ATAF1 to the *NCED3* promoter (between position –1136 and –1265 bp) was analyzed using ChIP-qPCR on *35S:ATAF1-HA* over-expressing (ATAF1-HA) and Col-0 wild-type plants. A non-binding control (NB) 1 kb downstream of the ATAF1–*NCED3* binding site, and a positive TFL2 TF binding-site control (*FLOWERING LOCUS T* (*FT*), [35]) were included. The ChIP results obtained by three independent replicate experiments are represented as percentage of input (%IP), and the error bars indicate ±1 standard deviation. (F) Expression of ABA biosynthetic gene *NCED3* in *ATAF1* over-expressing plants compared to wild-type Col-0 and *ataf1* mutants. Expression level of *NCED3* was determined by qPCR in the indicated genotypes. Mean (±sem) relative expression units are displayed using *ACT2* as reference. Bars represent the mean of three biological replicates. Statistical analyses were performed using Student's *t*-test of the differences between individual means compared to Col-0 ($^{*}P < 0.05$, $^{**}P < 0.01$).

levels associated with *ATAF1* induction or over-expression.

Finally, we note that our unbiased PBM-approach recovered a binding-site similar to that reported for ANAC019 [17], and that functional redundancy has been reported for NAC TFs [49,50]. Overlapping polymorphisms within NAC binding sites, could also explain why residual *NCED3* and ABA levels are observed in *ataf1* mutant plants (Fig. 3D and F). This also includes the potentially physiologically relevant ATAF1–ATAF2 heterodimerization reported by Wu et al. ([24], and Supplementary Fig. S4). However, using PBM to uncover binding site preferences for all basic helix-loop-helix (bHLH) TFs from *Caenorrhabditis elegans*, none of the bHLH proteins that participate in heterodimeric interactions were shown to exhibit significant sequence-specific DNA binding on their own [51]. In our study, ATAF1 on it's own shows sequence specificity to DNA-binding (Fig. 1C). Knowing that dimerization-deficient NAC mutants do not bind DNA [17], this indicates that top-ranking oligomers in this study are *bona fide* targets of ATAF1 homodimers. In combination with stringent co-expression analysis using hundreds of genome-wide expression data sets, our data emphasize the value of *in vitro*-defined oligomers in estimating TF binding sites and identification of target genes. Ideally, probing all plant TFs using such analyses should uncover the complex transcriptional imprint required to fine-tune plant hormone homeostasis.

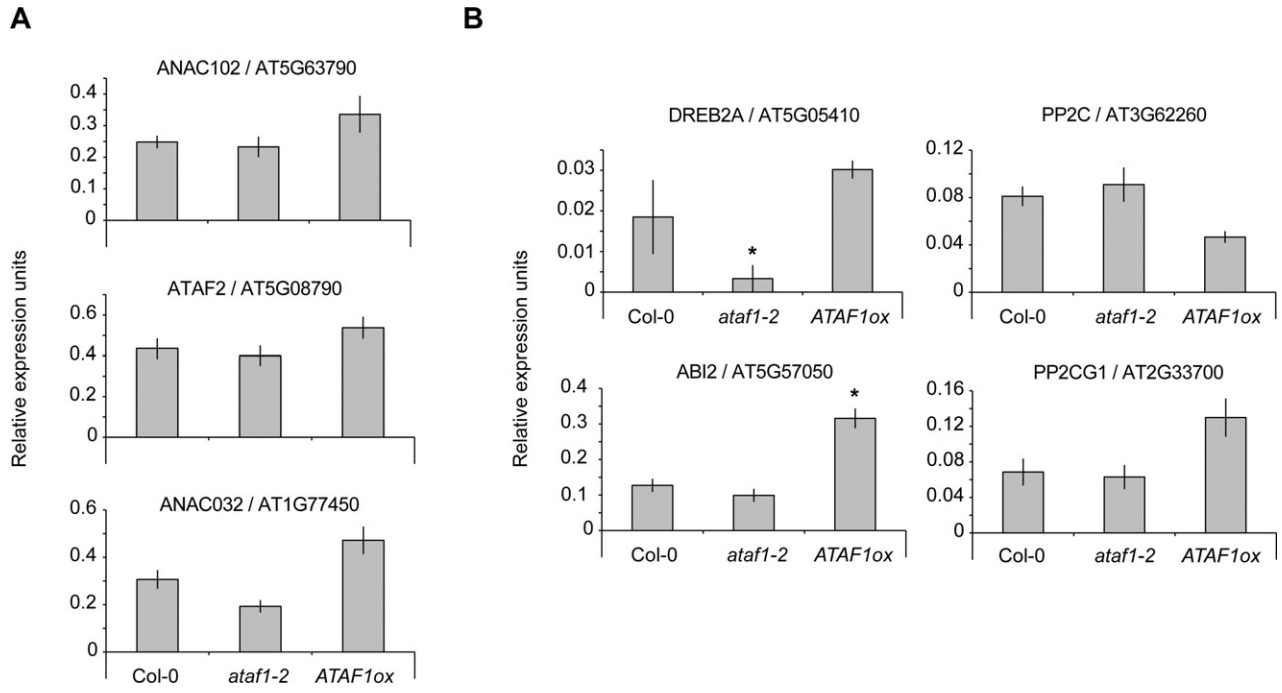

Fig. 4. Expression perturbations of *ATAF* subclade members and *ATAF1* co-expressed genes. (A) Expression of genes encoding ATAF subclade NAC TFs. Expression level of candidate genes was determined by qPCR in the indicated genotypes. Mean (± sem) relative expression units are displayed using *ACT2* as reference. Bars represent the mean of three biological replicates. (B) Expression of ATAF1 co-expressed genes in *ATAF1* over-expressing plants compared to wild-type Col-0 and *ataf1* mutants. Statistical analyses were performed using Student's *t*-test of the differences between individual means compared to Col-0 ($^*P < 0.05$).

Methods and materials

Plant materials and growth conditions

Arabidopsis thaliana wild-type accession Col-0 and *ataf1–2* mutant plants (T-DNA insertion line SALK-057618) [23], were grown on soil in controlled environment chambers under an 8 h light regime (150–170 $\mu E/m^2$ s) at 21 °C and 65% relative humidity.

Plasmid construction and plant transformation

The *ATAF1-HA* C-terminally tagged gene was generated by amplifying a full-length *ATAF1* cDNA obtained from ABRC with forward and reverse primers; AAAGAATTCATGTCAGAATTATTACAGTTGCC and CCGGGATCCCTAAGCGTAATCTGGTACGTCGTATGGGTAAGGCTTCTGCATGTAC, respectively, and cloned into pCAMBIA3300. Transformation of Col-0 plants was performed by the floral dip method [52] using *Agrobacterium tumefaciens* strain GV3101 (pMP90). Transgenic plants were selected by BASTA spraying, and homozygous T3 seeds from transformants expressing transgenes were used for subsequent analyses. The *35S:ATAF1-HA* construct used in this study complements the *ataf1* mutations [14], indicating that the addition of the HA epitope does not impair ATAF1 function.

Protein expression and purification

N-terminally GST-tagged, recombinant ATAF1(1–165) was cloned, expressed and purified as described [21].

Protein binding microarray

Microarray design, preparation, and PBM experiments were performed as described previously by Berger and Bulyk [53]. All experiments were performed using custom-designed "all 8-mer" arrays synthesized in a "4 × 44K" array format (Agilent Technologies, CA, USA) containing 4 copies of publicly available de Bruijn sequences [28,54]. Briefly, 200 nM of GST-ATAF1(1–165) protein was incubated on the microarray for 60′ at RT. TF–DNA interactions were detected by first incubating the array with a rabbit anti-GST polyclonal antibody (Invitrogen), followed by a Cy5 labeled anti-rabbit antibody (Jackson-Immuno, PA, USA). Blocking, protein binding and washing procedures were identical to standard PBM protocols [53].

Protein binding microarray data normalization and motif analysis

Microarrays were scanned using a SureScan scanner at a 2 μm resolution (Agilent Technologies, CA, USA), and spot intensities retrieved using Feature Extraction Software (Agilent Technologies, CA, USA). Data normalization and analysis were performed as described previously [53]. Resulting PWMs were graphically visualized using enoLOGOS [55].

Western blotting

Nuclear extracts were precipitated over-night with 80% acetone and proteins resuspended and boiled in SDS–PAGE loading buffer. Supernatants were separated by SDS–PAGE and proteins detected by Western blotting using monoclonal mouse anti-HA antibody (Sigma, MO, USA).

Yeast two-hybrid assays and qPCR analyses

Both methods were as described in [21]. Primers used to clone ATAF1(1–165) into pGBKT7 were AAGAATTCATGTCAGAATTATTACAGTTGCC and CCGGGATCCCCGCCTCTCGGTAGCTCC. Primers for pGADT7-ATAF1(1–165) and pGADT7-ATAF1(1–289) used forward primer AAGAATTCATGTCAGAATTATTACAGTTGCC. Reverse primers were CCGGGATCCCCGCCTCTCGGTAGCTCC and CCGGGATCCGTAAGGCTTCTGCATGTACATGAA, respectively. For quantitative real-time PCR (qPCR), *Actin2* (*ACT2*) was used as a reference. For *NCED3* we used AGCTCCTTACCTATGGCCAG and CGCTCTCTGGAACAAATTCATC. For endogenous *ATAF1* we used GTTGTTTACGGCGACGAAATC and

TAAAACGGTCTCGTGTTGCCATAA. For ectopic *ATAF1* we used GTTGTTTACGGCGACGAAATC and CGGCAACAGGATTCAATCTT. QPCRs were performed in triplicate for each individual line using Brilliant II SYBR Green qPCR kit (Stratagene, CA, USA) on an iCycler IQ (Bio-Rad, CA, USA). Quantification of CT (cycle threshold) values was achieved by calculating means of normalized expression using Q-gene software [56].

ChIP assay

Five-week old *35S:ATAF1-HA*, *35S:TFL2-HA* and Col-0 wild-type plants were harvested. The ChIP procedure was performed according to Reimer and Turck [57]. The DNA was sheared by sonication using a Mysonix sonicator (CT, USA) set to 3.5 output 10 × 10 s with 20 s. interval, and immunoprecipitated using anti-HA antibodies (Sigma, MO, USA). Each of the IPs was performed at least three independent times. For ChIP of *NCED3* we used CAGTTGTCTATTATCCTAGAAACCA and TGATGTAACACACCGAC. For the non-binding (NB) control we used; GGTTATAGAGGGAATTAAAAAGGG and GTCTCAAGTCTCAACTTTGAACC. For *FT* we used GCTCAAACATGTTGCTCGAA and TGCGATCAGTAAAATACACAGACA.

MS–LC for hormone quantifications

Quantifications were performed as described [58]. Statistical analyses were performed using Student's *t*-test ($^{**}P < 0.01$).

Accession numbers

The following accession codes were used: ATAF1, At1g01720; FL, At1g65480; NCED3, At3g14440.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fob.2013.07.006.

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