# PSB27: A thylakoid protein enabling Arabidopsis to adapt to changing light intensity

Xin Hou, Aigen Fu, Veder J. Garcia, Bob B. Buchanan, and Sheng Luan

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**Executive Summary**

The research activities associated with this project significantly contributed to the understanding of plant photosynthesis and its regulation by light intensity and other environmental factors. Using a combination of techniques including genetics, biochemical, and structural analysis, PI and his laboratory effectively investigated the function of proteins located in the thylakoid lumen, the innermost compartment, of chloroplast in the regulation of assembly of photosynthetic machines. This knowledge has potential to serve as a platform for engineering more effective photosynthesis in plants.

**The specific objectives, activities, and achievements:**

1. To dissect the mechanism of action for CYP38. Our studies suggest an "autoinhibitory" model for CYP38 action. We plan to test this model and determine the mechanistic details for CYP38 function by biochemical and genetic procedures. We have tested this hypothesis by using cyp38 mutant as a genetic model: when transformed by the full length of the CYP38 protein, the light-sensitive phenotype of the cyp38 mtuant was complemented. However, the CYP38 protein without the cyclophilin domain did not complement the mutant. The CYP38 protein lacking the N-terminal "autoinhibitory" domain also complemented the mutant, suggesting that the cyclophilin domain functions as a independent active unit in the process.

2. To determine the mechanism of action of FKBP20-2/FKBP16-3. Studies on FBKP16-3 and FKBP20-2 suggest that they may work together as a redox-dependent duo in PSII assembly. We will test this hypothesis using biochemical and genetic tools. We generated single mutants of each FKBP and tested their phenotype. While fkbp20-2 showed obvious phenotype under low light condition, the fkbp16-3 did not. We generated the double mutant and found that the two protein had some additive functions. The yeast two hybrid assays showed that FKBP20-2 and FKBP16-3 physically interact with each other.

3. To determine the function of lumenal FKBPs by multi-gene mutagenesis approach. Using new genetic knockout (KO) procedures especially CRISPR/CAS9 system, we plan to generate multi-gene KO models that will likely provide vital information on those FKBPs with functional redundancy. Using CRISPR/Cas9-based mutagenesis, we have generated several mutants that lack multiple FKBP proteins. The detailed analysis of these mutants terminated due to funding shortage.

4. Functional analysis of thylakoid lumen network. We will focus our effort on objectives 1-3. Objective 4 represents a long-term goal to establish a more comprehensive network of proteins in the thylakoid lumen and their function in the assembly and maintenance of photosynthetic complexes. We identified several other proteins in the thylakoid lumen that either interact with immunophilins or represent unique functional properties that are related to regulation of photosynthesis. Some of the proteins were investigated using genetics and biochemical tools.

**Publications**

Hou X, Fu A, Garcia VJ, Buchanan BB, Luan S. (2015) PSB27: A thylakoid protein enabling Arabidopsis to adapt to changing light intensity. *Proc Natl Acad Sci U S A.* 112(5):1613-8.

Seok MS, You YN, Park HJ, Lee SS, Aigen F, Luan S, Ahn JC, Cho HS (2014) AtFKBP16-1, a chloroplast lumenal immunophilin, mediates response to photosynthetic stress by regulating PsaL stability. *Physiol Plant*. 150(4):620-31.

Che Y, Fu A, Hou X, McDonald K, Buchanan BB, Huang W, Luan S (2013) C-terminal processing of reaction center protein D1 is essential for the function and assembly of photosystem II in Arabidopsis. *Proc Natl Acad Sci U S A.* 110(40):16247-52.

# PSB27: A thylakoid protein enabling *Arabidopsis* to adapt to changing light intensity

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**In earlier studies we have identified FKBP20-2 and CYP38 as soluble proteins of the chloroplast thylakoid lumen that are required for the formation of photosystem II supercomplexes (PSII SCs). Subsequent work has identified another potential candidate functional in SC formation (PSB27). We have followed up on this possibility and isolated mutants defective in the PSB27 gene. In addition to lack of PSII SCs, mutant plants were severely stunted when cultivated with light of variable intensity. The stunted growth was associated with lower PSII efficiency and defective starch accumulation. In response to high light exposure, the mutant plants also displayed enhanced ROS production, leading to decreased biosynthesis of anthocyanin. Unexpectedly, we detected a second defect in the mutant, namely in CP26, an antenna protein known to be required for the formation of PSII SCs that has been linked to state transitions. Lack of PSII SCs was found to be independent of PSB27, but was due to a mutation in the previously described *cp26* gene that we found had no effect on light adaptation. The present results suggest that PSII SCs, despite being required for state transitions, are not associated with acclimation to changing light intensity. Our results are consistent with the conclusion that PSB27 plays an essential role in enabling plants to adapt to fluctuating light intensity through a mechanism distinct from photosystem II supercomplexes and state transitions.**

photosystem | PSII supercomplex | photosynthesis | anthocyanin biosynthesis | reactive oxygen species

Photosynthetic light reactions entail the coordinated function of several large membrane complexes: photosystem I (PSI), photosystem II (PSII), cytochrome *b6f* complex, and $CF_0$-$CF_1$ complex. PSII catalyzes the initial step of photosynthesis, the light-dependent oxidation of water that yields molecular oxygen and reduced plastoquinone. The native form of PSII residing in the thylakoid membrane is believed to be organized into several types of supercomplexes (SCs), including the PSII core and the peripheral light harvesting complex II (LHCII), that play a primary role in the harvesting of light, transfer of excitation energy to the reaction center and regulation of light utilization. Several monomeric antenna proteins including CP24, CP26, and CP29 regulate the interaction of the PSII core with LHCII trimers (1–4). Regulation is achieved through photoprotective mechanisms that dissipate absorbed excess energy as heat in response to stress conditions such as high light intensity (5, 6).

In natural settings, both the intensity and the spectral quality of light vary extensively, sometimes within very short periods. The changes in light intensity result in imbalanced excitation of PSI and PSII, and with that may lower the efficiency of the photosynthetic light reactions. In acclimating to the changing conditions, plants modify their thylakoid proteins and reorganize their photosynthetic machinery (7, 8). In a rapid response, designated state transitions (9), LHCII associates reversibly with PSII or PSI. Under high light intensity, excessive activation of PSII increases the reduced plastoquinone pool and thereby activates protein kinase STN7 which, in turn, phosphorylates LHCII and prompts the migration of LHCII from PSII (state 1) to PSI (state 2) (10, 11). Oxidation of the plastoquinone pool by the higher activity of PSI then activates protein phosphatase PPH1 that dephosphorylates LHCII and promotes a return to the original association of LHCII with PSII (state 1) (12, 13). In this context, the formation of LHCII-PSII SCs is expected to be a prerequisite for state transitions and, therefore, essential for adaptation to changing light intensity. However, it remains to be rigorously tested if state transitions play a crucial role in plant adaptation to changing light intensity.

In the present study, we have identified an *Arabidopsis* mutant that lacked PSII supercomplexes and grew poorly under changing light intensity. The mutant harbored a T-DNA insertion in the gene encoding the thylakoid lumen protein, PSB27, implicating its function in both the assembly of PSII SCs and adaptation to changing light. A detailed comparative genetic and biochemical analysis confirmed the requirement for PSB27 in enabling plants to adapt to changing light, but, surprisingly, revealed that this adaptation is independent of PSII SC assembly. A second, previously unrecognized defect was localized in CP26, a protein unrelated to PSB27, that is linked to PSII SC assembly. These results prompt the conclusion that PSB27 plays a fundamental role in enabling plants to adapt to changes in light intensity independently of the formation of PSII SCs.

## Results and Discussion

**Identification of a Mutant with a Defect in the Assembly of PS II Supercomplexes.** Our earlier work suggests that FKBP20-2 and CYP38 chaperones function in the assembly of PSII SCs (14, 15). Specifically, these proteins appear to be localized to the chloroplast thylakoid lumen where a major portion of the PSII complex resides. These findings raise the possibility that additional luminal proteins may be required for PSII assembly. To gain information on this point, we have taken a systematic reverse genetic approach and isolated 58 *Arabidopsis* T-DNA insertion mutants in genes

### Significance

**Our results provide evidence that PSB27, a soluble protein of the chloroplast thylakoid lumen, is essential for enabling plants to adapt to changes in light intensity. Adaptation takes place independently of photosystem II supercomplexes and, based on work of others, of state transitions for the redistribution of light harvesting proteins. This finding opens the door to pursue the question of how photosynthesis adjusts to fluctuating sunlight and enables plants to grow under ever-changing environmental conditions.**

Author contributions: X.H., A.F., and S.L. designed research; X.H., A.F., and V.J.G. performed research; X.H., A.F., B.B.B., and S.L. analyzed data; and X.H., B.B.B., and S.L. wrote the paper.

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The authors declare no conflict of interest.

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encoding 30 luminal proteins (16, 17). The mutants were screened for defects in PSII complex organization by blue native gel electrophoresis. The mutant line with the most marked defect in PSII organization (SALK_004769) contained a T-DNA insertion in the 5′ UTR of the *PSB27* gene (At1g03600, Fig. S1*A*). As reported earlier (18), this mutant was devoid of *PSB27* mRNA determined by RT-PCR [Fig. S1*B*, compare wild type (WT) and mutant line (ML)] as well as the corresponding protein probed by SDS/PAGE and Western blot (Fig. S1*C*). Protein fractionation of subchloroplast compartments confirmed that PSB27 is located in the thylakoid lumen (Fig. S1*D*).

In an effort to localize the defect in PSII, we next conducted more quantitative blue native gel analysis with WT and mutant thylakoid membrane proteins and found pronounced differences in the abundance of individual PSII SC bands: notably, the mutant plants were almost completely devoid of PSII SCs that were readily detected in the WT (Fig. 1*A*). By contrast, the band representing the free forms of the corresponding LHC trimers (LHCII-T) showed a relative increase in abundance. When subjecting the blue native gel slices to the second electrophoresis dimension by SDS/PAGE, we observed a dramatic decrease in abundance of protein subunits associated with the PSII SCs (Fig. 1*B*, see red boxes).

To further visualize changes in the PSII complexes, we used immunoblots to examine the distribution and abundance of subunits of the four major thylakoid membrane complexes. To this end, the individual proteins resolved by 2D-SDS PAGE were transferred to a nitrocellulose membrane and probed with antibody against subunits of each of the four thylakoid membrane complexes: PSII: D1, D2, CP43, CP47, PsbO, and LHCII; PSI: PsaA/B, PsaD, and PsaF; cytochrome $b_6f$ complex: CytF, b6, and PetC; and $CF_0$-$CF_1$ complex: ATPα. The results confirmed that the mutant (ML) lacked the protein subunits in the PSII SCs that were observed in the WT (Fig. 1*C*). The subunit distribution pattern of other complexes including PSI, Cyt $b_6f$, and $CF_0$-$CF_1$ remained unchanged (Fig.1*C*).

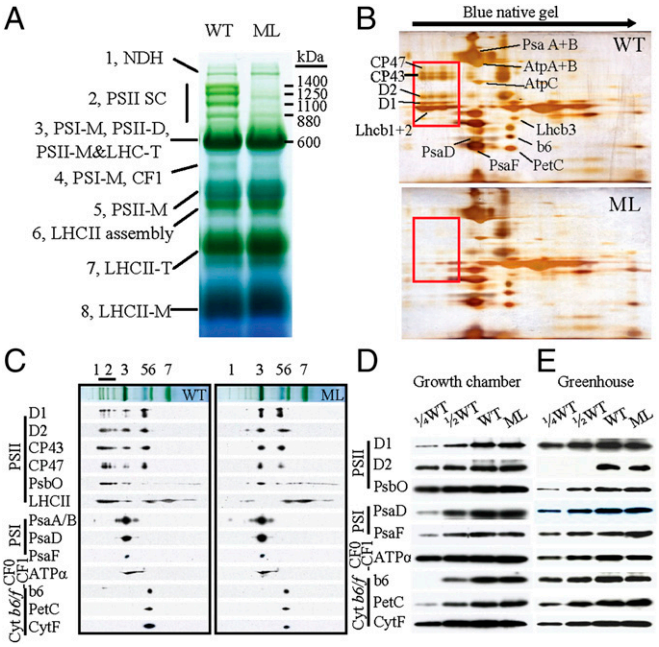


**Fig. 1.** Analysis of thylakoid membrane protein complexes. (*A*) Blue native gel analysis of thylakoid proteins of WT and ML plants (15 μg chlorophyll as loading standard). (*B*) Thylakoid proteins separated by BN gel in *A* were further subjected to SDS PAGE and silver-stained. The red frames indicate the individual protein components of the PSII SCs. Protein identification was according to Fu et al. (15). (*C*) 2D-SDS PAGE immunoblots of thylakoid proteins. Antibodies were applied as indicated. (*D*) 1D-SDS PAGE Western blots of thylakoid proteins (2.5 μg chlorophyll) from WT and ML plants grown for 4 wk in a growth chamber under constant light intensity. (*E*) 1D-SDS PAGE immunoblots of thylakoid proteins (2.5 μg chlorophyll) from WT and ML grown in a greenhouse for 4 wk. Antibodies applied and plants grown as indicated.

It was difficult to compare the abundance of each individual protein between WT and mutant using 2D Western blots because the gels were processed separately. To circumvent this difficulty, we quantified these proteins by loading WT and mutant samples on the same SDS/PAGE gel, followed by Western blot. The results showed no significant difference in the abundance of PSII subunits between the WT and mutant (ML) cultivated under growth chamber (Fig. 1*D*) or greenhouse conditions (Fig. 1*E*). Taken together, these results suggested although the total amount of individual PSII subunits was not affected, the mutant plants were incapable of assembling the individual subunits to form PSII SCs.

We noted that the mutant showed two new weak bands in the PSII SCs region of the blue native gel (Fig. 1*A*): one was located between 1,250 and 1,100 kDa and the other was just below the 880 kDa band of WT PSII SC. To determine whether these bands represented altered forms of the PSII SC or were unrelated to this photosystem, we performed blue native gel analysis of both WT and mutant (ML) with a larger amount of total protein (Fig. S2*A*). The major bands above the PSII dimer in both WT and mutant samples in Fig. S2*A* were isolated and subjected to SDS PAGE analysis. The results showed that the top band, which was identified as NDH (labeled WT-1 and ML-1 in Fig. S2*A*), and the bottom band, which was identified as PSI SC (19, 20) (labeled WT-6 and ML-4), displayed identical patterns of subunit composition in the WT and ML (Fig. S2*B*). The four major PSII SC bands of WT (WT-2–WT-5) all contained typical PSII subunits (red lettered a–d bands in Fig. S2*B*), whereas the two unique bands (ML-2 and ML-3) in mutant lacked these subunits. We further showed by mass spectrometry that these proteins (a–d bands in the WT sample in Fig. S2*B*) are true PSII subunits (Fig. S2*C*). Interestingly, both the 880-kDa band of the mutant (ML-3) and the upper band (ML-2), neither of which is obvious in the WT gel, displayed a subunit pattern similar to PSI SC (WT-6) instead of PSII SC (Fig. S2 *A* and *B*). This result again supports our conclusion that all detectable forms of PSII SCs are eliminated in the mutant.

**Mutant Line Is Deficient in Adaptation to Changing Light Intensity.** When cultivated in a growth chamber under normal growth conditions (Fig. 2*A*), the ML failed to show a noticeable phenotypic difference from WT in either soil (Fig. 2*E*) or MS medium (Fig. 2*I*) despite lacking PSII SCs. Similar results were reported previously (18). By contrast, when grown in the greenhouse (Fig. 2*B*), leaves of ML plants were much smaller and less green compared with WT (Fig. 2 *F* and *J*). Clearly, environmental differences (growth chamber vs. greenhouse) caused a dramatic change in mutant phenotype. To identify the factor responsible for such a difference, we cultivated the plants under several growth conditions—i.e., temperature, day length (8 h light/16 h dark for short day, 16 h light/8 h dark for long day), and light intensity (20 μmol $m^{-2}$ $s^{-1}$ to 250 μmol $m^{-2}$ $s^{-1}$). The results indicated that none of these factors was responsible for the observed difference in phenotype. We then realized that the growth chamber supplies light at constant intensity during the light cycle, whereas the greenhouse experiences light at fluctuating intensity during the day akin to a natural setting.

To pursue the possibility that change in light intensity led to altered growth of the mutant, we designed growth chamber conditions such that plants experienced a gradient of light intensity during the light cycle (Fig. 2*C*), mimicking greenhouse conditions (Fig. 2*B*). Indeed, the ML plants subjected to this light intensity regime clearly differed from WT, similar to those cultivated in the greenhouse (Fig. 2 *G* and *K*). When the light intensity was changed every hour from 50 to 150 μmol $m^{-2}$ $s^{-1}$ during the light cycle (Fig. 2*D*), growth of ML plants was also slow relative to WT

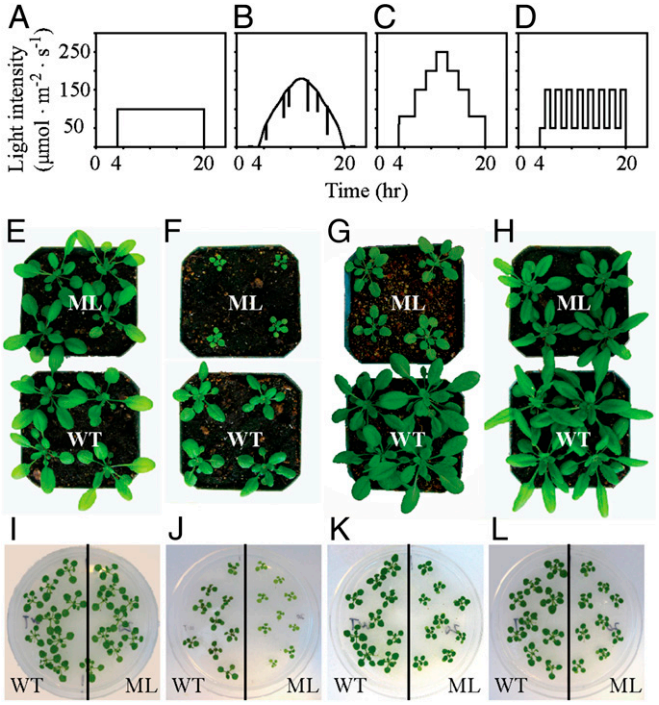


**Fig. 2.** Mutant line is compromised in light adaptation. (*A–D*) Light intensity regimes for plant growth. The light/dark cycle was 16 h light/8 h dark for all regimes. (*A*) Growth chamber conditions with constant light intensity (100 μmol $m^{-2}$ $s^{-1}$) during the light periods. (*B*) Greenhouse conditions with light intensity changes during the day time. (*C*) Growth chamber conditions with light intensity changes from 75 μmol $m^{-2}$ $s^{-1}$ to 250 μmol $m^{-2}$ $s^{-1}$ during the light periods. (*D*) Growth chamber conditions with light intensity switched between 50 μmol $m^{-2}$ $s^{-1}$ and 150 μmol $m^{-2}$ $s^{-1}$ every hour during the light periods. (*E–H*) WT and ML were grown in soil for 4 wk under the various light regimes in *A–D*. (*I–L*) WT and ML were grown on 1/2 MS medium for 10 d under light regimes in *A–D*. Growth conditions depicted in *A–D* were used, respectively, for growth in *E–H* and *I–L*.

(Fig. 2*H*). A similar phenotypic difference was observed with plants grown on agar plates containing 1/2 MS medium (Fig. 2*L*). These results suggested the ML plants have defects in adaptation to changing light intensity.

**Mutant Line Is Defective in PSII Function and Starch Biosynthesis.** The stunted growth of ML plants observed under changing light intensity suggests that the mutant had lower photosynthetic capacity under these conditions. To test this idea, photosynthetic parameters were determined by measuring chlorophyll fluorescence in leaves of whole plants. The ratio of variable fluorescence to maximum fluorescence ($F_v/F_m$), which represents the maximum photochemical efficiency of PSII (21), was lower in the ML than in the WT even with plants cultivated in the growth chamber which showed no visible phenotypic difference (Fig. 3*A*, *Upper*). The decrease in the $F_v/F_m$ ratio became more pronounced with plants grown in the greenhouse (Fig. 3*B*, *Upper*). ΦPSII, which measures the proportion of PSII-associated light harvesting, was also lower in the ML (Fig. 3*A*, *Lower*). Like the $F_v/F_m$ ratio, ΦPSII was further decreased when plants were grown in the greenhouse (Fig. 3*B*, *Lower*). Taken together, the chlorophyll fluorescence assays suggested that ML plants are compromised in PSII function.

The fluorescence results prompted us to test the possibility that PSII SC formation is needed for thylakoid membrane organization. If so, lack of PSII SCs could lead to defects in chloroplast morphology in mutant plants. To pursue this idea, we examined leaf sections of both WT and ML under the electron microscope. Under growth chamber conditions, the chloroplasts from ML and WT showed similar size, grana, and starch accumulation (Fig. 3*C*). By contrast, electron microscopy revealed a striking difference between WT and ML specimens grown in the greenhouse: The chloroplasts in wild type accumulated copious amounts of starch, whereas, consistent with its stunted growth, the ML was almost completely devoid of starch grains (Fig. 3*D*), indicating a much lower efficiency of starch biosynthesis under changing light conditions. This inefficiency could result, in part, from an impairment in the regulation of enzymes of starch biosynthesis due to defective PSII activity and the attendant reduction of thioredoxin (22).

**Mutant Line Accumulates Less Anthocyanin and Produces More ROS Under High Light Stress.** Because PSII deficiency and photoinhibition can lead to damage under high light, we investigated whether the mutant line is defective in adaptation to this condition. To this end, WT and ML were grown on 1/2 MS medium for

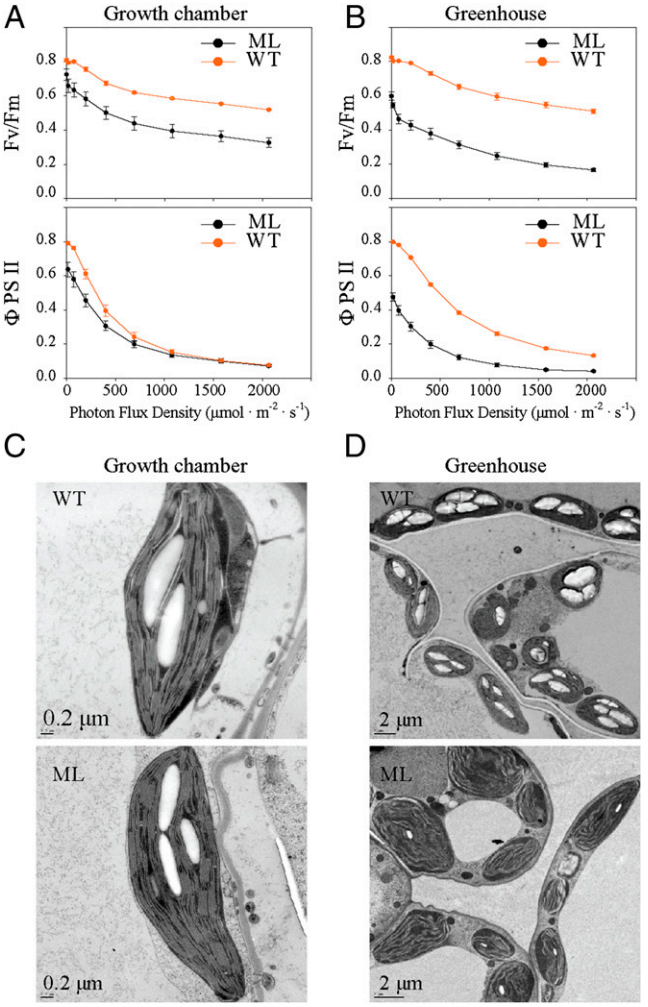


**Fig. 3.** Mutant line is deficient in utilization of light. (*A*) Chlorophyll fluorescence analyses of WT and ML plants grown under constant light intensity conditions in the growth chamber (80 μmol $m^{-2}$ $s^{-1}$ for 16 h and dark for 8 h) for 4 wk. (*B*) Chlorophyll fluorescence analyses of WT and ML plants grown under greenhouse conditions for 4 wk. $F_v/F_m$: the maximum efficiency of PSII photochemistry; ΦPSII: efficiency of PSII electron transport. Data represent mean ± SEM ($n = 4$). (*C*) Electron micrographs of chloroplasts from 4 wk-old WT and ML grown under constant light intensity conditions. (*D*) Electron micrographs of chloroplasts from WT and ML grown in greenhouse for 4 wk. Transmission Electron Microscope (FEI) was used to examine leaf cross sections as described (42).

10 d under low light (20 μmol $m^{-2}$ $s^{-1}$). These plants showed no obvious difference in phenotype. Further, when the plants were transferred to continuous high light (350 μmol $m^{-2}$ $s^{-1}$), both the WT and ML grew well. However, surprisingly, we observed a pronounced difference in leaf color within 2 d after the transfer to high light. WT plants turned dark purple, whereas the ML remained green (Fig. 4*A*, *Lower*), indicating that mutant is defective in the accumulation of anthocyanin, a pigment that serves as a "filter" for protection against high light (23). A similar pattern was observed when plants were cultivated in soil (Fig. 4*B*). The 4-wk-old, soil-grown plants showed no difference when grown under normal light intensity (60 μmol $m^{-2}$ $s^{-1}$) (Fig. 4*B*, Top). However, after transfer to high light (350 μmol $m^{-2}$ $s^{-1}$), a difference in the color of leaves of WT and mutant plants was clearly visible (Fig. 4*B*, *Lower*). In quantitative terms, WT rosettes accumulated 165 μg total anthocyanin per g fresh weight, whereas the ML accumulated only 18 μg, a drop of almost 90% (Fig. 4*C*).

To obtain insight into the anthocyanin deficiency, we applied real time q-PCR to examine the transcript levels of genes responsible for accumulation of the pigment, including two regulatory genes (*PAP1* and *PAP2*) and 12 biosynthesis genes (Table S1) (24). Each of these genes was induced by high light in both WT and ML (Fig. S3). However, the kinetics of mRNA accumulation differed between the two groups. In the mutant, transcript levels increased at the beginning and rapidly dropped to the basal level, whereas in WT, transcripts increased to a higher level, and were sustained for a longer time (Fig. S3 and Fig. 4*D*). The results suggest that the mutant can respond to a high light signal, but the response seems inadequate to sustain the production of anthocyanin.

The data above show that the mutant has a reduction in PSII function (Fig. 3 *A* and *B*). The mutant should, therefore, sustain more severe photoinhibition due to the formation of higher levels of reactive oxygen species (ROS). Moreover, the observed inhibition of anthocyanin biosynthesis in the ML may also have resulted from elevated levels of ROS as reported earlier (24, 25).

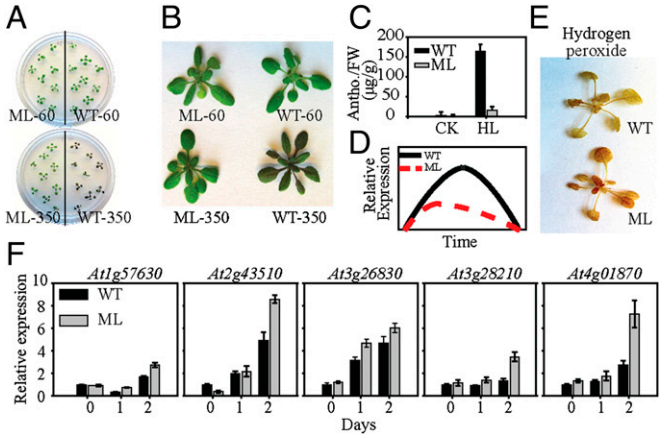


**Fig. 4.** Mutant line is defective in light-induced anthocyanin accumulation and displays enhanced ROS accumulation. (*A*) WT and ML plants were grown on 1/2 MS medium under constant low light conditions (20–30 μmol $m^{-2}$ $s^{-1}$) for 10 d, and then transferred to higher light intensity (60 μmol $m^{-2}$ $s^{-1}$, upper plate or 350 μmol $m^{-2}$ $s^{-1}$, lower plate) for 2 d. (*B*) WT and ML were grown in soil under constant light intensity (16 h light/8 h dark, 60 μmol $m^{-2}$ $s^{-1}$) for 4 wk, and then were transferred to high light conditions (350 μmol $m^{-2}$ $s^{-1}$) for 2 d. (*C*) Anthocyanin content of plants in *B*. Data represent mean ± SEM ($n = 4$). (*D*) Composite curves of gene expression patterns of WT and ML after high light treatment. Expression levels of individual genes are shown in Fig. S3. (*E*) DAB (3, 3-diaminobenzidine) staining for hydrogen peroxide in high light treated (350 μmol $m^{-2}$ $s^{-1}$ for 2 d) plants of WT and mutant. (*F*) The relative expression levels of ROS marker genes measured by real-time RT-PCR before and after high light (350 μmol $m^{-2}$ $s^{-1}$) treatment. The results are shown as mean ± SEM from three experiments.

To follow up on this possibility, we measured hydrogen peroxide in WT and ML using 3, 3-diaminobenzidine (DAB) stain. Elevated levels of $H_2O_2$ were observed in the mutant compared with WT plants following high light treatment (Fig. 4*E*). These results were confirmed in experiments with ROS-responsive gene markers (25) (Fig. 4*F*). The mRNA levels of five ROS marker genes were similar in WT and ML before the treatment. However, after exposing plants to high light for two days, the expression level of each of the five marker genes was significantly higher in ML compared with WT. The results support the conclusion that the excessive $H_2O_2$ production taking place in high light inhibits anthocyanin biosynthesis as previously reported (24, 25).

**Lack of CP26 Causes Deficiency of PS II Supercomplexes.** To confirm the link between phenotypic changes in the T-DNA mutant and the function of PSB27, we transformed the mutant with the wild type PSB27 gene driven either by the 35S promoter or native promoter. Several phenotypic changes in the mutant were fully complemented by PSB27, including defects in light adaptation, photosynthetic capacity and anthocyanin biosynthesis. The transgenic plants also grew similarly to WT under greenhouse conditions (Fig. S4 *A* and *B*) and accumulated comparable amounts of anthocyanin after high light treatment (Fig. S4*B*, *Right*). In like manner, the chlorophyll florescence parameters representing PSII capacity ($F_v/F_m$) and efficiency (ΦPSII) were recovered in the complemented plants grown under greenhouse conditions (Fig. S4*C*).

However, to our surprise, the defect in PSII SCs remained in the complemented plants (Fig. S4*D*). For a better resolution of PSII antenna components, the thylakoid proteins were, therefore, separated by Tris-Tricine SDS PAGE. This procedure revealed that a protein band of 26 KDa was missing in ML (Fig. S4*E*). Mass spectrometry identified the missing protein as CP26, a monomeric antenna protein shown earlier to be required for the assembly of PSII SCs (1, 4). Lack of CP26 would explain why the mutant failed to form PSII SCs and also why PSB27 was unable to complement this defect. In short, the CP26 defect was unrelated to PSB27 function. As a consequence, we followed up by examining the genomic sequence of the *CP26* gene in the mutant and found a point mutation in the first exon of *CP26*, which caused the absence of the corresponding mRNA and protein (Fig. S4 *E*–*G*).

**PSB27, but Not PSII Supercomplex, Is Critical for Growth Adaptation to Changing Light Intensity.** Although finding the CP26 defect in the mutant line was unexpected, it provided an ideal genetic system to compare the contribution of PSII SCs and PSB27 to light intensity adaptation. To this end, we segregated the *cp26* and *psb27* mutations by backcrossing ML with WT. The F2 population of plants was genotyped to identify the *cp26* and *psb27* single mutants. As seen in Fig. 5, the *psb27* mutant, but not the *cp26* mutant, showed retarded growth under greenhouse conditions (Fig. 5 *A* and *B* middle) and produced less anthocyanin under high light (Fig. 5*B*, *Right*). The *cp26* mutant, again not *psb27* mutant, was deficient in PSII SCs, which could be rescued by expressing a wild type copy of *CP26* gene in the mutant (Fig. 5 *C* and *D*).

We then compared *psb27,cp26* with state transition mutants including *stn7* (10), *stn8* (26), and *stn7 stn8* double mutants (27). After growing for 4 wk in the greenhouse, *cp26* and WT plants were comparable, the state transition mutants were slightly smaller than WT (particularly in the double mutant), whereas *psb27* displayed marked growth retardation (Fig. 6*A*). A similar phenotypic difference was observed with plants grown on 1/2 MS medium plates for 10 d (Fig. 6*B*). The phosphorylation of LHCII and CP43 which is regulated by STN7 and STN8 suggests that state transitions (10, 26), were not affected in *psb27* mutant (Fig. 6*C*). The results suggest that PSB27 plays a fundamental role in enabling plants to adapt to change in light intensity. Furthermore, PSII SCs or state transitions do not appear to significantly

contribute to light adaptation at least under the conditions used in this study.

**Concluding Remarks.** The present experiments have uncovered a protein of the chloroplast thylakoid lumen, PSB27, that is required for adaptation to changing light intensity. Using an *Arabidopsis* mutant lacking PSB27, we found that, although not essential under conditions of constant light intensity, this protein was required for normal growth under changing light regimes in either a natural setting or a growth chamber. The stunted growth observed under changing light was associated with lower PSII efficiency and defective starch accumulation. In response to high light exposure, mutant plants also displayed enhanced ROS production and reduced synthesis of the protectant anthocyanin although such changes did not affect plant growth.

We observed that the monomeric antenna protein CP26, a component of PSII SCs, is required for the assembly of the photosynthetic apparatus. An *Arabidopsis* mutant lacking CP26 was found to be essentially devoid of PSII SCs. Although it was reported that the LHCII S trimer could associate with the PSII core

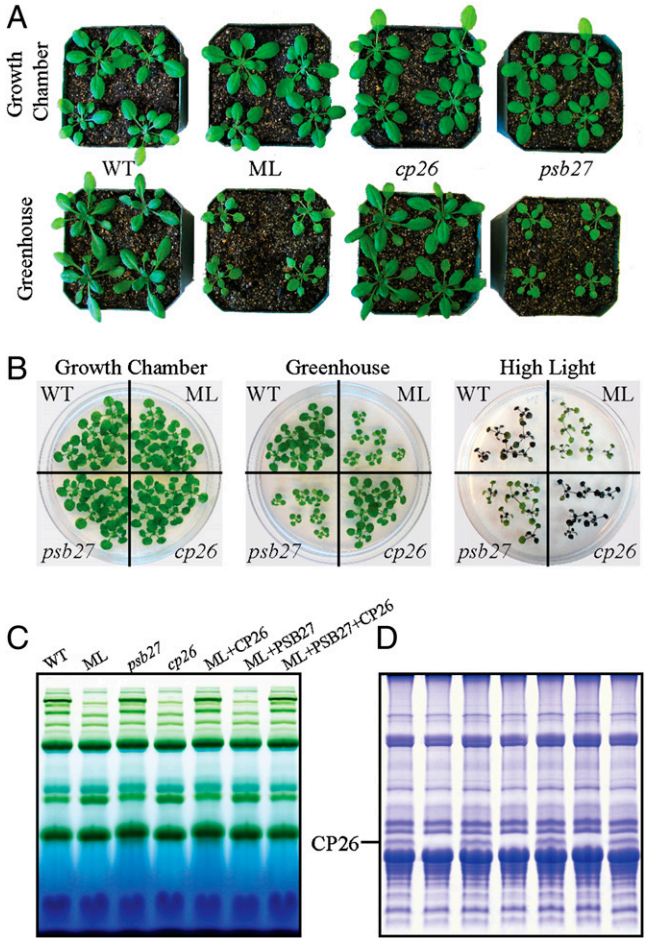


**Fig. 5.** Genetic linkage of *cp26* and *psb27* mutant alleles with plant growth defects. (*A*) The original T-DNA ML containing both *cp26* and *psb27* mutant alleles was compared with the WT and with segregated single mutants of *cp26* and *psb27* under constant light intensity in a growth chamber (*Upper*) or under varying light intensity in a greenhouse (*Lower*). (*B*) The plants were grown on 1/2 MS medium in growth chamber (*Left*) or greenhouse (*Center*) for 10 d. The same plants grown under constant low light for 10 d were then transferred to high light (350 μmol $m^{-2}$ $s^{-1}$) for 2 d (*Right*). (*C*) Blue native gel analysis showing PSII SCs of thylakoid proteins of WT, ML, *psb27*, *cp26*, ML complemented with CP26 or/and PSB27. (*D*) Tris-Tricine SDS PAGE analyses showing CP26 proteins of thylakoids of the same plants in *C*.

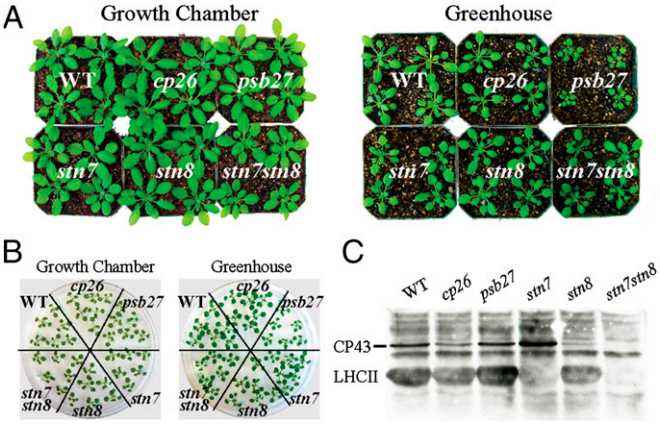


**Fig. 6.** Comparison of *cp26*, *psb27*, *stn7*, *stn8*, and *stn7 stn8* double mutant in their adaptation to light conditions. (*A*) WT, *cp26*, *psb27*, *stn7*, *stn8*, and *stn7 stn8* double mutant were grown in soil in growth chamber (*Upper*) and greenhouse (*Lower*) for 4 wk (the growth conditions were as in Fig. 2 *A* and *B*). (*B*) The same plants were grown on 1/2 MS medium for 10 d under light regimes in Fig. 2*A* (Growth Chamber) and Fig. 2*B* (Greenhouse). (*C*) phosphorylation of thylakoid proteins. Total proteins of 2-wk-old seedlings were separated by 12% SDS PAGE and immunoblotted with anti-phosphothreonine antibodies.

in the absence of CP26 (28), our results suggest that this association is not stable, consistent with the sucrose gradient results (1).

The impact of state transitions on energy balance between PSI and PSII has been established for the green alga *Chlamydomonas* (29). However, state transitions play a less prominent role in land plants than in green algae. Growth is only marginally affected in *Arabidopsis* mutants impaired in state transitions (27, 30), even in fluctuating light (10). Our results have shown that state transition mutants also display minor, if any, phenotypic change under conditions that severely inhibit growth of *psb27* mutant plants. Our results suggest that land plants adapt to changing light intensity through a PSB27-dependent mechanism that is independent of both state transitions and PSII SCs. Thus, based on our results it seems possible that the recently reported effects of PSB27 on PSII SC formation and state transitions (31) may have been the result of the *cp26* point mutation that was present together with the *psb27* T-DNA insert in the mutant line that was used (SALK_004769).

A final point, PSB27 is present in cyanobacteria as well as land plants (16, 32). In cyanobacteria PSB27 is tightly associated with the thylakoid membrane via an N-terminal lipid modification that is absent in PSB27 from land plants (33). Therefore, PSB27 of *Arabidopsis* is present exclusively in the soluble phase of the thylakoid lumen (Fig. S1*D*). This difference in location may, in part, be responsible for functional differences of PSB27 in the two groups of organisms. In cyanobacteria, the membrane-bound PSB27 facilitates the assembly of the manganese cluster of PSII (34–38) by interacting tightly with the CP43 core protein in the assembly of intermediates. In land plants, on the other hand, PSB27 functions in acclimation to changing light. Elucidation of further details of PSB27 function may well add insight into the evolution of oxygenic photosynthesis.

## Materials and Methods

**Plant Materials and Growth Conditions.** *Arabidopsis thaliana*, ecotype Columbia-0, was used in this study. The T-DNA insertion mutant line (SALK_004769, locus At1g03600) was obtained from the *Arabidopsis* Resource Center (Columbus, OH). For soil-grown plants, sown seeds were cold-treated for 2 d, and then transferred to the indicated growth conditions. For plate-grown plants, surface-sterilized seeds were planted on 1/2 MS medium with 0.8% phytagel, cold-treated for 2 d, and grown under the same conditions as soil-grown plants.

**Blue Native PAGE and 2D-SDS PAGE.** Blue native gel electrophoresis was performed as described in ref. 14. For 2D-SDS PAGE, lanes of blue native gel were excised with a razor blade and incubated in 2× SDS sample buffer containing 2.5% (vol/vol) β-mercaptoethanol for 15 min at 25 °C and then for 15 min at 70 °C. Each lane with denatured proteins was placed on top of a 12% SDS PAGE gel and subjected to second dimension separation (14).

**Immunoblot Analysis.** Protein samples corresponding to equal amounts of chlorophyll were separated on 12% SDS PAGE gels and transferred to nitrocellulose membranes, followed by Western blot analysis (39). After blocking nonspecific binding with 5% milk, the blot was subsequently incubated with antibodies generated against the indicated proteins and detected using SuperSignal West Pico Chemiluminescent Substrate kit (Pierce).

**Chlorophyll Fluorescence Measurements.** Chlorophyll fluorescence was measured with a FMS2 fluorometer (Hansatech) attached to intact leaves that had been dark adapted overnight. The $F_0$, $F_m$, and $F_s$ were measured and calculated as described (14). $F_v/F_m$ was calculated as $(F_m - F_0)/F_m$, and ΦPSII was defined as $(F'_m - F_s)/F'_m$.

**RNA Isolation and Quantitative Reverse Transcription-PCR.** Total RNA was isolated from leaves using an RNeasy Plant Mini Kit (Qiagen). cDNA was synthesized using SuperScript II Reverse Transcriptase kit (Invitrogen). Real-time quantitative RT-PCR was performed with a DNA Engine Opticon System (MJ Research) using DyNAmo HS SYBR Green qPCR Kit (New England BioLabs). *Actin2* gene was used as the endogenous control. Relative expression levels were determined as described (39).

**Anthocyanin and Hydrogen Peroxide Measurements.** Anthocyanin were extracted in water from 0.2 g leaves by grinding in liquid $N_2$. After centrifugation at 16,000g, the supernatant was diluted with 0.4 M sodium acetate (pH 4.5) and 25 mM potassium chloride (pH 1.0). Total monomeric anthocyanin was measured by recording the $A_{520}$ as outlined in Giusti and Wrolstad (40).

For hydrogen peroxide staining, plants were vacuum-infiltrated with 0.1 mg/mL 3,3-diaminobenzidine in 50 mM Tris-acetate buffer, pH 5.0. Samples were incubated for 24 h at room temperature in the dark. To remove chlorophylls, the stained samples were transferred to 80% ethanol.

**Mutant Complementation.** The coding region of *PSB27* gene was amplified by RT-PCR from total RNA and cloned in the vector pCB2004H to produce a construct that expresses PSB27 protein under the control of CaMV 35S promoter (39). For complementation by genomic DNA, a 2.2-kb genomic DNA fragment containing the coding region, 1.4-kb upstream, and 220-bp downstream sequences was amplified by PCR and cloned in the vector pCB2004. For complementation of *cp26* mutation, a 2.5-kb genomic DNA fragment containing the *CP26* gene and the 1.1-kb promoter was cloned in the vector pCB2004. The primers are shown in Table S1. These constructs were transferred into mutant plants using the floral dip method (41).

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