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State transitions and light adaptation require chloroplast thylakoid protein kinase STN7

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Photosynthetic organisms are able to adjust to changing light conditions through state transitions, a process that involves the redistribution of light excitation energy between photosystem II (PSII) and photosystem I (PSI)^{1,2}. Balancing of the light absorption capacity of these two photosystems is achieved through the

reversible association of the major antenna complex (LHCII) between PSII and PSI (ref. 3). Excess stimulation of PSII relative to PSI leads to the reduction of the plastoquinone pool and the activation of a kinase^{4,5}; the phosphorylation of LHCII; and the displacement of LHCII from PSII to PSI (state 2). Oxidation of the plastoquinone pool by excess stimulation of PSI reverses this process (state 1). The *Chlamydomonas* thylakoid-associated Ser-Thr kinase Stt7, which is required for state transitions, has an orthologue named STN7 in *Arabidopsis*⁶. Here we show that loss of STN7 blocks state transitions and LHCII phosphorylation. In *stn7* mutant plants the plastoquinone pool is more reduced and growth is impaired under changing light conditions, indicating that STN7, and probably state transitions, have an important role in response to environmental changes.

Although the phosphorylation of LHCII was observed many years ago^{7,8}, the search for kinases involved in this process in vascular plants has not yet been successful^{9,10}. The *Arabidopsis* genome contains two genes, *STN7* and *STN8*, that display significant sequence identity with the *Chlamydomonas* gene encoding the chloroplast Stt7 protein Ser-Thr kinase⁶. To determine the function of these proteins two *Arabidopsis* lines with T-DNA insertions in these genes were obtained from the Salk Institute collection. After self-crosses, homozygous lines for three T-DNA insertions (Supplementary Fig. S1) were identified by polymerase chain reaction (PCR) on genomic DNA by using appropriate primers as described in the Methods (data not shown). RT-PCR with specific primers for *STN7* and *STN8* was performed with RNA from the wild type and from the *stn7* and *stn8* mutants. Fragments corresponding to *STN7* and *STN8* with the expected size and sequence could be amplified from the wild-type RNA but not from the RNA of the mutants, indicating that the expression of the *STN7* and *STN8* genes is blocked in these lines (Supplementary Fig. S1).

In land plants, 15–20% of LHCII is mobile during state transitions and is reversibly displaced between PSII and PSI (ref. 3). To determine whether state transitions are affected in *stn7*, fluorescence measurements were performed as described^{11,12}. The maximum fluorescence signal, F_m , was measured on an intact leaf with a saturating flash using a pulse amplitude modulation fluorimeter. The leaf was subsequently illuminated with blue light in order to excite preferentially PSII, and the stationary fluorescence yield was recorded. After 15 min, far-red light was added to the blue light. This led to the stimulation of PSI and the transition to state 1 (Fig. 1a, b). After 15 min of blue and far-red light treatment, the maximal fluorescence in state 1 (F_{m1}) was determined. Then the far-red light was switched off to promote the return to state 2 under blue light excitation, and the maximal fluorescence in state 2 (F_{m2}) was determined after 15 min. Because the intensity of the light used to induce state transitions in Fig. 1 was not sufficient to elicit photo-inhibition as verified by F_v/F_m (where F_v is variable fluorescence) measurements (data not shown), we conclude that the observed changes in F_m are caused by state transitions alone.

Transition from state 1 to state 2 can be measured by the changes in maximal fluorescence ($(F_{m1} - F_{m2})/F_{m1} \times 100$) (ref. 8). In the wild-type strain this value was 10% (Fig. 1a). A similar value was obtained with heterozygous *stn7/stn7* plants (data not shown). In contrast, in the homozygous *stn7/stn7* mutant, state transitions were undetectable (Fig. 1b). Thus the *stn7* mutation is recessive, as expected from a loss-of-function mutation. Similar measurements with the *stn8* homozygous mutant indicated that it is not significantly affected in state transitions (data not shown). The double mutant *stn7/stn8* displayed the same phenotype as *stn7*. The wild-type phenotype was restored after *stn7* plants were transformed with the wild-type *STN7* gene (Fig. 1c).

To determine the changes in fluorescence both in PSII and PSI, low-temperature fluorescence emission spectra were measured at 77 K under state 1 and state 2 conditions. The spectra were normalized at 685 nm, corresponding to the peak of PSII fluorescence. In

wild-type plants a transition from state 1 to state 2 was accompanied by a large increase in relative PSI fluorescence at 730 nm, indicating a redistribution of the light excitation energy from PSII to PSI (Fig. 1g). In contrast, only a small increase in relative PSI fluorescence occurred in the *stn7* mutant, confirming that transition to state 2 is vastly reduced and that this mutant is blocked in state 1, similar to the *stt7* mutant of *Chlamydomonas* (Fig. 1h).

The presence of a putative NH₂-terminal transit peptide suggested that the *STN7* kinase is localized within the chloroplast, as is the case with the *Stt7* orthologue in *Chlamydomonas*⁶. To verify this localization, we transformed the *stn7* mutant with a construct carrying the *STN7* complementary DNA tagged with the haemagglutinin (HA) epitope at its 3' end under the control of the endogenous *STN7* promoter. Transformants obtained with this construct had a wild-type state transition phenotype, indicating that the tagged protein retains its activity (Fig. 1d). Immunoblotting of purified soluble and membrane chloroplast fractions with anti-HA antiserum revealed that STN7-HA was present in the chloroplast membrane fraction, with the expected size of 58 kDa (Supplementary Fig. S2A). Treatment of chloroplast membranes with calf intestinal phosphatase increased the mobility of the band, suggesting that the STN7 protein is phosphorylated (Supplementary Fig. S2A). The chloroplast localization of STN7 was further confirmed by transient expression of STN7 fused to green fluorescent protein (GFP) in isolated *Arabidopsis* protoplasts. Moreover, the amino-terminal 88 amino acids of STN7 are sufficient to target GFP into chloroplasts (Supplementary Fig. S2B).

The current model of state transitions assumes that phosphorylation of LHCII is required for the transition from state 1 to state 2 (refs 12, 13). Thr residues in the N-terminal part of the products of *Lhcb1* and *Lhcb2* are phosphorylated under state 2 conditions⁵. If the *stn7* mutant is blocked in state 1, it should fail to phosphorylate

LHCII under state 2 conditions. To test this prediction, LHCII phosphorylation was measured under state 1 and state 2 conditions in the wild-type and *stn7* strains. State 1 was obtained by maintaining the plants in the dark¹⁴ and state 2 was induced by light (80 μmol m⁻² s⁻¹, 30 min). Thylakoid membranes were isolated and the proteins were fractionated by polyacrylamide gel electrophoresis (PAGE) and immunoblotted with anti-phosphothreonine antibodies. A marked increase in LHCII phosphorylation was observed for the wild type but not for *stn7* under state 2 conditions (Fig. 2a). Moreover, phosphorylation of the D1 and D2 proteins was enhanced under these conditions for both the wild type and *stn7*. The fact that the increase in D1/D2 phosphorylation still occurred in *stn7* under state 2 conditions indicates that the STN7 kinase is specifically involved in LHCII phosphorylation. Protein kinase activity was also determined with isolated thylakoids using [γ-³²P]ATP for labelling. State 1 was obtained in the dark and gave rise to a weak phosphorylation of LHCII. Upon induction of state 2 by light for 20 min, LHCII was strongly phosphorylated in wild-type but not in *stn7* thylakoids (Fig. 2b). Phosphorylation of D1/D2 and PsbH was also markedly increased in wild-type thylakoids and only slightly less so in *stn7* thylakoids, indicating that these different substrates are phosphorylated by another kinase. The identity of the phosphorylated LHCII band was confirmed by its enrichment in the purified LHCII fraction (data not shown).

To test further the role of the STN7 kinase in state transitions, the conserved Lys 167 within domain II of the HA-tagged STN7 kinase was changed to Arg or Gln by site-directed mutagenesis. The gene of this altered kinase was introduced into the *stn7* mutant by transformation. No restoration of state transitions was observed, although the mutant protein accumulated (Fig. 1d–f, i). This clearly demonstrates that the activity of the STN7 kinase is required for state transitions. Thus, the *Chlamydomonas* *Stt7* and *Arabidopsis* *STN7* kinases are not only structurally but also functionally related. However, it is not yet known whether these kinases phosphorylate LHCII directly or whether additional kinases are involved in this process.

Mutants deficient in cytochrome *b₆f* or PSI activity are deficient in state transitions^{15,16}. To test whether accumulation of these complexes was affected in *stn7*, immunoblots were performed with proteins from wild type and the *stn7* mutant using antisera directed against the Rieske and PsaA proteins, which are representative subunits of these complexes. Moreover, immunoblots with antibodies against D1 (PSII), AtpA, LHCII and LHCI were used. In all cases no difference between wild type and *stn7* was observed (Supplementary Fig. S4). Because the TAK kinase also appears to be involved in state transitions^{17,18}, we verified by immunoblotting with TAK antisera that its expression is not altered in the *stn7*

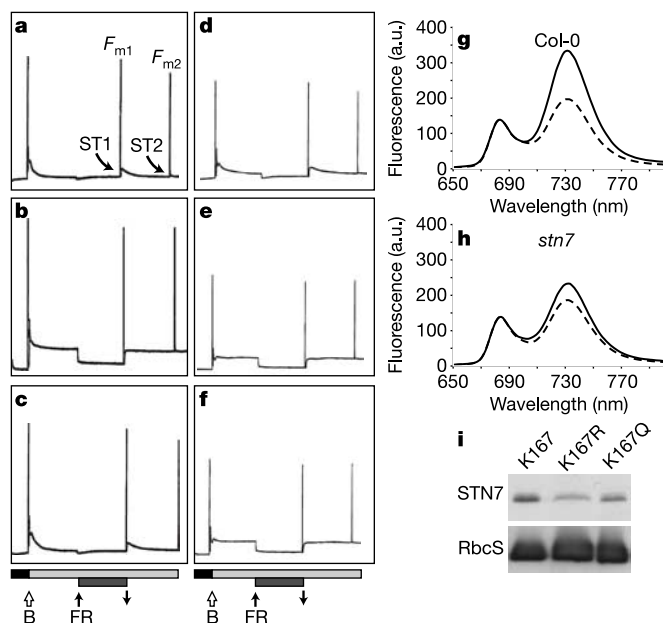


Figure 1 The STN7 protein kinase of *Arabidopsis* is required for state transitions. **a–f**, Blue light (B) and blue light supplemented with far-red light (FR) were used to induce transitions to state 2 and state 1, respectively. F_m (maximal fluorescence) was measured with a saturating 0.8-s flash at room temperature. Upward arrow, light switched on; downward arrow, light switched off. **a**, Col-0 (wild type); **b**, *stn7*; **c**, *stn7*-R, *stn7* rescued with *STN7*; **d–f**, *stn7* transformed with *STN7*-HA, *STN7*-HA-K167R and *STN7*-HA-K167Q, respectively. **g, h**, Low-temperature emission spectra of thylakoids from Col-0 (**g**) and *stn7* (**h**) in state 1 (dashed lines) and state 2 (solid lines). a.u., arbitrary units. **i**, Immunoblots with total extracts from *stn7* transformed with *STN7*-HA, *STN7*-HA-K167R and *STN7*-HA-K167Q with anti-HA and RbcS antisera.

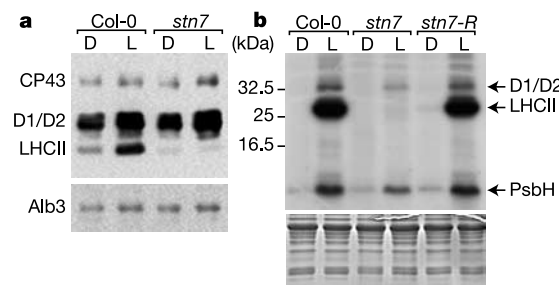


Figure 2 Phosphorylation of LHCII is diminished in *stn7* under state 2 conditions. **a**, Thylakoid membrane proteins extracted from Col-0 and *stn7* in the dark (state 1) or in the light (state 2) were separated by PAGE and immunoblotted with an anti-phosphothreonine antiserum. Equal protein loading was checked with antiserum against the thylakoid protein Alb3. **b**, Thylakoid membrane proteins extracted from wild-type and *stn7* leaves were incubated with [γ-³²P]ATP in the dark (state 1) or in the light (state 2). Proteins were separated by PAGE and autoradiographed.

mutant (Supplementary Fig. S4). On the basis of the observation that loss of the TAK and STN7 kinases leads to different phenotypes and that in contrast to STN7, the TAK kinase is involved in the phosphorylation of other thylakoid proteins besides LHCII, it is unlikely that these kinases act in the same pathway.

Because the main component in non-photochemical chlorophyll fluorescence quenching is energy-dependent (qE)¹⁹, it was important to check that the observed changes in fluorescence of the *stn7* mutant are not due to alterations in qE. Wild-type and *stn7* plants were grown either at a PFD (photon flux density) of 60 or 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and detached leaves were subsequently challenged with increasing light intensities. There was no significant difference in non-photochemical chlorophyll fluorescence quenching (NPQ) between wild-type and mutant plants under both conditions (Fig. 3a, b). Moreover, we confirmed previous studies showing that the qE-deficient mutants are able to perform state transitions²⁰ (data not shown). Taken together these results indicate that state transitions and qE operate independently from each other.

Although state transitions were discovered more than thirty years ago^{1,2}, their exact function is still not clear. The proposal that this process could have a role in protection against high light levels seems less likely given the fact that the LHCII kinase is inactivated by high light levels²¹. Another proposal is that state transitions may be involved in optimizing the photosynthetic yield and thus growth under low light conditions. However, growth of *stn7* mutant plants was not different compared to that of wild type when plants were subjected to an 8 h light/16 h dark regime either at a low (60 $\mu\text{mol m}^{-2} \text{s}^{-1}$) or higher PFD (160 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Photosynthetic parameters were determined from detached leaves of these plants by measuring chlorophyll fluorescence and CO₂ assimilation under increasing light intensities. In *stn7* leaves, the plastoquinone pool was more reduced as shown by the increase in $1 - q_p$ (Fig. 3c, d), where q_p is photochemical quenching. This is probably

due to the fact that excitation energy could not be redistributed to PSI through state transitions. An increase in plastoquinone reduction was also reported in the *Arabidopsis* strain lacking PsaH, which is deficient in state transitions^{22,23}. Although CO₂ assimilation rates of *stn7* and wild-type leaves were similar in plants grown at 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$, this was no longer the case when plants were grown at 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 3e, f). Under these conditions the maximum rate of CO₂ fixation (P_{max}) was reduced but there was no significant change of the quantum yield of CO₂ fixation (initial slope in Fig. 3e). These observations are compatible with the fact that there was no significant change in growth between wild type and *stn7* under low light conditions. The lowering of P_{max} may result from the loss of control of the plastoquinone redox state in *stn7*, which may perturb the adaptation processes to light, which are known to be complex. Thus, the STN7 kinase may also have an important role in the regulation of the redox state of the plastoquinone pool by balancing the light absorption between the two photosystems.

In order to determine the impact of state transitions on plant growth, plants were placed under changing light conditions. In a first experiment, plants were grown under 8 h light/16 h dark photoperiods in which the PFD was changed every hour from 50 to 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during the light period. In another experiment the plants were subjected to a 12 h light/12 h dark regime with alternating 1 h PSII and PSI light cycles during the light phase (see Methods). Under both conditions growth of the *stn7* mutant was impaired relative to wild-type plants as further confirmed by fresh and dry weight measurements (Fig. 4). Notably, flowering of the *stn7* plants grown under changing PSII and PSI light cycles occurred earlier (44 ± 3 days) than in the wild-type plants (51 ± 4 days). When the periods of the light cycles were increased, the differences in growth between wild-type and mutant plants became less pronounced. It is likely that under these conditions other more long-term mechanisms of adaptation involving changes in the expression of photosynthetic genes²⁴ become prominent. Our results differ from those obtained with *Arabidopsis* plants that lack PsaH and have state transition deficiencies. In this case growth was not impaired although oxygen evolution was diminished by 14%²³. These differences could be due to the particular growth conditions used and/or to the different primary lesions in these plants.

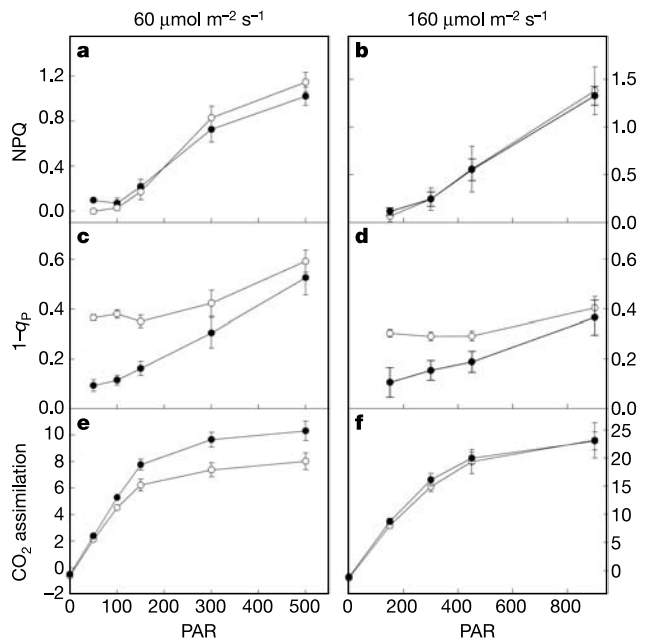


Figure 3 Measurements of photosynthetic parameters for *stn7* detached leaves. Col-0 and *stn7* were grown under an 8 h/16 h light/dark regime at 60 or 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during the light period. Measurements of chlorophyll fluorescence parameters and CO₂ assimilation were performed with various light intensities as indicated. Gas conditions were 1.2% O₂, 750 $\mu\text{l CO}_2 \text{l}^{-1}$ air. **a, b**, NPQ (qE); **c, d**, $1 - q_p$; **e, f**, CO₂ assimilation (expressed in $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$). Col-0, filled circles; *stn7*, open circles; PAR, photosynthetic active radiation ($\mu\text{mol m}^{-2} \text{s}^{-1}$). Standard deviations were determined from five and six independent measurements for plants adapted to 60 and 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively.

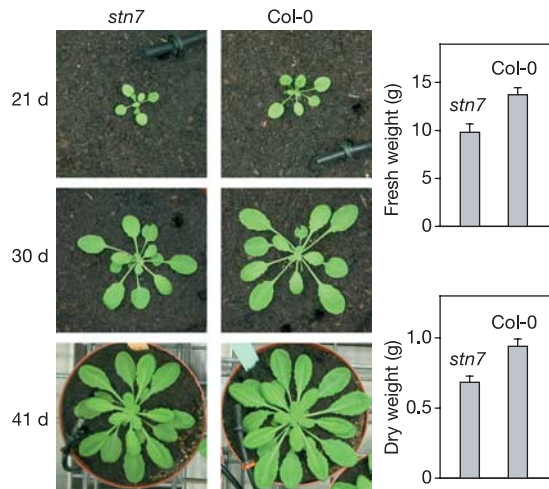


Figure 4 Growth of the *stn7* mutant is impaired under changing light conditions. Col-0 and *stn7* plants were grown under an 8 h/16 h light/dark regime. After one week at 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$, plants were alternatively illuminated for 1 h at 50 and 1 h at 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Pictures were taken after 21, 30 and 41 days. Plant fresh and dry weights were determined after 50 days. Standard deviations were determined from eight and seven measurements for *stn7* and Col-0, respectively.

As much as 80% of the LHC antenna is mobile in *Chlamydomonas* during state transitions²⁵, whereas in land plants the mobile fraction of LHCII is only 15–20%³. In spite of this relatively low value, our study shows that in land plants the STN7 kinase, and probably state transitions, are important for adaptation and that in their absence growth is significantly impaired under conditions in which light quality and quantity change frequently. This points to the importance of state transitions in a natural environment where plants are often subjected to light fluctuations of this sort. □

Methods

Plant material

Arabidopsis thaliana (L.) ecotype Columbia (Col-0) was used for all experiments. Plants were grown under controlled conditions of light (50 or 160 μmol m⁻² s⁻¹; 8 h or 12 h photoperiods, 23/20 °C day/night, and relative air humidity of 50–70%).

All physiological and biochemical analyses were performed with rosette leaves harvested before flowering. We obtained the T-DNA insertion lines in the Columbia background for At1g68830 (SALK 073254) and At5g01920 (SALK 060869 and SALK 064913) from the Salk Institute (see Supplementary Information for the characterization of these lines and for the DNA, RNA, protein and chlorophyll analyses).

State transitions and NPQ

State transitions and NPQ were measured as described^{11,26} (see Supplementary Information for details).

Photosynthetic measurements

Photosynthetic gas exchange and chlorophyll fluorescence measurements were simultaneously performed on detached leaves using a LI-6400 portable photosynthesis system equipped with a 6400-40 fluorometer (LI-COR Biosciences) (for details see Supplementary Information). PSII light was obtained with cool white fluorescent lamps (Osram L18W/20) with orange 105 Lee filters and PSI light was obtained with red fluorescent lamps (Osram L18W/60) with red 027 Lee filters. Chlorophyll fluorescence emission spectra of thylakoid membrane suspensions were recorded in liquid nitrogen (77 K) as described^{27,28} (see Supplementary Information).

In vivo and in vitro phosphorylation of the LHCII antennae

Leaves from dark-acclimated plants, floating on water, were exposed to low light (80 μmol m⁻² s⁻¹) or kept in the dark for 30 min²⁹. Thylakoid membranes were isolated from the dark-incubated and illuminated leaves as described²⁷ in the presence of 10 mM NaF to inhibit phospho-LHCII phosphatase activity. Thylakoids were re-suspended in assay buffer consisting of 50 mM HEPES-KOH pH 7.5, 100 mM sucrose, 5 mM NaCl, 10 mM MgCl₂ and 10 mM NaF at a final chlorophyll concentration of 0.4 mg ml⁻¹.

After dark adaptation, thylakoids were isolated from plants according to ref. 30, and re-suspended in storage buffer (100 mM sorbitol, 5 mM MgCl₂, 5 mM NaCl and 50 mM HEPES/KOH pH 7.5). They were used as substrate for the kinase assay. Thylakoid membrane proteins equivalent to 8 μg of chlorophyll were subjected to a 20 min light induction (80 μmol m⁻² s⁻¹) at 25 °C in the presence of 10 μCi [γ -³²P]ATP (Amersham 3,000 Ci mmol⁻¹), 0.4 mM ATP and 10 mM NaF in 100 μl of storage buffer²⁹. Reactions were terminated by centrifugation, washing twice in storage buffer and addition of denaturing sample buffer, and were electrophoresed on 12% polyacrylamide-SDS gels, and finally analysed with a phosphorimager.

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Functional cartography of complex metabolic networks

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High-throughput techniques are leading to an explosive growth in the size of biological databases and creating the opportunity to revolutionize our understanding of life and disease. Interpretation of these data remains, however, a major scientific challenge. Here, we propose a methodology that enables us to extract