Fibrillin expression is regulated by abscisic acid response regulators and is involved in abscisic acid-mediated photoprotection

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Fibrillins are lipid-binding proteins of plastids that are induced under abiotic stress conditions. In response to environmental stress, plants generate abscisic acid (ABA) as an endogenous signal. We show that ABA treatment and fibrillin accumulation enhance the tolerance of photosystem II toward light stress-triggered photoinhibition in *Arabidopsis*. ABA induces fibrillin accumulation, and the ABA response regulators ABI1 and ABI2 regulate fibrillin expression. The abundance of fibrillin transcripts was specifically reduced in the ABA-insensitive *abi1* mutant but not in the *abi2* mutant. However, leaves of *abi2* revealed in comparison to WT and *abi1* enhanced fibrillin levels, pointing to a posttranscriptional control mechanism. Protein interaction analysis identified the protein phosphatase ABI2 to target the preprotein of fibrillin. Interaction was abrogated either by deleting the signal peptide of prefibrillin or by the single amino acid exchange present in the phosphatase-deficient abi2 protein. Thus, ABI1 and ABI2 seem to control fibrillin expression that is involved in mediating ABA-induced photoprotection.

ABI1 | ABI2 | abiotic stress | photoinhibition | light stress

Fibrillins are plastid-associated lipid-binding proteins that are ubiquitous in plants (1, 2). They have been primarily characterized from chromoplasts of tomato and pepper fruits and are known to accumulate during abiotic stress in plastids e.g., inflicted by high light, cold, and drought (3–5), and also during pathogen infection (6). The family of fibrillin-like proteins is unique to plants, and the members contain a hydrophobic domain that associates with or anchors within lipids (7). Fibrillins associate with stromal lamellae of thylakoids and fibrillic carotenoid-containing structures of chromoplasts (3, 8). A model for the fibrillic structures predicts a layer of fibrillin shielding polar lipids and carotenoids (1). In potato, the fibrillin C40.4 protein is specifically associated with the photosystem II (PSII) light-harvesting complex with a presumptive role in the modulation of photosynthetic efficiency (9). A posttranslational control of fibrillin accumulation by abscisic acid (ABA) was indicated by studies on ABA-deficient tomato that failed to accumulate fibrillin despite normal transcript levels (5). Although fibrillin is induced in response to abiotic stress, its role in stress responses and the molecular mechanism regulating its accumulation are still elusive.

ABA plays a major role in regulating plant growth and development and mediating adaptations to environmental stress such as cold, drought, and salinity (10). ABA regulates ion channel activities involved in osmoregulation and stomatal closure and influences gene expression at the transcriptional and posttranscriptional levels (11, 12). Key regulators of diverse ABA-mediated responses are ABI1 and ABI2. Both proteins are members of a larger family of plant protein phosphatases 2C (PP2Cs), several of which act negatively in a partially redundant manner on ABA responses (13–15). The Arabidopsis mutants *abi1-1 (abi1)* and *abi2-1 (abi2)* show a dominant ABA-insensitive phenotype conferred by an identical amino acid exchange within the catalytic domain that results in a strongly reduced protein phosphatases activity of abi1 ($ABI1^{Gly180Asp}$) and abi2 ($ABI2^{Gly168Asp}$) (16–19). Little is known about the substrates and cellular regulators of ABI1 and ABI2. Protein microinjection analysis revealed a competition of ABI1 and abi1 for common binding sites and an action downstream of Ca^{2+} in the ABA signal pathway (20). An interacting protein of ABI1 is the homeodomain transcription factor AtHB6 (21). AtHB6 is up-regulated by ABA and constitutes part of a negative feedback loop involved in the adjustment of plant's sensitivity to ABA. Furthermore, ABI1 and ABI2 physically interact with a SNF1-like protein kinase that forms a calcium-responsive negative regulatory circuit together with the calcium binding protein CaBP5 (22). The central role of ABI1 in ABA signal transduction is reflected by the finding that >90% of ≈1,300 identified ABA-responsive genes are deregulated in *abi1* (23). The massive ABA-evoked readjustment of gene expression is integrated into the plant's adaptive responses to environmental challenges (10, 24).

In this study, we establish a molecular link between the ABA signal pathway and fibrillin accumulation in *Arabidopsis*. Regulation of fibrillin expression involves the two ABA response regulators ABI1 and ABI2 that act at the transcriptional and posttranscriptional level, respectively. Fibrillin and ABA enhance the tolerance of PSII toward light stress-triggered photoinhibition.

Results

Identification of Prefibrillin as an Interacting Partner of ABI2. The key regulatory functions exerted by ABI1 and ABI2 in diverse ABA responses include regulation of gene expression, ion channel activity, and growth and development. Hence, both protein phosphatases are promising starting points for the elucidation of the integrative network of ABA signaling. We used the yeast-two-hybrid system to identify proteins interacting with ABI2. Fusion of ABI2 to the GAL4 DNA-binding domain did not reveal significant transcriptional autoactivation (Fig. 1*A*) in contrast to previous results with ABI1 in yeast (21). Screening of *Arabidopsis* cDNA libraries for interactors of ABI2 in yeast resulted in the identification of six clones that showed *lacZ* activation and histidine autotrophy in dependence on the expression of the cDNA fusion protein. DNA sequence analysis revealed that two cDNA fusions were identical and encoded the fibrillin precursor protein (At4g04020), of which the last three carboxyl-terminal amino acid residues were missing. The preprotein encompasses 318 amino acid residues with a transit peptide of 55 amino acid residues (25). Fibrillin shares high structural similarity with a second gene of *Arabidopsis*, fibrillin2 (At4g22240, 76% amino acid identity), and members of the protein family of other species such as the homologue of rapeseed (AAK57564, 79%), tobacco (T03635, 72%), tomato (CAA75658,

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Abbreviations: ABA, abscisic acid; FD, fibrillin-down-regulated lines; FU, fibrillin-up-regulated lines; PP2C, protein phosphatases 2C; PSII, photosystem II.

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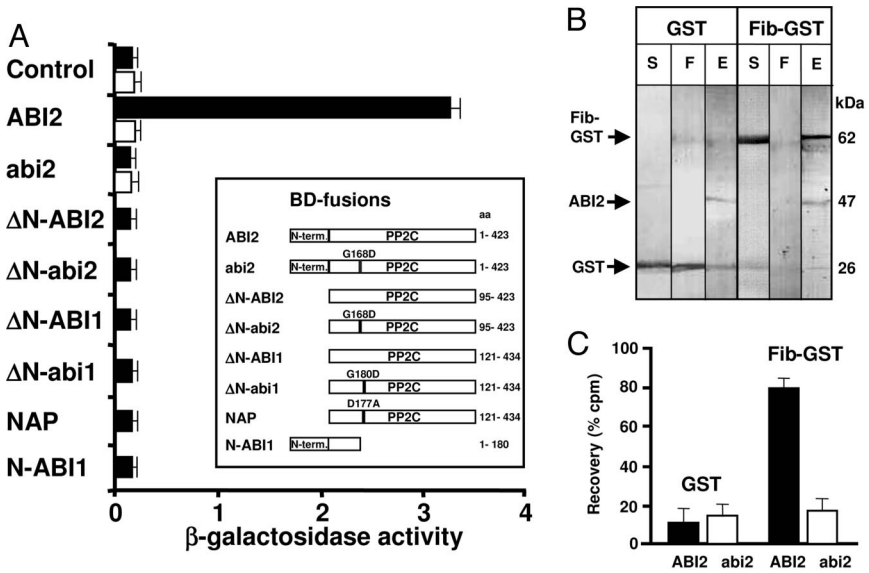

Fig. 1. Interaction of prefibrillin and fibrillin with ABI2. (*A*) The specificity of ABI2 interaction with prefibrillin was examined in the yeast two-hybrid system with the fibrillin preprotein and matured protein fused to the GAL4 activation domain (AD). The analysis included different ABI1 and ABI2 variants fused to the GAL4 DNA binding domain (BD fusions), which are schematically represented with the number of amino acid residues (*Inset*). The catalytic domain (PP2C) and the amino-terminal part (N-term) are highlighted. The single amino acid change of mutant abi1 and abi2 are presented as well as the mutation in the catalytically nonactive PP2C (NAP). Yeast cells expressing ABI2 and a nonchimeric AD domain were used as a control. Interaction of prefibrillin (filled bars) or fibrillin (open bars) is indicated by transactivation of the β-gal reporter above control level. Reporter activity is given in Miller units and was calculated as the mean value of three independent experiments (±SD). (*B*) Prefibrillin and ABI2 interaction *in vitro*. Purified GST and prefibrillin-GST fusion protein (Fib-GST) were analyzed with immobilized ABI2 by interaction chromatography. Comparable aliquots of fractions from the applied sample (S), flow through (F). and eluate of ABI2 and bound proteins (E) were analyzed for the presence of GST or Fib-GST by Western blot analysis with anti-ABI1-GST antibody that weakly cross-reacted with ABI2. Arrows indicate the positions of immunodetected proteins and the predicted M_r is indicated. (*C*) Discriminative binding of fibrillin to ABI2 and mutant abi2 analyzed by pulldown assay. Immobilized ABI2 and abi2 were incubated with radiolabeled Fib-GST or GST as control. The values present the recovery of initial label (10 kcpm) associated with the PP2C protein of two independent experiments (±SD).

64%), potato (T07825, 64%), cucumber (T10179, 64%), and pepper (S56633, 61%).

To further characterize the specificity and structural requirements of the interaction, different variants of ABI1 and ABI2 were fused to the binding domain and were analyzed in yeast (Fig. 1*A*). Truncation of the amino-terminal domain of ABI2 (ΔNABI2, amino acids 95–423), or the single point mutation in abi2, which results in catalytic deficiency, abolished the interaction. In addition, all variants of ABI1 tested failed to show significant interaction with fibrillin. Furthermore, deletion of the transit peptide of prefibrillin abrogated the interaction with ABI2 indicating that the ABI2 fusion protein binds to the preprotein rather than to the mature fibrillin.

The interaction was examined *in vitro* by both affinity chromatography and pulldown assays with purified preparations of ABI2 and prefibrillin. The fibrillin precursor protein was linked to the GST, and the resulting fusion protein (FIB-GST) was tested for binding to ABI2 tethered onto resin material by virtue of a carboxyl-terminal histidine tag. Subsequent chromatography of FIB-GST over the ABI2-coated resin resulted in efficient retention of the fusion protein but not of the GST control (Fig. 1*B*). Elution of ABI2 and other bound proteins yielded 83% of applied FIB-GST, whereas 24% of applied GST was recovered in that fraction. In the second experiment, the discrimination of ABI2 versus abi2 was analyzed in the interaction with prefibrillin by using a pulldown assay. ABI2- and abi2-coated beads were incubated with radiolabeled FIB-GST and GST, and subsequently the binding efficiency was determined. The majority of applied FIB-GST (78%) was associated with the immobilized ABI2, whereas only 17% of the preprotein was recovered with abi2-coated beads (Fig. 1*C*). The recovery of FIB-GST with abi2-coated beads is close to the values for the negative controls that consisted of the combinations GST with ABI2 or abi2 and yielded 10% and 14%, respectively. Taken together, the results strongly argue for a specific binding of ABI2 to the fibrillin preprotein *in vitro* and *in vivo*.

Gene Expression of Fibrillin Is Controlled by ABA and Depends on ABI1. Fibrillins are inducible proteins, and their expression is up-regulated by drought, cold, and oxidative and salt stress (3–6). These stress conditions involve ABA as a signal, and, hence, we examined whether ABA is able to regulate fibrillin gene expression. Exposure of *Arabidopsis* seedlings to ABA (30 μM) resulted in moderate enhancement of transcript levels within 3 h and with induction levels of ≈3-fold (Fig. 2*A*). Analysis of the dose–response dependence showed detectable up-regulation at 1 μM ABA reaching saturation at ≈10 μM ABA. Interestingly, analysis of ABA-insensitive *abi1* and *abi2* plants revealed a clear dependence of fibrillin transcript levels on abi1 but not abi2 (Fig. 2*B*). In the *abi1* mutant background, the mRNA abundance of fibrillin was lowered by a factor of >10 in comparison with WT and the *abi2* mutant. The basal transcript levels present in WT and *abi2* plants was recovered in abi1 in the presence of ≈100 μM exogenous ABA.

The observed difference in fibrillin transcript levels could reflect ABA-stimulated promoter activity or enhanced mRNA stability. To discern the action of ABA, a fibrillin promoter-luciferase construct was transfected into *Arabidopsis* protoplasts for transient analysis. ABA-responsive reporter expression was enhanced 2- to 3-fold in the presence of 1–30 μM ABA (Fig. 2*C*). Exposure of transfected protoplasts to light stimulated the fibrillin promoter as well, and a combined treatment of light and ABA further activated luciferase expression (Fig. 2*D*). However, coexpression of dominant-acting abi1 efficiently blocked ABA-mediated reporter induction in agreement with an ABA-inducible and ABI1-dependent gene expression of fibrillin.

Functional Analysis of Fibrillin. The emerging interaction of the ABA response pathway with fibrillin expression prompted us to examine

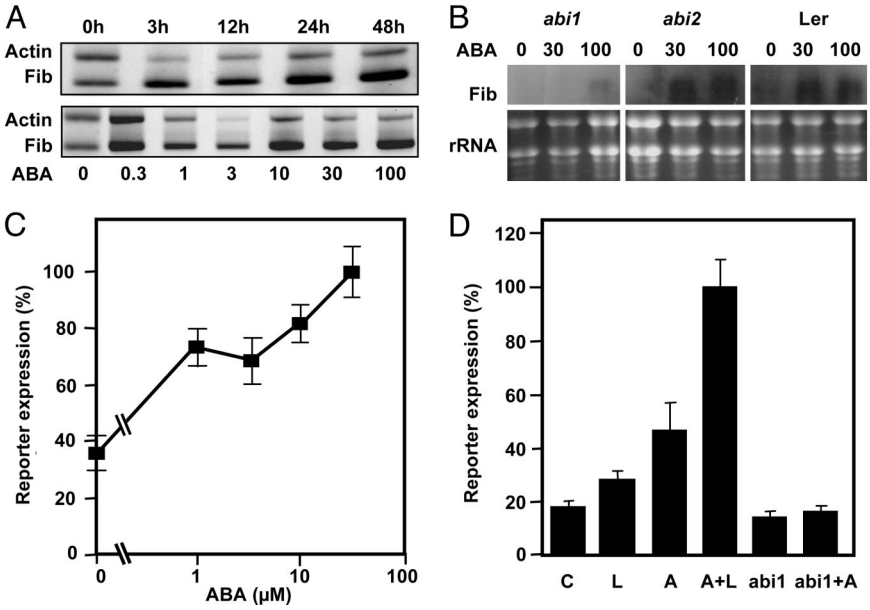

Fig. 2. Regulation of fibrillin expression by ABA. (*A*) RT-PCR analysis of fibrillin transcripts from seedlings exposed to exogenous ABA (30 μM) for different time (*Upper*) and dose–response dependence (*Lower*; 0–100 μM ABA, 24 h). cDNA of actin was coamplified as a standard. (*B*) Dependence of fibrillin expression on ABI1. Total RNAs were isolated from *Arabidopsis* seedlings (Ler) and the mutants *abi1* and *abi2*. Before RNA isolation, seedlings were treated with ABA for 24 h. Fibrillin transcripts were detected by Northern blot analysis (*Upper*). Ethidium bromide staining was used to visualize rRNA abundance (*Lower*). (*C*) *Arabidopsis* protoplasts were transfected with a reporter construct in which luciferase expression is controlled by the fibrillin promoter. Luciferase activity was determined in dependence of exogenous ABA and has been normalized to β-glucuronidase coexpressed from a constitutive promoter. The expression level at 30 μM ABA was arbitrarily set at 1. (*D*) Protoplasts analyzed as in *C* were either nontreated as control (C), challenged to light (L; 100 μmol·m^{-2}·s^{-1}), ABA (A; 30 μM), or a combination of both during phenotypic expression. In addition, protoplasts expressing abi1 in the absence (abi1) and the presence of 100 μM ABA (abi1+A) were analyzed for luciferase expression. The expression level in the combined light and ABA regime was set at 1.

a possible link between fibrillin, light stress, and ABA. A photoprotective role of ABA has been postulated for chloroplasts under cold conditions (26). Furthermore, fibrillin is known to accumulate during high-light conditions (5), and fibrillin affects photosynthetic efficiency (9). Taken together, the findings provided a hint that fibrillin and ABA might be involved in the plant response toward light stress. Therefore, we analyzed the effect of ABA and fibrillin on light-triggered photoinhibition of PSII by using plants with deregulated fibrillin accumulation. *Arabidopsis* plants of ecotype RLD with elevated or lowered levels of fibrillin were successfully generated by up- and down-regulation of fibrillin transcript abundance (Fig. 3). Two fibrillin-up-regulated lines (FU) and two fibrillin-down-regulated lines (FD) were randomly selected. Analysis revealed a 6-fold and >20-fold enhanced level of fibrillin transcripts in FU6 and FU7, respectively, whereas the level was lowered by a factor of 2 and 5 in the FD2 and FD7 compared with the control (Fig. 3*A*). ABA challenge (30 μM for 24 h) increased the fibrillin mRNA level in control plants but not in FD and FU lines. RT-PCR analysis discriminative for fibrillin and the homologous fibrillin2 revealed the down-regulation of only fibrillin transcripts (Fig. 3*B*). Immunoblot analysis with a fibrillin-specific antibody confirmed that altered transcript levels resulted in correspondingly altered protein levels (Fig. 3*C*).

The contribution of fibrillin and ABA toward photoprotection of PSII was examined by high-fluence irradiation of leaves in the absence or presence of ABA. Maximal photochemical efficiency of PSII (F_v/F_m) was determined after light stress and subsequent recovery period in the dark to allow for reversible adaptation processes. A light stress-dependent reduction of F_v/F_m values is interpreted as photoinhibition of PSII (27). Photoinhibition in leaves of *Arabidopsis* clearly depended on the duration of light stress and already was observed after 30 min of high-intensity illumination. A light stress of 1 h followed by the recovery period resulted in a drop of maximal photochemical PSII activity by 35% in leaves of RLD (Fig. 4*A*; see also Table 1, which is published as supporting information on the PNAS web site). In the analysis, the transgenic lines with deregulated fibrillin expression differed considerably in the degree of photoinhibition. Leaves of fibrillin-overexpressing lines revealed enhanced phototolerance, whereas those of fibrillin-down-regulated plants were more photoinhibited than RLD. The rate of photoinhibition after 1 h of light stress corresponded to ≈0.15% min^{-1} and 0.11% min^{-1} for PSII in FU6 and FU7, respectively, whereas the values were 2- to 3-fold higher in leaves of FD2 and FD10 corresponding to 0.33% min^{-1} and 0.37% min^{-1}, respectively ($P < 0.01$). Leaves of RLD revealed an intermediate rate of 0.22% min^{-1}, which was still 2-fold higher than the inactivation rate of the FU7 line.

Preincubation of detached leaves with exogenous ABA (25 μM for 4 h) before high fluence exposure changed the light stress-triggered photoinhibition in WT plants. Phototolerance was now improved to a level indistinguishable from the previously more phototolerant FU lines (Fig. 4*B* and Table 1). Interestingly, the phototolerance of FU lines was not further improved by ABA. ABA did not change the decline of F_v/F_m values in FD leaves, which were light-stressed up to 1 h ($P < 0.05$); however, longer periods lead to a partial recovery. Fibrillin levels were induced 4-fold and 2-fold within 2 h of exposure to ABA and light, respectively (Fig. 4*C*). Fibrillin accumulation occurred also in the FD line, however, at a reduced rate (Fig. 4*D*).

The results are consistent with an ABA-induced and fibrillin-dependent protection of the photosynthetic machinery. To gain insight into the role of ABI2 in that process, leaves of *abi2* and *ABI2* plants of the ecotype Landsberg (Ler) were analyzed for light-induced inactivation of photochemical PSII activity (Fig. 5*A*; see also Table 2, which is published as supporting information on the PNAS web site). Light stress up to 2 h led to an almost linear decline of F_v/F_m values at an inactivation rate of 0.27% min^{-1} and 0.13% min^{-1} in control and *abi2* leaves, respectively. The effective quan-

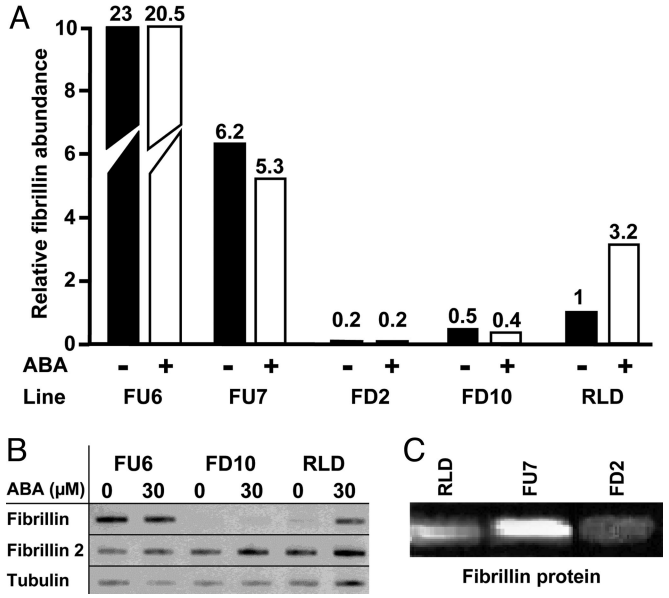

Fig. 3. Altered fibrillin expression in *Arabidopsis*. (*A*) Analysis by real-time RT-PCR. Transgenic *Arabidopsis* lines with deregulated fibrillin expression were generated by either up-regulation (FU lines) or down-regulation of transcript abundance (FD lines). Seedlings of FU and FD lines and a transgenic control (RLD) were exposed to ABA (30 μM) or water as a control for 24 h before transcript analysis. The abundance of fibrillin transcripts was normalized to actin transcripts. (*B*) Specificity of fibrillin transcript deregulation. Seedlings of FU6 and FD10 lines were examined for transcript levels of fibrillin and the homologous fibrillin2 by RT-PCR analysis. Tubulin transcripts were analyzed as a control. (*C*) Protein levels of fibrillin immunodetected in the membrane fraction of leaf extracts (100 μg protein per lane) from WT (RLD), FU7, and FD2 plants by using fibrillin-specific antibodies.

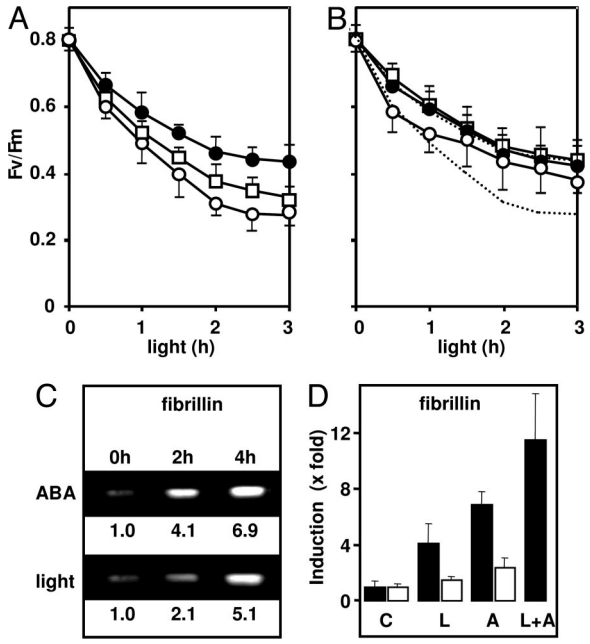

Fig. 4. Influence of fibrillin expression and ABA on phototolerance of PSII. (*A* and *B*) High-intensity light-induced photoinhibition of PSII in the absence (*A*) or presence of ABA (*B*). Detached leaves of RLD control plants (□), fibrillin-deregulated FU7 (●), and FD2 (○) were preincubated with or without exogenous ABA (25 μM for 4 h) in the presence of light (70 μmol·m^{-2}·s^{-1}) and then exposed to variable duration of high-intensity light (1,400 μmol·m^{-2}·s^{-1}). Subsequently, the leaves were kept in darkness to allow for reversible adaptation processes. Maximum photochemical efficiency of PSII was measured as the ratio of variable to maximum chlorophyll a fluorescence (F_v/F_m). The exposure-dependent decline of F_v/F_m is interpreted as photoinhibition. The two dotted lines shown in *B* indicate the data graph for RLD and FU7 presented in *A* to help in the visualization of ABA-induced changes. Analysis of FU6 and FD10 lines yielded data comparable with the results shown for FU7 and FD2, respectively, and were omitted. The data depicted are mean values from three independent experiments, each comprising five leaves, and only one direction of SD is indicated for reasons of clarity. (*C* and *D*) Fibrillin accumulation induced by ABA (A; 25 μM) and light (L; 1,400 μmol·m^{-2}·s^{-1}) in RLD (*C*) and comparison between wild type and FD2 transgenic (*D*). Fibrillin levels were quantified by the immunosignal that was normalized via the chlorophyll content of extracts to leaf fresh weight. The induction factor is presented and factor 1 corresponds to 0.2 and 0.1 μg fibrillin per gram of fresh weight for RLD and FD2, respectively.

tum yield after 2 h of light stress remained clearly higher in leaves of *abi2* compared with WT ($P < 0.01$), corresponding to a higher extent of photochemical quenching and reduced nonphotochemical quenching (28) in mutant leaves (see also Fig. 6, which is published as supporting information on the PNAS web site). Whether the markedly improved phototolerance of the mutant may reflect differences in fibrillin accumulation was addressed by immunoanalysis. Protein extracts of leaves revealed 4.7-fold higher fibrillin levels in leaves of *abi2* plants compared with Ler (Fig. 5*B*). Preincubation of Ler leaves in the presence of ABA (20 μM) or high concentration of mannitol (0.5 M) for 4 h increased fibrillin abundance by a factor of 2.2 and 3.7, respectively. A minor induction of fibrillin by osmotica and ABA also was observed in *abi2*, resulting in ≈1.3-fold and 1.7-fold elevated levels, respectively.

Discussion

Abiotic stress conditions inflicted by cold, salt, and drought affect a plant's water status and trigger ABA signaling (10, 24). The ABA signal mediates reduction of water loss by closing stomata, and this response generates a major dilemma during ongoing photosynthesis (29). CO_2 deficiency due to impaired gas exchange can lead to misdirection of the light-driven electron transport to the acceptor O_2, causing the generation of toxic superoxide radicals and other reactive oxygen species (30, 31). This scenario may lead, in turn, to photooxidation and membrane damage. Thus, protection of chloroplast functionality under these stress conditions is of paramount importance for plants. One of the plastidic proteins induced during abiotic stress is fibrillin. Our analysis revealed that fibrillin accumulation contributes to the protection of PSII against light stress and that fibrillin accumulation depends on the ABA signal pathway.

Regulation of Fibrillin Expression at the Transcriptional Level by ABA. The role of ABA in transcriptional regulation of fibrillin has been elusive in several species of *Solanaceae* (3–6). In *Arabidopsis*, however, fibrillin expression is unequivocally regulated by ABA. Fibrillin transcript levels increased rapidly and sensitively in the presence of ABA, and transcript abundance was strongly reduced in the ABA-insensitive *abi1* but not in *abi2* seedlings, indicating a selective control of the ABA response regulators on transcript levels. Genomewide expression analysis revealed frequent down-regulation of ABA-inducible genes in the *abi1* mutant, even in the absence of elevated ABA levels (23). A recovery of basic expression levels for fibrillin in *abi1* required at least 100-fold higher ABA concentrations than are sufficient to elicit a response in WT seedlings. Consistent with a role of this PP2C in controlling ABA-dependent transcript levels, ABA-mediated activation of the fibrillin promoter was abrogated by ectopic expression of abi1 known to block ABA signaling (32). The down-regulated transcript level of *abi1*, however, did not result in reduced protein levels of fibrillin (data not shown) as observed for FD lines, suggesting an additional mechanism of compensation that may be provided by the massive alteration in gene expression of the mutant (23).

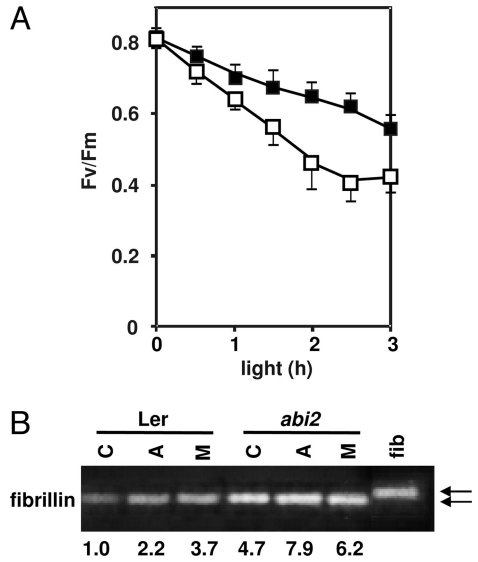

Fig. 5. Phototolerance affected by *abi2*. (*A*) Leaves of *abi2* revealed enhanced tolerance of PSII to high intensity light. The experiment was performed as mentioned in Fig. 4. The F_v/F_m ratios of Ler (□) and *abi2* (■) are presented. (*B*) Fibrillin was immunodetected in the protein fraction of *ABI2* (Ler) and *abi2* leaves. Proteins were extracted from excised leaves incubated in the absence (C) and presence of 20 μM ABA (A) or 0.5 M mannitol (M). Samples of the protein fractions (100 μg protein per lane) were analyzed by fibrillin-specific antibodies. The immunoreactive band of $M_r \approx 29$ kDa corresponds to the fibrillin (marked by an arrow). The fibrillin-specific signal detected in the fraction from pepper fruits (fib) with a M_r of ≈31 kDa served as a control. The strength of the immunospecific signal is expressed relative to the signal obtained with the control Ler.

Genome-comprehensive profiling of *Arabidopsis* transcripts identified coexpression of fibrillin and ABI2 in leaves, reproductive organs, and, at low levels, in seeds and roots (www.arabidopsis.org/info/expression/ATGenExpress.jsp). Histochemical analysis of *Arabidopsis* plantlets expressing a reporter gene under the control of a fibrillin promoter fragment revealed predominant expression in the mesophyll and guard cells (data not shown). Consistent with the fibrillin promoter activity, cell-specific transcript analyses detected coexpression of fibrillin and ABI2 in guard cells and mesophyll (33), lending support for the presence of both components in common cells, a prerequisite for interaction of prefibrillin and ABI2 *in planta*.

Posttranslational Control of Fibrillin Accumulation by ABI2. A physical interaction of ABI2 with prefibrillin is supported by *in vivo* analysis in yeast and by *in vitro* experiments. The protein interaction was highly discriminative against the point-mutated abi2 and ABI2 lacking the amino-terminal extension that characterizes the amino-terminal part as a novel interaction domain of plant PP2Cs (34). The interaction of ABI2 with prefibrillin, rather than fibrillin, emphasizes the importance of the signal peptide for binding. The finding is compatible with a cytosolic interaction site. The deregulated fibrillin accumulation in *abi2* provides genetic evidence for interaction. Interestingly, fibrillin transcript levels were comparable in the mutant and wild type. However, >4-fold higher levels of fibrillin were observed in *abi2*. Similarly, the analysis of a tomato deficient in ABA biosynthesis revealed normal fibrillin transcript levels but a failure to accumulate the protein during drought stress (5). Taken together, the observations and the fact that ABA levels are not reduced in the *abi2* mutant (35) argue for a posttranscriptional or posttranslational regulation of fibrillin accumulation by both ABA and ABI2. In light of the specific binding of ABI2 to prefibrillin, it seems likely that it is, in fact, a posttranslational control step exerted by ABI2 and regulated by ABA.

The failure of abi2 to interact with prefibrillin and the hyperaccumulation of fibrillin in the mutant suggest a phosphatase-dependent negative control exerted by ABI2. The analysis of *abi2* heterozygous plants revealed intermediate increases of fibrillin levels, also in FD lines, indicating a codominant action of abi2 on fibrillin accumulation (data not shown). The findings are not compatible with a simple model in which ABI2 targets freely the transit peptide of prefibrillin, e.g., for dephosphorylation. Clearly, the elucidation of the regulatory mechanism requires future analyses.

Fibrillin and Light Stress. Our analysis of *Arabidopsis* has unraveled an important role of ABA and fibrillin in establishing tolerance of PSII toward light stress. Plants with deregulated fibrillin expression revealed a clear dependence of photoinhibition on fibrillin levels. The light-triggered reduction of maximal photochemical efficiency of PSII was determined after an extended recovery period, which is interpreted as "irreversible" photoinhibition (27). Pretreatment of leaves with ABA or elevated levels of fibrillin accomplished either by fibrillin overexpression or by the *abi2* mutation resulted in enhanced phototolerance. ABA may act in numerous ways to contribute to photoprotection, e.g., by stimulating the xanthophyll cycle or redirecting gene expression of components contributing to photoprotection (23, 26, 30). However, fibrillin seems to be a major player in this adaptive response. ABA quickly induces fibrillin accumulation in *Arabidopsis* leaves, and preinduced fibrillin levels provide enhanced tolerance. ABA and light both stimulate fibrillin accumulation. Most importantly, preinduced fibrillin levels and ABA do not act additively in providing phototolerance of PSII, suggesting a common response pathway. Analysis in potato identified a specific association of fibrillin with the light-harvesting complex of PSII with a presumptive role in the modulation of photosynthetic efficiency (9). How the modulation is accomplished remains open. The observed photoprotection could involve the scaffold function of fibrillin for lipids and lipophilic substances (1, 7, 36) and reflect a fibrillin-mediated support of the PSII repair system that is a critical component in a plant's response to light stress (37). As a consequence of this protective role, preinduced fibrillin levels in overexpressor lines and in the *abi2* mutant resulted in higher PSII efficiency under light stress and decreased nonphotochemical quenching compared with control plants.

In conclusion, our data establish previously undescribed links between the ABA response pathway, fibrillin, and light stress by showing that fibrillin accumulation is involved in providing ABA-mediated photoprotection of PSII. The ABA response regulators ABI1 and ABI2 control transcript abundance and accumulation of fibrillin, respectively. Hence, fibrillin gene expression and action seem to be tightly interwoven with steps of ABA signal transduction.

Methods

Molecular Biological Analysis. The different GAL4 DNA-binding fusions used in the protein interaction analysis, the *in vitro* binding assays, and primers used for PCR are further detailed in *Supporting Methods*, which is published as supporting information on the PNAS web site. The analyses were essentially carried out as reported for ABI1 (21). The *Arabidopsis* cDNA library established in the pACT prey vector was provided by the DNA Stock Center at *Arabidopsis* Biological Resource Center, (Columbus, OH). Arabidopsis cDNA inserts were amplified by PCR with the forward primer (5′-AGATCTGGAATTCGGATCCTC-3′ and the reverse primer 5′-TTAAGCTTAGATCTCTCGAGGCCCGAAG-3′) and cloned via the EcoRI and HindIII sites at their 5′ ends, respectively, for DNA sequence analysis (ABI310 Genetic Analyser; Applied Biosystems). For deletion of the transit peptide of prefibrillin (amino acids 1–53) a different forward primer (5′-

CGGAATTCCATCCGAGCGACGGACATC-3′) was used for amplification of a truncated cDNA fragment to replace the 0.7-kb EcoRI-BglII fragment of prefibrillin cDNA in the pACT vector. Fibrillin transcript levels were analyzed by real-time PCR (Carl Roth, Karlsruhe, Germany). Transient gene expression was analyzed in protoplasts after 16 h of phenotypic expression in the dark and in the presence of ABA unless otherwise stated. Reporter constructs and conditions were as reported in ref. 21. Briefly, polyethylene glycol-mediated DNA transfections were performed by using plasmid DNA of 35S-GUS (20 μg) and fibrillin promoter-firefly luciferase (LUC) (20 μg) constructs per 5×10^5 *Arabidopsis* mesophyll protoplasts. In some cases, the 35S-abi1 construct (10 μg) was cotransfected. LUC activities were assayed in a luminometer (Berthold Technologies, Bad Wildbad, Germany) during a 90-s integration period of light emission. The fibrillin promoter-LUC construct was generated by fusing a BamHI-HindIII 1.7-kb fibrillin promoter fragment into the unique BamHI-HindIII sites of pBI221 (38). The fibrillin promoter was amplified from BAC T24H24 (DNA Stock Center at *Arabidopsis* Biological Resource Center) with the forward primer 5′-TTAAAGCTTATGAAACATCGTCAGATC-3′ and the reverse primer 5′-AATGGATCCTGTGTTTGTTCTTCAGAGAAACC-3′. ABA-regulated expression was normalized for glucuronidase activity. The 35S-abi1 expression cassette was generated by replacing the XbaI-Ecl136II GUS gene fragment of pBI221 with the BamHI-Eco147I *abi1* cDNA fragment (18).

Transgenic Plants and Physiological Characterization. *Arabidopsis* plants, accession RLD, were transformed via *Agrobacterium tumefaciens* strain C58pGV3850 (39). Transgenic FU and FD *Arabidopsis* lines were generated by transformation with pBI121-35S-fibrillin and pBI121-35S-asfibrillin, which were constructed by replacing the GUS gene of pBI121 (38) with the blunted EcoRI-HindIII fibrillin cDNA fragment in sense and antisense orientation into the blunted SmaI-Ecl136II sites of the vector. For physiological characterization, *Arabidopsis* plants were grown in pots on perlite/soil mixture at 23°C under long-day conditions with 16-h light (250 μmol·m^{-2}·s^{-1}). Detached rosette leaves from 4-week-old plants, fully developed and of comparable size, were floated on half-strength liquid MS medium with adaxial sides facing upward. The temperature was controlled at 23°C (Haake, Karlsruhe, Germany). Incubation was carried out in the presence or absence of ABA, or 0.5 M mannitol at a light intensity of 70 μmol·m^{-2}·s^{-1} for 4 h before exposure to high-light conditions (1,400 μmol·m^{-2}·s^{-1}). Light was passed through an infrared-absorbing layer of water. After a variable period of light stress, leaves were kept in darkness (45 min), and maximal photochemical activity of PSII (F_v/F_m) was measured by using a PAM-2000 (Heinz Walz, Effeltrich, Germany) under atmospheric conditions (40). A stress-dependent reduction of F_v/F_m values was interpreted as photoinhibition of PSII (27, 41). For statistical analysis, Student's *t* test was used.

Immunochemical Analysis. Proteins of leaf extracts for immunochemical analysis were obtained and analyzed as described in ref. 42. Protein concentration was determined in extracts to assure loading of equal amounts of protein. Leaves WT, mutant, and transgenic plants were exposed to 2 h of high light conditions before fibrillin analysis unless otherwise indicated. Polyclonal antibodies directed against pepper fibrillin were used (1), and cross reactivity of the antibody to *Brassicaceae* (43) was confirmed by testing the specific recognition of fibrillins obtained either from pepper fruits and by heterologous expression of *Arabidopsis* prefibrillin in *Escherichia coli*. Bound antibody was detected with a secondary anti-rabbit antibody conjugated to horseradish peroxidase by using SuperSignal West Femto (Pierce) as a substrate. Peroxidase activity was measured by light emission recorded by an intensified charge-coupled device camera (ORCAII ERG; Hamamatsu Photonics, Hamamatsu City, Japan) equipped with a Schneider Xenon 0.95/25 objective (Kreuznach, Germany) in a dark box.

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