Photosynthetic Electron Transport Determines Nitrate Reductase Gene Expression and Activity in Higher Plants*

Received for publication, March 26, 2002, and in revised form, September 12, 2002
Published, JBC Papers in Press, September 18, 2002, DOI 10.1074/jbc.M202924200

Irena Sherameti, Sudhir K. Sopory‡, Artan Trebicka§, Thomas Pfannschmidt¶, and Ralf Oelmüller¶

From the Institut für Allgemeine Botanik, Lehrstuhl für Pflanzenphysiologie, Dornburger Strasse 159, 07743 Jena, Germany

The influence of photosynthetic electron flow in chloroplasts on the expression and enzyme activity of the cytosolic nitrate reductase (NR) was studied. Using light sources that predominantly excite either photosystem I (PSI) or photosystem II (PSII), we modulated photosynthetic electron transport in tobacco, Arabidopsis, and Lemma sprouts. In all instances, oxidation of components of photosynthetic electron flow by PSI light correlated with an increase in NR activity and/or transcription. This is confirmed by experiments with electron transport inhibitors 3-(3′,4′-dichlorophenyl)-1,1′-dimethyl urea and 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone. In addition, a Lemn a mutant deficient in the cytochrome b6/ f complex failed to respond to the different light sources and exhibited a constitutively high level of NR activity. These data indicate that NR is activated by the oxidized state of an electron transport component located after the plastoquinone pool. An involvement of the cytoplasmic photoreceptor phytochrome A in this light regulation could be excluded, since an Arabidopsis phytochrome A mutant exhibited a wild-type like response. The observation that NR activity in the cytoplasm and the expression of its gene in the nucleus is controlled by signals from photosynthetic electron flow adds a new facet to the intracellular cross-talk between chloroplasts and the nucleus.

Nitrate is the major source of nitrogen for all living organisms. It is reduced by nitrate reductase (NR), the key enzyme of the nitrate-assimilating pathway. NR catalyzes the rate-limiting step in this process (1) and generates nitrite in the cytoplasm of a plant cell, which is translocated into the plastids for further reduction and metabolism (2–6). Expression of nitrate reductase and its activity is highly regulated by a variety of environmental and cell-internal factors, such as nitrate or ammonium (2, 3, 7–11), the circadian rhythm (12–15), sucrose or glucose (16–18), the CO2 concentration (19–21), the developmental stage of the plastids (22–26), and especially light (12, 27–31). Most of the energy for nitrate assimilation in a cell derives from photosynthesis, and a few studies suggest that photosynthesis could be also involved in the light regulation of NR activity and NR gene expression (3, 32–34). However, our present understanding of this photosynthetic control is weak. Further complexity derives from the observation that NR is regulated at various levels. NR transcription is controlled via one or several responsive elements in the promoter (3, 5, 9, 35–39), and signal transduction mutants with abnormal NR transcription have been identified (40). Stimulation of NR gene expression leads to a rapid accumulation of the NR mRNA, followed by an increase in the protein level (41, 42). The protein has a short half-life, which allows an efficient down-regulation of the nitrate metabolism under unfavorable conditions (42, 43). In addition, NR can be rapidly inactivated by phosphorylation (44–47), and this scenario involves 14-3-3 proteins (48). Apparently the N-terminal part of the enzyme is essential for this regulation (49).

To which extent and why plastids exert their photosynthetic control on NR activity in the cytosol and NR gene expression in the nucleus is mainly unknown. This study monitored for the first time the changes in expression of the chimeric NIA2 promoter::UIDA gene fusions in transgenic tobacco seedlings, the NIA2 transcript accumulation in Arabidopsis and NR activity in three different species (Lemma, Arabidopsis, and tobacco) in parallel after physiological modulation of photosynthetic electron transport. Using photosystem-specific excitation in combination with electron transport inhibitor experiments on whole seedlings we demonstrate that the efficiency of photosynthetic electron flow affects the transcriptional rate and transcript pool size of the nuclear gene as well as the NR enzyme activity in a coordinated manner underlining the importance of photosynthesis as regulator of nitrate assimilation pathway components in plastids, cytosol, and nucleus.

EXPERIMENTAL PROCEDURES

Growth of Tobacco and Arabidopsis Seedlings—All tobacco (wild-type and transgenic lines, Samsun NN) and Arabidopsis (var. Columbia, or Landsberg in comparison with the phytochrome A mutant in the same background) seedlings were grown in Petri dishes with 0.5× strength Murashige and Skoog (MS) medium (50) supplemented with 2% sucrose in a temperature controlled growth chamber at 22 °C. Seedlings were either kept in PSI or PSII light for 16 days or they were transferred from one light source to the other 96 h before harvest. The spectral quality and light quantity of the PSI- and PSII-light sources (51, 52) as well as the characterization of the acclimation process of the...
tobacco seedlings to the two light sources have been described earlier (53). The acclamation of Lemma sprouts and of Arabidopsis (Landsberg erecta) seedlings has been analyzed in this study (see below). White light control plants were illuminated continuously for 18 days with 30-watt white stripe lamps (OSRAM) with a photon flux density of 100 μE. After 18 days, the seedlings were harvested and prepared for the determination of NR or β-glucuronidase (GUS) activity.

The electron transport inhibitors DCMU and DBMIB (Sigma) have been applied to wild-type and transgenic plants as described (53). Control seedlings were treated with the solvent without inhibitors. DBMIB treatment was repeated every 3–4 days during the last 96 h of experimentation. Stock solutions of DCMU were 10 mM in 50% ethanol and of DBMIB 100 mM in 10% Me₅SO in ethanol. Sublethal effects of usual inhibitor concentrations (10 μM for DCMU and 25 μM for DBMIB) have been determined and described earlier (53). The different inhibitor concentrations were prepared by dilution in sterile water directly prior to use. For RNA isolation, Arabidopsis wild-type seedlings were grown for 14 days under PSI or PSII light and were then switched to the respective other light source for 8 h. In inhibitor experiments plants were treated with 10 μM DCMU or 25 μM DBMIB just before this light switch. Untreated controls remained 14 days and 8 h under PSI or PSII light. Growth of Lemma aquinocialis—The propagation, growth conditions, growth media, and temperature were described by Appenroth et al. (54), except that the medium was supplemented with 50 mg glucose. Wild-type and mutant strains (1073, see Ref. 55) were treated identically. After 2 weeks in white light, sprouts were transferred to darkness for 3 days before shifting them to PSI or PSII light for 48 h. After acclimation, sprouts were transferred to the respective other light source for an additional 48 h, and NR activity was assayed.

Isolation of the NIA2 Promoter, Generation of Transgenic Plants, F3 Generation—The NIA2 promoter from Arabidopsis was isolated using classical strategies. A genomic Arabidopsis library was constructed in pBluescript and a positive phage was isolated using a NIA2 cDNA fragment as a probe. Sequence analysis confirmed that the phage contained the NIA2 sequence. Using PCR, we amplified a 3.6-kb fragment of the 5′-flanking region. At the 5′-end we designed a primer that ends directly before the ATG codon. After the complete sequence of the Arabidopsis genome became available, our results were confirmed: NIA2 is located on chromosome 1 and our 5′-flanking region corresponds to the BAC F28L22 sequence 59370–62005. The DNA fragment was inserted into the Smal site of pBluescript II (56) and transformed into Nicotiana tabacum (Samson NN) via Agrobacterium tumefaciens (57). 35 transgenic lines were regenerated after selection on kanamycin (100 μg/ml). F3 seedlings of 6 lines that showed the highest expression level of the reporter gene were used for these studies.

Physiological Characterization of Transgenic Lines—For the initial characterization of the transgenic lines, F3 seedlings were grown on solidified MS medium with MS medium for 4 days in either light or darkness. The media were either not supplemented or supplemented with nitrate (15 mM), cytokinin (N6-benzylaminopurine, 10 mM), abscisic acid (10⁻⁵ M), sucrose (2%), or norflurazon (10⁻⁴ M). For electron transport inhibitor experiments seedlings were sprayed with DCMU or DBMIB as described above.

GUS Staining—Seedlings were harvested and immediately put into X-gluc solution (50 mg X-gluc; 1 ml of dimethylformamide; 4.9 ml of 50 mM sodium phosphate, pH 7.0; 250 μl of Me₂SO; 500 μl of potassium hexacyanoferrate (III) (100 mM); 500 μl of potassium hexacyanoferrate (II) (100 mM)) and incubated overnight at 37°C. After washing with water, the seedlings were incubated in 10% ethanol and stored at 4°C. For GUS staining of root hairs, seeds were germinated and seedlings were grown in liquid MS medium to avoid hair damage. Fluorescence Measurements—In vivo Chl fluorescence parameters were measured with a pulse amplitude-modulated fluorometer (PAM101/103; Heinz Walz, Effeltrich, Germany). Arabidopsis seedlings and Lemma sprouts were arranged densely, so that the fluorescence of several seedlings/sprouts could be measured simultaneously under the emitter/detector unit. Fluorescence parameters were determined as described previously (53). The steady-state fluorescence F₀ was calculated as F₀ = Fm' − Fp − Fp'. Fluorescence-quenching parameter Fp (photochemical quenching) was calculated as Fp = (Fm' − F₀)/(Fm − F₀) (58). The effective quantum yield of PSI (Φ PSI) was calculated as Φ PSI = (Fm' − Fp')/(Fm − Fp') (58).

RNA Preparation and Quantitative RT-PCR—Total RNA from 3–5 g of leaf material was isolated following a protocol modified from Chomczynski and Sacchi (60) using the TRizol reagent (Invitrogen Life Technologies). RT-PCR analysis was performed by reverse transcription of 5 μg of total RNA with gene-specific reverse primers (see below) for Arabidopsis NIA2 and 18S rRNA genes using a first strand cDNA synthesis kit (K1631; MBI Fermentas, St. Leon-Roth, Germany) followed by 20 PCR cycles. Gene-specific primer pairs for amplification of NIA2 and actin genes were as follows: NIA2 forward primer, 5’–ATG GCC GCC TCT GTA GAT AAT CGC CC–3’; reverse primer, 5’–CTT CGT GAC ATG GGG TGG TAA TCA CGG–3’; 18S forward primer, 5’–GTT AGG CGA TTG GCT AAC ATT GTC TGC–3; reverse primer, 5’–GAG ACA CCA ACA GTT CCT CTT CTG CG–3’. PCR products were separated on 1.5% agarose gels and stained with ethidium bromide, and visualized bands were quantified with the ImageMaster Video system (Amersham Biosciences).

Enzyme Assays—The NR and GUS assays were described earlier (14, 57). In both instances the system of reference was an equal amount of fresh weight.

Sugar Determination—Sucrose was determined with an analytical kit from Roche Molecular Biochemicals (716260, Ingelheim, Germany).

Statistics—All NR activity data are based on seven independent experiments. The GUS values are based on independent experiments with the F3 seedling populations of six independent lines; all experiments were repeated seven times.

RESULTS

Expression and Regulation of the Arabidopsis NIA2 Promoter—UIDA Gene Fusion in Tobacco—To analyze the regulation of NR gene expression under various environmental conditions as well as its spatial expression characteristics in whole plants, we generated transgenic tobacco plants with a chimeric Arabidopsis NIA2 promoter::UIDA gene fusion (see “Experimental Procedures”). The isolated 3.6-kb promoter region differed only in 3 positions in the far upstream region from the sequence, which is now available in the data bank (GenBank™, acc. no. F28L22, see “Experimental Procedures”). Initial primer extension analyses identified 3 major transcription start sites (59, 88, and 152-bp upstream of the ATG codon), the most prominent one being located 88-bp upstream of the ATG codon (data not shown), which is consistent with previous results (36). F3 seedlings from six independent transgenic lines were used for initial physiological and histological studies (Table I). GUS gene expression was significantly stimulated by light. The application of cytokinin, abscisic acid, sucrose, norflurazon, DCMU, and DBMIB had only small effects on the NIA2 promoter-driven reporter gene expression in the dark, only nitrate could activate it significantly. Additional illumination, however, led to a stimulation of promoter activity in each case. This becomes especially apparent when combinations of nitrate, sucrose, and cytokinin treatments were used (data not shown). As only exception, norflurazon treatment of

| Treatment | Darkness | White light |
|-----------|----------|-------------|
| None      | 100      | 175         |
| Nitrate   | 275      | 566         |
| Cytokinin | 133      | 341         |
| Abscisic acid | 83      | 200         |
| Sucrose   | 82       | 600         |
| Norflurazon | 116     | 75          |
| DCMU      | 89       | 185         |
| DBMIB     | 128      | 160         |

Table I: GUS activity in tobacco lines harboring NIA2 promoter::UIDA gene fusions

Seeds were grown on half-strength MS medium in either darkness or white light for 18 days, and whole seedlings were used for determination of the GUS activity. The medium was supplemented with nitrate, cytokinin, abscisic acid, sucrose, or norflurazon. As control unsupplemented medium was used. DCMU and DBMIB were sprayed directly on the seedlings. Results are based on seven independent experiments with six independent primary transformants. Activity of untreated dark-grown plants was taken as 100%, and all other values were expressed relative to it. Maximal S.E. was ± 8%. For experimental details, see “Experimental Procedures.”
seedlings, which completely prevents plastid biogenesis (61), resulted in an inhibitory effect either when applied alone or in combination with other positive regulators (data not shown). These results demonstrate that the transgene responds similarly to the applied regulatory compounds or signals as described in other studies with NR (see the Introduction) and confirm at least partial transcriptional regulation of NR.

Further GUS staining experiments indicate that the promoter is active in shoots and roots (Fig. 1). If seedlings are grown under conditions that allow extensive root formation and subsequent analysis of GUS staining (see “Experimental Procedures”), the high expression level in root hairs becomes obvious. Irrespective of the system of reference (fresh weight or protein content), 20% of the total GUS activity in white light-grown seedlings is found in roots, while more than 40% is detectable in roots of etiolated seedlings. More detailed studies uncovered that the activity in shoots is up-regulated by light and the functional stage of the plastids while the GUS level in the roots is more or less constitutively expressed (data not shown). This suggests that light regulation of NR in shoots is limited to photosynthetic active tissue. Therefore, all subsequent physiological studies were performed solely with cotyledons from seedlings grown under different light conditions.

Transgenic tobacco lines with PSI promoter::UIDA reporter gene constructs have been demonstrated to represent a useful tool to investigate photosynthetic redox signaling pathways between plastids and the nucleus (53). Therefore the tobacco lines with the NIA2 promoter::UIDA gene fusion offered the opportunity to determine in an identical experimental setup whether light regulation of the Arabidopsis NIA2 promoter activity is coupled to photosynthetic electron transport. In this setup transgenic tobacco seedlings were grown under light sources favoring either PSI or PSII. Such light sources induce imbalances in the excitation of the two photosystems, and shifts between them can be used to generate oxidation or reduction signals from photosynthetic electron transport (51, 52, 62). Furthermore, in this system exogenous application of sublethal concentrations of electron transport inhibitors DCMU and DBMIB, which do not block, but limit the photosynthetic electron transport allow to confirm a coupling of photosynthetic electron transport and transgene promoter activity (53).

Redox Regulation of NIA2 Transcript Accumulation—To analyze if the regulation of the NIA2 promoter activity is reflected in transcript abundance and if the transgene expression in tobacco correlates with the situation in Arabidopsis we monitored the NIA2 transcript pool size by RT-PCR in Arabidopsis seedlings, which were grown under the same conditions as the transgenic tobacco lines. The NIA2 RT-PCR product (Fig. 3, lane 2) exhibited essentially the same regulation as the transgenic tobacco lines. The NIA2 transcript size in leaves of 18-day old tobacco seedlings harboring an Arabidopsis NIA2 promoter::UIDA gene fusion with a PSI promoter activity is coupled to photosynthetic light stimulated and PSII light inhibited the Arabidopsis NIA2 promoter activity (Fig. 2). Conversely, PSI light grown plants (PSII plants) which were shifted to PSII light (PSI → II plants) showed a decrease in the NIA2 promoter activity while PSI light grown plants (PSI plants) exhibited the opposite reaction after a shift to PSI light (PSI → I plants). We then added DCMU and DBMIB in the same way as described previously (53) in order to manipulate the redox signal generated by the photosynthetic electron transport. Both inhibitors had no or only small effects on the NIA2 promoter activity in PSII plants but activates it significantly in PSI plants. These data are consistent with that obtained in the light-shift experiments and demonstrate that NR transcription is coupled to photosynthetic electron transport.

Redox Regulation of Nitrate Reductase Enzyme Activity—Nitrate reductase is also highly regulated at the level of the plastids and the nucleus (53). Therefore the tobacco lines with the NIA2 promoter::UIDA gene fusion we determined that PSI light stimulated and PSII light inhibited the Arabidopsis NIA2 promoter activity (Fig. 2).

Fig. 1. GUS staining of transgenic tobacco seedlings harboring a NIA2 promoter::UIDA gene fusion. Comparison of a white light-grown seedling (left) with an etiolated seedling. Bottom, GUS staining of root hairs of a white light-grown seedling.
enzyme activity. To elucidate if the observed redox regulation extends to the enzyme activity tobacco has been grown under the different light regimes and NR activity was determined (Fig. 4). Under PSI light NR activity was higher than under PSII light. Furthermore, shifts between these light sources resulted in corresponding changes of NR activity, i.e. a PSI → II light shift repressed the enzyme activity whereas a PSII → I light shift increased it. These results are consistent with those obtained at the transcriptional level.

To obtain additional support for the coupling of NR activity to photosynthetic electron transport, which did not rely on inhibitor experiments, we included a mutant of *L. aequinoctialis* (1073) that lacks the cytochrome b6f complex because of a mutation in the Rieske protein (55). In this mutant all redox-reactive compounds located after the plastoquinone pool are always oxidized. Furthermore, light regulation of NR activity was shown to be mediated by phytochrome A (*phyA*) (63). To prove the independence of the observed photosynthetic control from the cytosolic photoreceptor we decided to test the response of a *phyA*-deficient *Arabidopsis* mutant to the PSI and PSII light sources. Since both organisms have not been used in our experimental setup before we first had to determine the regular response of the respective wild-types to PSI and PSII light treatments. We checked the respective acclimation of wild-type *Arabidopsis* seedlings and *L. aequinoctialis* sprouts by determining characteristic changes in Chl fluorescence parameters, which were obtained by standard pulse amplitude modulated fluorescence measurements (Table II) as reported in previous studies with mustard and tobacco seedlings (52, 53). No significant changes in the maximal quantum yield were observed after acclimation of the plants to the different conditions. However, PSI plants show a higher level in steady state fluorescence Fs than PSI plants, which is known as a typical acclimation response (53). The Fs/Fm ratio therefore is high in PSI plants and decreases after acclimation to PSII light, and the opposite reaction was observed for PSII plants after acclimation to PSI light. As a consequence the reduction state of the first electron acceptor of PSII, QA, expressed as 1–qP, is significantly higher in PSI and PSII → I plants than in PSI and PSII → II plants. By contrast, the effective quantum yield φPSII is higher in PSII plants than in PSI plants and changes accordingly after the shift to the alternate light source. These data indicate that *Arabidopsis* and *Lemna* readily acclimate to light quality.

We then determined the NR activity in these organisms after acclimation to the PSI and PSII light sources. In *L. aequinoctialis* wild-type sprouts NR was found to be substantially decreased under PSII light as compared with PSI seedlings (Fig. 5). Shift experiments from one light source to the other demonstrated that 2 days are sufficient to acclimate the NR activity to the new light conditions. The *Lemna* mutant 1073, however, failed to respond to the different light conditions and showed a constitutively high level of NR activity under all illumination conditions, comparable to the activity level detectable in PSI light acclimated wild-type sprouts. Thus, oxidation of compounds associated with the photosynthetic electron flow after the PQ pool appears to be accompanied by an increase in the NR activity in the cytoplasm.

Fig. 6 demonstrates that this is also observed for *Arabidopsis* seedlings. NR activity was always stimulated by PSI light and repressed by PSII light. For the shifted seedlings, the redox state of the plastoquinone pool was also modulated by the inhibitors DCMU or DBMIB (53), which were applied directly before the light shifts. In all instances, NR activity increased to values comparable to that from the PSI seedlings (data not shown). It is concluded that the redox state of the plastoquinone pool is not involved in NR activity in the cytoplasm and that redox-reactive compounds located after the plastoquinone pool in the electron transport chain are crucial for NR regulation. The same experiments performed with the *phyA* mutant led to comparable results (Fig. 6), again illumination with PSI light activated NR activity while PSII light repressed it. Since this response pattern is identical to the wild-type seedlings it is concluded that the observed redox regulation of NR activity is independent from *phyA*-mediated light regulation.

**DISCUSSION**

We showed that acclimation of three different plant species to light sources favoring either PSI or PSII excitation dramatically affected NR activity and/or gene expression. In all instances oxidation of photosynthetic electron transport components after the plastoquinol oxidation site, either by the PSI light source, by the application of inhibitors, or by a mutation...
Experimental Procedures.

Plants were acclimated to either PSI or PSII light or they were shifted to the respective other light source for 4 days. Respective growth conditions are given in the left column, fluorescence parameters determined, and species investigated given on top of each column. Values represent means of 3-5 different plants out of three independent experiments. Fluorescence parameters and measurements are described under "Experimental Procedures."

Table II: Chlorophyll fluorescence analysis of Arabidopsis (Landsberg erecta) seedlings and Lemna sprouts after acclimation to different light qualities

| Light | Fv/Fm | Fs/Fm | 1-qP | φ PSII |
|-------|-------|-------|-------|---------|
|       | Arabidopsis | Lemna | Arabidopsis | Lemna | Arabidopsis | Lemna | Arabidopsis | Lemna |
| PSI   | 0.78 ± 0.01 | 0.79 ± 0.02 | 0.168 ± 0.01 | 0.257 ± 0.05 | 0.268 ± 0.001 | 0.291 ± 0.003 | 0.523 ± 0.017 | 0.625 ± 0.015 |
| PSI → II | 0.80 ± 0.006 | 0.78 ± 0.05 | 0.097 ± 0.01 | 0.081 ± 0.06 | 0.154 ± 0.005 | 0.112 ± 0.09 | 0.634 ± 0.022 | 0.716 ± 0.09 |
| PSII | 0.80 ± 0.01 | 0.82 ± 0.03 | 0.099 ± 0.002 | 0.046 ± 0.025 | 0.063 ± 0.006 | 0.071 ± 0.003 | 0.605 ± 0.019 | 0.759 ± 0.01 |
| PSII → I | 0.79 ± 0.01 | 0.79 ± 0.005 | 0.17 ± 0.013 | 0.254 ± 0.001 | 0.204 ± 0.032 | 0.143 ± 0.02 | 0.562 ± 0.005 | 0.659 ± 0.04 |

Fig. 5. Effects of PSI and PSII light as well as light shift experiments from PSI to PSII (and vice versa) on nitrate reductase activity in wild-type and mutant sprouts of L. aequinoctialis. After 2 weeks in white light, sprouts were transferred to darkness for 3 days before acclimating them to PSI or PSII light for 96 h. For the PSI → PSII and PSII → PSI shift experiments, sprouts were first kept in one light quality for 48 h before transferring them to the other light source for additional 48 h. For experimental treatments, see "Experimental Procedures." Results are based on seven independent experiments, and bars represent S.E.

Fig. 6. Effects of PSI and PSII light and PSI → PSII (and vice versa) light shifts on nitrate reductase activity in leaves of 18-day old A. thaliana seedlings. WT, wild-type seedlings of Landsberg erecta; PhyA, the isogenic phytochrome A mutant (allele phyA-201). For details, see "Experimental Procedures" and text. Results are based on seven independent experiments, and bars represent S.E.

in the cytochrome b6f complex, resulted in an increase in NR activity in the cytoplasm. The responsible redox control parameter could not be identified, however the inhibitor studies clearly demonstrate that the redox state of the plastoquinone pool cannot be the origin of the signal, because application of DBMIB which blocks the electron transport after the plastoquinone pool, has the same effect on NR and GUS activities as DCMU which blocks before the plastoquinone pool. Therefore, the redox sensor should be located after the Qb site of the cytochrome b6f complex and must activate NR activity in a more oxidized state. One might argue that in planta treatments with DBMIB may lead to wrong results since DBMIB in very high concentrations is known to bind at the Qb site of PSI, however, in our experiments this is unlikely since the used concentration was shown to inhibit electron transport only partially (53). This is supported by the constitutively high and unregulated NR activity in the Lemna mutant lacking the cytochrome b6f complex (55), which suggests that the redox signal(s) originate even downstream from the cytochrome b6f complex. Alternatively, one might speculate that if the redox signal originates from a component in the cytochrome b6f complex, the lack of this complex in the mutant leads to the complete loss of any regulation resulting in permanent high NR activity.

Application of DCMU and DBMIB usually results in a decline of the response of interest, which makes it difficult to exclude side effects. In our studies, DCMU and DBMIB treatment of dark-grown seedlings had only little effect on NIA2 promoter activity suggesting no major side effects of the inhibitors at least at the concentrations used here. However, NR activity and expression are stimulated by both inhibitors. In addition, also in the Lemna mutant, in which the electron flow is completely blocked, a significant increase in the NR activity can be observed in comparison to the isogenic wild type. Stimulation rather than inhibition of the NR activity and/or transcription in response to the inhibitors therefore indicates that the effects are specific. Interestingly, DCMU and DBMIB had no effect on GUS gene expression when dark-grown tobacco seedlings were set into light. This could mean that the light-induced NIA2 promoter activity during greening of seedlings does not depend on photosynthesis and is mediated by other light receptors such as phytochromes. Another possibility, however, could be that the intensity of the white light source (which is 3-5 times of that of the PSI or PSII light sources) is so high that sublethal inhibitor concentrations does not generate a significant signal. Treatment of seedlings with 100 μM DCMU resulted in a 30% increase in GUS activity (data not shown) suggesting that this second possibility might be true.

In Arabidopsis, NR activity is the result of two gene products, NIA1 and NIA2. It remains to be determined whether both genes respond identically to the redox signal. Our data demonstrate that the total NR activity is regulated comparably to the expression of the NIA2:UIDA gene fusion, and previous studies have shown that NIA1 and NIA2:reporter gene fusions in transgenic tobacco respond similarly to exogenously applied agents such as nitrate (36). Thus it is very likely that the
Photosynthetic Control of Nitrate Reductase

regulatory mechanisms controlling the expression of both genes are similar.

The importance of light for NR regulation has been investigated in many studies (63, 64), however, until now there is still confusion as to whether light is an absolute requirement and what the primary photoreceptor is for such a regulation. Gowri and Campbell (8) have shown that nitrate can induce NR mRNA in etiolated and light-grown maize leaves, and Cheng et al. (16) proposed that light can be replaced by sucrose. Thus it was argued that light is not obligatory when nitrate and sufficient carbohydrates are available. Furthermore, in many studies it remained open whether phytochromes or components deriving from photosynthesis are crucial for NR regulation (63). Our data clearly demonstrate that phytochrome A in *Arabidopsis* is not involved in the NR regulation analyzed in this study and that the role of the photosynthetic electron flow for NR expression is probably more significant than anticipated thus far. It remains to be determined whether NR activity also responds to different irradiances. The PSI light used here has a lower photosynthetic active radiation than the PSII light, but nonetheless has an activating effect. Regulation of NR differs from other nuclear-encoded redox-controlled genes investigated in this study in that the PSI light source activates NR. Pfannschmidt et al. (53) have recently demonstrated that light sources preferentially exciting PSI stimulate the expression of nuclear genes for PSII components. These differences might be explained by the fact that the nitrate assimilation pathway is predominantly dependent upon the availability of reduction equivalents, whereas expression of PSI genes is regulated by an acclimation process, which optimizes light harvesting under unfavourable PS stoichiometry.

The transport of the redox signal from the thylakoid membrane to the cytoplasm at present is difficult to explain and may involve additional factors. In any case, triose phosphates generated during photosynthesis appear to be transported into the cytoplasm and provide the reducing equivalents for NR activity through their oxidation by triose phosphate dehydrogenase. Two systems, the dihydroxyacetone phosphate/phosphoglycerate shuttle and the malate/oxalacetate shuttle, have been proposed to provide NADH for nitrate reduction (see Ref. 64). Whether these pools are plastid target sites for regulation is presently unclear. An additional putative transmitter could be sucrose. Cheng et al. (16) have shown that 2% sucrose can replace light in eliciting an increase of NR mRNA accumulation in dark-adapted green *Arabidopsis* plants and that a 2.7-kbp region of the 5′-flanking sequence of the *NIA1* gene is sufficient to confer the light or sucrose response. Our physiological conditions differ from those of Cheng et al. (16) in that we kept the seedlings continuously on 2% sucrose under low light conditions. In addition, we could not detect any significant difference in the internal sucrose concentrations under our conditions (PSI plants: 1.19 ± 0.07 mg/g fresh weight; PSII plants: 1.22 ± 0.05 mg/g fresh weight). Thus, the involvement of bulk sucrose in the plastids and cytoplasm as a specific transmitter for the efficiency of the photosynthetic electron transport appears unlikely. In addition, Oswald et al. (34) reported that a plastid-derived redox signal can override the sugar-regulated expression of nuclear-encoded photosynthesis genes suggesting that photosynthetic redox signals may act independently from the sugar status of the cell. However, they found no significant effect of DCMU on NR transcript accumulation in an *Arabidopsis* cell culture. In the study NR mRNA abundance was found to be high under 3% sucrose and low after sugar repletion, and application of DCMU did not affect this regulation. It is difficult to reconcile this observation with our finding that DCMU application raises NR expression and activity. However, a conceivable explanation could be that 3% sucrose results in such a high NR induction that it masks any effect of the inhibitor even after sugar repletion whereas the constant presence of 2% sucrose in our study allows the variation of NR expression and activity by photosynthetic redox signals. This suggests that in the case of NR sugar signals can override photosynthetic redox signals, which represents the opposite type of regulation as observed for nuclear photosynthetic genes. This is consistent with the fact that NR is positively regulated by sugar signals while photosynthesis genes are negatively regulated. The exact range in which these signals cooperate or inhibit each other has to be analyzed in the future in a well defined physiological system.

What is the reason for the photosynthetic control of NR expression and activity by the PSI and PSII lights used in this study? Recently, Wollman (65) proposed that the progress in the field of State I/State II transitions offers a new view of photosynthesis as a flexible energy conversion system in which State I behaves as a carbon fixation device whereas State II operates more likely as an ATP generator. This view provides an attractive explanation for our observations. In State I (which is reached under PSI light) linear electron flow and generation of reducing equivalents are promoted thus allowing the reduction of assimilated nitrate beside CO₂. In State II (which is reached under PSII light) ATP generation is preferred, and nitrate (and CO₂) reduction is decreased.

It has been previously reported that NR activity and transcription increases with increasing CO₂ concentrations suggesting that nitrate and CO₂ reduction are correlated. It was interpreted that a simultaneous increase in CO₂ fixation and nitrate assimilation allows for a faster plant growth (26). Our investigations show that nitrate assimilation appears to be correlated to the light energy distribution between the photosystems that provide an acclimation of the plant cell metabolism to the energy supply by photosynthesis. The amount of reduced NADPH does not seem to be the signaling parameter since the *Lemna* mutant lacking the cytochrome *b₅f* complex is not able to produce this compound but shows increased nitrate activity. This is consistent with observations in barley where a high nitrate reductase activity was found to be not strictly linked to a reduced ferredoxin pool nor to high Calvin cycle activity (33). Therefore we propose as working hypothesis that the signal is generated between the PQ pool and the reducing side of PSI, which regulates nitrate reductase activity and expression under low light conditions when photosynthetic energy and reducing power supply are limited and therefore have to be used economically. Further investigations that will clarify these interactions are under way.

Acknowledgments—We thank R. Reimann and K.-J. Appenroth for help with the NR assay and the optimization of the *Lemna* growth conditions.

REFERENCES

1. Caboche, M., and Rouze, P. (1990) Trends Genet. 6, 187–191
2. Crawford, N. M. (1995) Plant Cell 7, 859–866
3. Daniel-Vedele, F., and Caboche, M. (1996) C. R. Acad. Sci. Paris, Life Sci. 319, 961–968
4. Sivasankar, S., and Oaks, A. (1996) Plant Physiol. Biochem. 34, 609–620
5. Campbell, W. H. (1999) Annu. Rev. Plant Physiol. Plant Mol. Biol. 50, 277–303
6. Kaiser, W. M., Weiner, H., and Huber, S. C. (1999) Plant Physiol. 120, 385–390
7. Crawford, N. M., Campbell, W. H., and Davis, R. W. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 8073–8076
8. Gowri, G., and Campbell, W. H. (1989) Plant Physiol. 90, 792–798
9. Campbell, W. H. (1996) Plant Physiol. 111, 355–361
10. Aislabie, M., Travis, R. L., Reins, D. W., and Huffaker, R. C. (1997) Plant Physiol. 101, 612–619
11. Baghuran, N., and Sopory, S. K. (1999) Biochem. Mol. Biol. Int. 47, 239–249
12. Pilgrim, M. L., Caspar, T., Quail, P. H., and McClung, C. R. (1983) Plant Mol. Biol. 23, 349–364
13. Deng, M.-D., Moureau, T., Leydecker, M.-T., and Caboche, M. (1990) Planta 180, 257–261
Photosynthetic Electron Transport Determines Nitrate Reductase Gene Expression and Activity in Higher Plants

Irena Sherameti, Sudhir K. Sopory, Artan Trebicka, Thomas Pfannschmidt and Ralf Oelmüller

J. Biol. Chem. 2002, 277:46594-46600.
doi: 10.1074/jbc.M202924200 originally published online September 18, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M202924200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 64 references, 18 of which can be accessed free at http://www.jbc.org/content/277/48/46594.full.html#ref-list-1