

Chlororespiration and cyclic electron flow around PSI during photosynthesis and plant stress response

DOMINIQUE RUMEAU¹, GILLES PELTIER² & LAURENT COURNAC²

¹Laboratoire d'Ecophysiologie Moléculaire des Plantes and ²Laboratoire de Bioénergétique et Biotechnologie des Bactéries et Microalgues, CEA Cadarache, DSV, IBEB, SBVME, UMR 6191 CNRS/CEA/Université Aix-Marseille, Saint Paul lez Durance F-13108, France

ABSTRACT

Besides major photosynthetic complexes of oxygenic photosynthesis, new electron carriers have been identified in thylakoid membranes of higher plant chloroplasts. These minor components, located in the stroma lamellae, include a plastidial NAD(P)H dehydrogenase (NDH) complex and a plastid terminal plastoquinone oxidase (PTOX). The NDH complex, by reducing plastoquinones (PQs), participates in one of the two electron transfer pathways operating around photosystem I (PSI), the other likely involving a still uncharacterized ferredoxin-plastoquinone reductase (FQR) and the newly discovered PGR5. The existence of a complex network of mechanisms regulating expression and activity of the NDH complex, and the presence of higher amounts of NDH complex and PTOX in response to environmental stress conditions the phenotype of mutants, indicate that these components likely play a role in the acclimation of photosynthesis to changing environmental conditions. Based on recently published data, we propose that the NDH-dependent cyclic pathway around PSI participates to the ATP supply in conditions of high ATP demand (such as high temperature or water limitation) and together with PTOX regulates cyclic electron transfer activity by tuning the redox state of intersystem electron carriers. In response to severe stress conditions, PTOX associated to the NDH and/or the PGR5 pathway may also limit electron pressure on PSI acceptor and prevent PSI photoinhibition.

Key-words: chloroplast; FQR; NADH dehydrogenase; PGR5; photosystem; PTOX.

Abbreviations: FNR, ferredoxin-NADP oxidoreductase; FQR, ferredoxin-plastoquinone reductase; NDH, NAD(P)H dehydrogenase; PQ, plastoquinone; PTOX, plastid terminal oxidase; ROS, reactive oxygen species.

INTRODUCTION

Major reactions of oxygenic photosynthesis consist in a vectorial electron transfer from water to NADP⁺ involving

Correspondence: L. Cournac. Fax: +33 4 42 25 62 65; e-mail: laurent.cournac@cea.fr

protein complexes present in thylakoid membranes, namely photosystem II (PSII), the cytochrome *b₆/f* complex, photosystem I (PSI) and ferredoxin NADP⁺ reductase, connected with soluble carriers such as PQs, plastocyanin and ferredoxin (Fd). Besides this major pathway (the so-called 'Z' scheme), alternative electron transfer pathways, involving non-photochemical reduction or oxidation of PQs at the expense of stromal electron donors or acceptors have been proposed based on functional measurements. These additional reactions cover two main concepts, one based on the cycling of electrons around PSI (see for reviews Fork & Herbert 1993; Bukhov & Carpentier 2004; Johnson 2005) and the other on electron transfer reactions (chlororespiration, Bennoun 1982) from stromal reductants to O₂ through the PQ pool (see for reviews Nixon 2000; Peltier & Cournac 2002; Bukhov & Carpentier 2004). The existence of such pathways has been supported by the discovery of new molecular components of thylakoid membranes, including a plastidial NDH complex (NDH), a PTOX and a key component of cyclic electron reactions around PSI (PGR5). Growing evidence has accumulated on the enhanced expression of some of these new components in response to environmental stress conditions, and on the discovery of numerous mechanisms for the regulation of their expression and activity. Physiological studies on mutants or transformants affected in the expression of these complexes have contributed to a better understanding of their role during photosynthesis. This review will focus on the most recent data concerning new components of alternative electron transfer reactions of oxygenic photosynthesis and discuss their function during photosynthesis under changing environmental conditions.

COMPONENTS

PQ reduction

Cyclic electron transfer around PSI implies reduction of the intersystem electron transfer chain at the expense of electron donors resulting from PSI activity. Among stromal electron carriers, Fd and NADPH appear as obvious candidates for participating in such an electron pathway, and several studies have shown that both can deliver electrons into the photosynthetic electron transport chain at the level of PQ (Bukhov & Carpentier 2004).

The existence of a functional plastidial NDH complex involved in PQ reduction has been deduced from the study of different tobacco transformants inactivated in plastid *ndh* genes and lacking the NDH complex (Burrows *et al.* 1998; Kofer *et al.* 1998; Shikanai *et al.* 1998; Horvath *et al.* 2000). Among the subunits encoded by the chloroplast genome, none is homologous to subunits which in heterotrophic bacteria constitute the diaphorase part of the complex carrying out NADH oxidation. Three nucleus-encoded subunits (NDHM, N and O) identified in purified NDH complexes (Funk, Schafer & Steinmuller 1999; Rumeau *et al.* 2005) have homologs in cyanobacteria (Prommeenate *et al.* 2004; Battchikova *et al.* 2005). It was recently shown that mutants affected in the corresponding genes are impaired in PQ reduction in the same manner as chloroplast null mutants (Rumeau *et al.* 2005). A homolog of cyanobacterial subunit NDHL has been found in the nuclear genome of higher plants (Battchikova *et al.* 2005). Based on the fact that both their presence is necessary for NDH complex assembly and that they are degraded in NDH-deficient strains, other components (CRR3 and CRR7) have also been hypothesized as subunits of the complex (Munshi, Kobayashi & Shikanai 2005; Muraoka *et al.* 2006). However, none of these additional subunits do show any known motif, which could be attributed to pyridine nucleotide binding or catalysis. The nature of the catalytic subunits, as well as the nature of stromal electron donors to the NDH complex, still remains to be elucidated. In particular, NADH has been reported as a preferential substrate of the NDH complex (Sazanov, Burrows & Nixon 1998b; Rumeau *et al.* 2005), which implies that either a transhydrogenase or several metabolic steps are needed for this complex to drive a cyclic electron transfer around PSI.

An Fd-dependent, antimycin A-sensitive PQ reduction activity has also been evidenced and named FQR for 'ferredoxin-plastoquinone reductase' (for review, see Bendall & Manasse 1995). A genetic approach in

Arabidopsis thaliana based on screening mutants impaired in non-photochemical quenching of chlorophyll fluorescence (NPQ), identified *PGR5* (*pgr* stands for 'proton gradient regulation') as an essential component of the antimycin A-dependent cyclic pathway (Munekage *et al.* 2002). However, *PGR5* product does not exhibit any known features of electron transfer enzymes, and its involvement in FQR activity could be indirect. The exact nature of FQR and the role of *PGR5* remain to be established.

FNR has been proposed as an enzyme susceptible for mediating electron donation between Fd and PQ, notably via an association with *cyt b₆f* (Zhang, Whitelegge & Cramer 2001). In fact, there are at least three FNRs located in the maize chloroplast, among which two co-purify with *cyt b₆f* (Okutani *et al.* 2005). FNR has also been proposed as able to link with the NDH complex (Guedeny *et al.* 1996; Quiles & Cuello 1998) where it could be a part of the diaphorase moiety.

Other enzymes such as type-II NAD(P)H dehydrogenases (NDH-2) (Corneille *et al.* 1998; Yamane *et al.* 2000) could also be involved in PQ reduction, but the molecular identity of such enzymes is still unknown.

PQ oxidation

Potential enzymatic components of PQ oxidation activity include PTOX, a chloroplast-targeted quinol oxidase, homolog to mitochondrial alternative oxidase (AOX), whose reactivity relies on non-heme iron (Carol *et al.* 1999; Wu *et al.* 1999). Based on the homology between AOX and PTOX, and experiments using ROS scavengers, it was proposed that PTOX used O₂ and that the final product of the reaction was H₂O (Cournac *et al.* 2000). Because NDH and PTOX co-localize in the stroma lamellae (on the stromal side, Fig. 1) (Lennon, Prommeenate & Nixon 2003), both could be involved in the chlororespiratory pathway, although direct evidence of electron transfer between NDH and PTOX is still lacking.

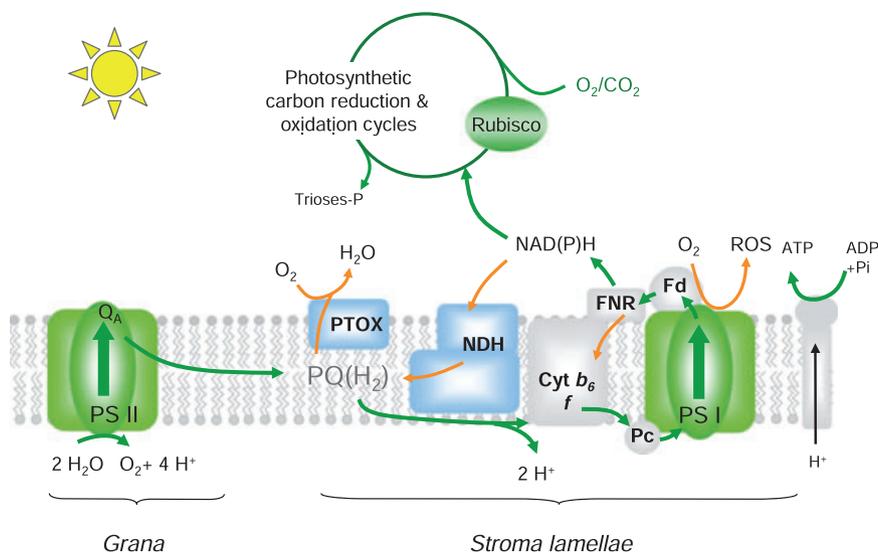


Figure 1. Electron transfer reactions during oxygenic photosynthesis. Granal thylakoids contain photosystem II (PSII) complexes and the cytochrome *b₆f* complex (not shown on the figure), whereas stroma lamellae contain photosystem I (PSI) complexes, ATPases, the cytochrome *b₆f* complex, the NDH complex and PTOX. Rubisco, ribulose 1·5-bisphosphate carboxylase/oxygenase.

PSII component cytochrome b559 is also probably involved in PQ oxidation, at least at the vicinity of PSII: it has been shown to mediate PQH₂ oxidation *in vitro* (Kruk & Strzalka 2001) and a mutant in which cytochrome b559 is mutated shows an over-reduction of PQ pool in the dark (Bondarava *et al.* 2003). The existence of a thylakoid-located peroxidase branched on the PQ pool using H₂O₂ as a substrate has also been proposed from *in vitro* studies (Casano *et al.* 2000). Alternatively, non-enzymatic PQ oxidation may also occur and generate ROS, in particular H₂O₂ (Mubarakshina, Khorobrykh & Ivanov 2006), reduced PQ, particularly semiquinones being susceptible of direct interaction with O₂ (Khorobrykh & Ivanov 2002).

CHLOROPLAST BIOGENESIS AND SENESCENCE

The observation that high amounts of NDH subunits are present in etioplasts (Fischer, Funk & Steinmüller 1997; Guera, de Nova & Sabater 2000; Lennon *et al.* 2003), led to the hypothesis that 'chlororespiratory' components may serve to energize the plastid membrane and favour synthesis and/or insertion of photosynthetic complexes during the greening process. However, the absence of any obvious phenotype related to greening in NDH-deficient transformants suggests that this role is not essential.

In contrast, the role of PTOX appears crucial during the greening process. PTOX is involved in carotenoid processing (phytoene desaturation) and its absence results in a severe variegation phenotype as the absence of carotenoids renders chloroplasts susceptible to irreversible bleaching, especially in high light (Carol *et al.* 1999; Wu *et al.* 1999). Based on *in situ* hybridization and reporter gene experiments, it was shown that PTOX gene expression is not strictly connected with carotenoid accumulation as it has been detected in all tissues and organs throughout development (Aluru *et al.* 2001). However, a possible function of PTOX in a chlororespiratory activity of non-green plastids has not been clearly established.

Based on the observation that an NDH-deficient transplastomic tobacco shows a 30 d delay in leaf senescence with respect to wild type, it was proposed that the electron transfer pathway involving the NDH complex and a plastid peroxidase would be involved in programmed cell death occurring during leaf senescence (Zapata *et al.* 2005). Such a striking phenotype has not been reported in any other of the NDH-deficient mutants studied so far, but in view of these results, a detailed comparison of leaf life cycles between available wild type and NDH mutant plants may be worth conducting.

FUNCTION OF CHLORORESPIRATORY COMPONENTS DURING PHOTOSYNTHESIS

C₃ photosynthesis

Several reports suggest a significant contribution of cyclic electron transfer in the normal operation of C₃

photosynthesis, which is enhanced in conditions such as low CO₂, high light and induction phase of photosynthesis during dark to light transition (Harbinson & Foyer 1991; Golding, Finazzi & Johnson 2004; Joliot & Joliot 2005; Miyake *et al.* 2005). Indeed in these conditions, acidification of thylakoid lumen – inducing NPQ and triggering ATP synthesis – has been considered to require alternative mechanisms in addition to linear electron flow, such as cyclic electron transport or oxygen photoreduction (Heber & Walker 1992; Bendall & Manasse 1995). The question as to whether such activities are important or not for photosynthetic function has been assessed by reverse genetic approaches.

Inactivation of the NDH complex did not lead to a decrease in photosynthesis or growth under optimal conditions in air (Burrows *et al.* 1998; Kofer *et al.* 1998; Shikanai *et al.* 1998; Horvath *et al.* 2000). By studying the effect of antimycin A on a *ndhB* knockout mutant, Joet *et al.* (2001) concluded to the existence of two parallel cyclic pathways around PSI, one sensitive to antimycin A, the other involving the NDH complex, which would in certain extent compensate each other and whose contribution would appear higher in photorespiratory conditions. Simultaneous impairment of these pathways leads to a severe inhibition of photosynthesis, even in optimal conditions (Joet *et al.* 2001). The *pgr5* mutant, which is deficient in the antimycin A-sensitive pathway, showed a reduced electron transport rate under high irradiance (Munekage *et al.* 2002). Furthermore, a severe decrease in electron transport and photoautotrophic growth was observed in double mutant *crr2 pgr5* deficient in both PGR5 and NDH complex (Munekage *et al.* 2004). This is in accordance with previous findings (Joet *et al.* 2001) and sustains the view that although FQR and NDH activities are individually dispensable in optimal conditions for photosynthesis, at least one component of cyclic electron flow (CEF) is needed for photosynthesis operation.

It has been suggested that due to the low abundance of the complex (Burrows *et al.* 1998), NDH-mediated electron flows are too low to account for bioenergetically significant ATP production (Joliot & Joliot 2005). It has been proposed that, together with PTOX activity, they could rather have a regulatory role (Joet *et al.* 2002b; Peltier & Cournac 2002). By poisoning the redox state of the PQ pool in thylakoid domains performing CEF around PSI (through FQR for instance), they would modulate its efficiency (Nixon 2000; Peltier & Cournac 2002). This is basically in accordance with Johnson (2005) who proposes a model in which cyclic electron transfer is essentially Fd-dependent and is triggered by NADP reduction. Interestingly, overexpression of Fd induces a stimulation of PQ reduction, CEF and NPQ, and a slight increase in oxidative stress tolerance, but hardly impacts CO₂ fixation (Yamamoto *et al.* 2006). On the other hand, it was found that the half-time of CEF as investigated by photoacoustic methods is significantly affected by NDH deficiency (Joet *et al.* 2002a). The importance of FQR and NDH in PQ reduction may vary from one species to another: for instance, although delayed luminescence (correlated to PQ reduction after far red illumination) was

found mainly dependent on FQR in tobacco, it appeared more strongly affected by NDH deficiency in *Arabidopsis* (Havaux, Rumeau & Ducruet 2005).

Based on the fact that FNR has been reported to occur as a soluble enzyme in the stroma, and also associated with PSI, cyt *b₆f* and/or NDH, Bojko, Kruk & Wieckowski (2003) have suggested that this enzyme may modulate the partition between linear and cyclic photosynthetic electron pathways. Such a view is in accordance with kinetic measurements (Breyton *et al.* 2006). These authors additionally proposed that such a partition could be essentially dependent on Fd as an electron carrier and regulated by the redox state of the NADP pool. Recently, Lintala *et al.* (2007), studying *A. thaliana* mutants deficient in one of the chloroplast FNR isoforms, concluded that this deficiency affected both linear and cyclic electron transfer capacities, with a significant impact on CO₂ fixation. They further proposed that dimerization between the isoforms LFNR1 and LFNR2 was important for membrane attachment and that this process could be a way to regulate the partition between FNR activities involved in linear and cyclic electron transfer. Overexpression of FNR does not impact photosynthesis parameters but induces higher tolerance to oxidative stress, which was essentially interpreted through a hypothetical antioxidant role of free FNR (Rodriguez *et al.* 2007).

The role of PTOX in photosynthesis has been much less investigated, in part because the striking phenotype of PTOX-deficient mutants makes such studies difficult. PTOX has been proposed to serve as a 'safety valve', preventing over-reduction of the electron transfer chain in excess light (Aluru *et al.* 2006). Overexpressing mutants have then been constructed in order to tackle the role of PTOX in the 'normal' course of photosynthesis (Joet *et al.* 2002b; Rosso *et al.* 2006). This overexpression has a significant impact during the photosynthesis induction phase where PQ reduction state appears lower than in wild type (possibly because of the addition of an electron exit which is active before activation of CO₂ fixation). Few effects of the overexpression are detected at steady state (only some slight but significant decrease in qP and qN, electron transfer rate being unaffected) (Joet *et al.* 2002b; Rosso *et al.* 2006).

C₄ photosynthesis

In maize and sorghum, higher amounts of NDH complex in bundle sheath chloroplasts than in mesophyll chloroplasts suggest that the NDH complex could contribute to ATP generation through its involvement in cyclic electron transport around PSI (Kubicki *et al.* 1996; Darie *et al.* 2006). Comparing different C₄ species, NDH was found highly expressed in mesophyll cells in the NAD-malic enzyme species, and in bundle sheath cells in NADP-malic enzyme species, that is, in each case in the cell types which exhibit the highest ATP requirements. These results strengthen the hypothesis that cyclic electron transfer via or dependent on NDH plays a central role in supplying the ATP needed for driving the CO₂-concentrating mechanism in C₄

photosynthesis (Takabayashi *et al.* 2005). Conversely, expression profiles of PGR5 do not correlate well with C₄ cell types (Takabayashi *et al.* 2005).

INVOLVEMENT OF CHLORORESPIRATORY PATHWAYS IN PHOTOSYNTHESIS ADAPTATION TO STRESS CONDITIONS

Several studies proposed that chlororespiratory components may be involved in protective or adaptive mechanisms of plant in response to environmental stress such as heat, high light or water stress. These conclusions are based on physiological studies showing an increased activity of non-photochemical reduction of PQs and on the higher expression of chlororespiratory enzymes under particular stress conditions. In some cases, the phenotype of plastid transformants deficient in the NDH complex confirms the involvement of this complex to the stress response.

Heat stress

Heat stress significantly enhances the dark reduction of PQs (Havaux, Greppin & Strasser 1991; Havaux 1996; Sazanov, Burrows & Nixon 1998a; Bukhov, Samson & Carpentier 2000; Bukhov & Carpentier 2004) and increases the transthylakoid proton gradient which was interpreted though a stimulation of CEF around PSI (Havaux 1996; Bukhov *et al.* 2000). In response to heat stress, both NDH complex and PTOX amounts increase (Quiles 2006). High temperatures negatively affect photosynthetic CO₂ fixation at different levels depending on stress intensity. In response to mild heat stress conditions, the activity of photorespiration increases (Jordan & Ogren 1984) and the ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) activation state decreases, Rubisco activase being particularly sensitive to elevated temperature (Salvucci & Crafts-Brandner 2004a,b). More severe heat stress conditions impair PSII activity, PSI being more resistant to high temperatures (Havaux 1996). Mild temperature stress therefore results in a higher ATP demand, CO₂ fixation requiring more ATP when photorespiration is active (Osmond 1981) and Rubisco activase also needing ATP. The higher ATP demand under mild heat stress conditions would result in a higher NADPH/ATP ratio favouring non-photochemical reduction of the PQ pool from stromal donors which in turn would activate the NDH-mediated cyclic electron pathway. This would help dissipating excess energy and provide additional ATP to maintain active CO₂ fixation. In the absence of NDH-mediated cyclic electron pathway, like in tobacco transformants deficient in the NDH complex, the incapacity to equilibrate the NADPH/ATP ratio at high temperature would favour ROS generation (Wang *et al.* 2006). However, the increase in non-photochemical reduction of PQs observed in response to heat stress was not affected in tobacco transformants deficient in the NDH complex (Sazanov *et al.* 1998a; Yamane *et al.* 2000).

This indicates that when the electron pressure is high, another pathway is operating. This pathway may involve

either the FQR and PGR5-dependent pathway, or a single subunit flavoenzyme containing NADH dehydrogenase (NDH-2) (Corneille *et al.* 1998; Yamane *et al.* 2000). Additional experiments are needed to elucidate the nature and determine the molecular components involved in electron transfer reactions triggered in response to heat stress.

Water deficit

In response to water shortage, higher plants close the stomata to limit water loss by transpiration. This lowers internal CO₂ concentration and results in an increased activity of photorespiration. In these conditions, the ATP requirement of photosynthetic CO₂ fixation is increased. Although growth of tobacco mutants defective in the NDH complex was shown to be unaffected under normal conditions, a defect in photosynthesis induction (Burrows *et al.* 1998) and a growth phenotype (Horvath *et al.* 2000) were observed under conditions of stomatal closure. This effect was interpreted through an involvement of the NDH-mediated cyclic electron pathway around PSI in supplying the extra ATP required in conditions of mild water stress characterized by CO₂ limitation (Horvath *et al.* 2000). A possible role of chlororespiratory components, especially PTOX, in conditions of severe water deficit, when ROS are produced, remains to be investigated.

High light and chilling

High light may affect differentially photosynthesis depending on temperature conditions. Under normal temperature ranges, high light induces PSII damage and photoinhibition. Paradoxically, this process might also be considered a protective mechanism, as it prevents over-reduction of the photosynthetic electron transfer chain and as it can be rapidly reversed because of the high turnover rate of PSII and efficiency of its repair cycle. When high light illumination is coupled to low temperatures, PSI inhibition may occur (Sonoike 1996). Increased NDH complex amounts have been reported in barley (Casano, Martin & Sabater 2001; Guera *et al.* 2005) and in oat plants (Quiles & Lopez 2004; Quiles 2006). Increased amounts of PTOX and of a NDH subunit were recently reported in a high mountain species adapted to high light and low temperature (Streb *et al.* 2005). PTOX amounts also increased in oat plants in response to high light illumination (Quiles 2006). Tobacco transformants deficient in the NDH complex were first reported to be high light sensitive (Endo *et al.* 1999), but this result was not confirmed by later studies and may result from side effects of gene disruption (Barth & Krause 2002). No difference in the photoinhibition response of NDH-deficient tobacco transformants was observed either at normal or low temperatures (Barth & Krause 2002). Similarly, Wang *et al.* (2006) concluded to a limited role of the NDH complex in the protection of the photosynthesis apparatus to oxidative damage generated at low temperature. Moreover, a transcriptomic study reported an increased expression of PTOX in transgenic *Arabidopsis*

lines lacking catalase and ascorbate peroxidase (Rizhsky *et al.* 2002). Therefore, in contrast to high temperature stress which results in a significant increase in NDH-mediated electron flow from reduced stromal components, high light or chilling stresses do not induce similar effects.

Possible mechanisms involving the NDH complex and PTOX during stress response

Generally, two main functions have been proposed for chlororespiratory components in stress response.

- 1 ATP supply and energy dissipation by cyclic electron transport around PSI. High temperature and water stress both induce an increase in ATP demand that may be fulfilled by CEF around PSI. The NDH complex through its involvement in cyclic electron around PSI, likely participates to energy dissipation and ATP supply under high temperature and water stress conditions. Although the stress dependency of NDH and PTOX has been widely studied, little is known concerning the stress dependency of PGR5. This will be an interesting question to address in the future as both NDH and PGR5 have been proposed to be involved in two complementary cyclic electron pathways around PSI (Munekage *et al.* 2004). Quite interestingly, growth impairment of the *Arabidopsis pgr5* mutant under high illumination (Munekage *et al.* 2002) is suppressed at high CO₂ concentration (Munekage *et al.*, unpublished results). PTOX appearing as an electron sink, its contribution to cyclic electron transfer is not obvious. However, by allowing a fine-tuning of the redox state of intersystem electron carriers, PTOX may help CEF to be fully operational (Joet *et al.* 2002b).
- 2 PTOX as a safety valve to prevent over-reduction of PSI rather than PSII acceptors. PTOX has been suggested to act as an electron safety valve that would prevent over-reduction of PSII acceptors and avoid photoinhibitory damages at PSII (Niyogi 2000; Peltier & Cournac 2002; Streb *et al.* 2005). Over-reduction of PSII first quinone acceptor (Q_A) and intersystem electron carriers was effectively lowered in plants overexpressing AtPTOX, but this effect was only observed during dark to light transients (Joet *et al.* 2002b). However, PTOX overexpression did not confer any particular resistance to PSII photoinhibition either in tobacco (Joet *et al.* 2002b) or in *Arabidopsis* plants (Rosso *et al.* 2006). Therefore, expression of PTOX by itself is not sufficient protection against PSII photoinhibition and it has been proposed that PTOX cannot be considered a significant electron valve (Rosso *et al.* 2006). Actually, PSII activity can be regulated by various mechanisms including non-photochemical quenching or state transitions, therefore avoiding over-reduction of PSII acceptors. Even in case these regulatory mechanisms would not be efficient enough, photoinhibition of PSII would be an ultimate protective mechanism, recovery being achieved rather rapidly because of the rapid turnover of PSII. The situation becomes much more critical as far as PSI photoinhibition is concerned, this process being

slowly reversible (Sonoike 1996). When the NADPH/ATP ratio is high (because of a high ATP demand due to high temperature or water stress for instance), a high electron pressure occurs on PSI acceptors, thus triggering CEF around PSI. In contrast to PSII, mechanisms proposed to regulate CEF are scarce. To avoid such dramatic effects, plants have developed detoxification mechanisms including the water–water cycle. A possible function of the chlororespiratory pathway (electron transfer reactions involving a concerted activity of the NDH complex and PTOX) could be to reduce the electron pressure on PSI acceptors by recycling electrons to the PQ and ultimately to PTOX. This hypothesis is sustained by the protective effect on PSI photoinhibition that was reported in *Arabidopsis* lines overexpressing PTOX (Rosso *et al.* 2006). Interestingly, a transcriptomic study reported an increased expression of PTOX in transgenic *Arabidopsis* lines lacking catalase and ascorbate peroxidase, indicating that under certain circumstances, PTOX may compensate a deficiency in detoxifying enzymes (Rizhsky *et al.* 2002).

Another mechanism has been proposed to involve chlororespiratory components in stress adaptation. Based on the constitution of an *in vitro* electron transport system from NADH to H₂O₂ supported by the NDH complex and a plastoquinol peroxidase, Casano *et al.* (2000) proposed that chlororespiration may serve as an H₂O₂ detoxification pathway within chloroplasts. Although this mechanism has not been demonstrated *in vivo*, increases in NDH complex

amounts and in thylakoid peroxidase activity observed in response to photooxidative or oxidative stresses have often been considered to support such a role (Casano *et al.* 2000, 2001).

REGULATION OF CHLOROPLAST NDH COMPLEX SYNTHESIS AND ACTIVITY

Very little is known about possible regulation mechanisms underlying the biosynthesis of the different nuclear-encoded proteins participating in chlororespiration and CEF around PSI. A possible translational or post-translational mechanism has been proposed to generate an increase in PTOX in plastid mutants devoid of PSII (Baena-Gonzalez *et al.* 2003).

Conversely, data available on the regulation of the NDH complex biosynthesis are varied, scattered and basically reflective of the complexity and the multiplicity of plastid gene expression regulation steps (Fig. 2) mainly post-transcriptional events, including RNA trimming, intron splicing, RNA editing and RNA stability (for reviews, see Sugita & Sugiura 1996; Barkan & Goldschmidt-Clermont 2000; Monde, Schuster & Stern 2000). Functional assembly of NDH subunits into the thylakoid membrane is a coordinated mechanism as it has been demonstrated that mutant plants deficient for a single subunit present a loss of the whole set of NDH subunits (Burrows *et al.* 1998; Kofer *et al.* 1998; Hashimoto

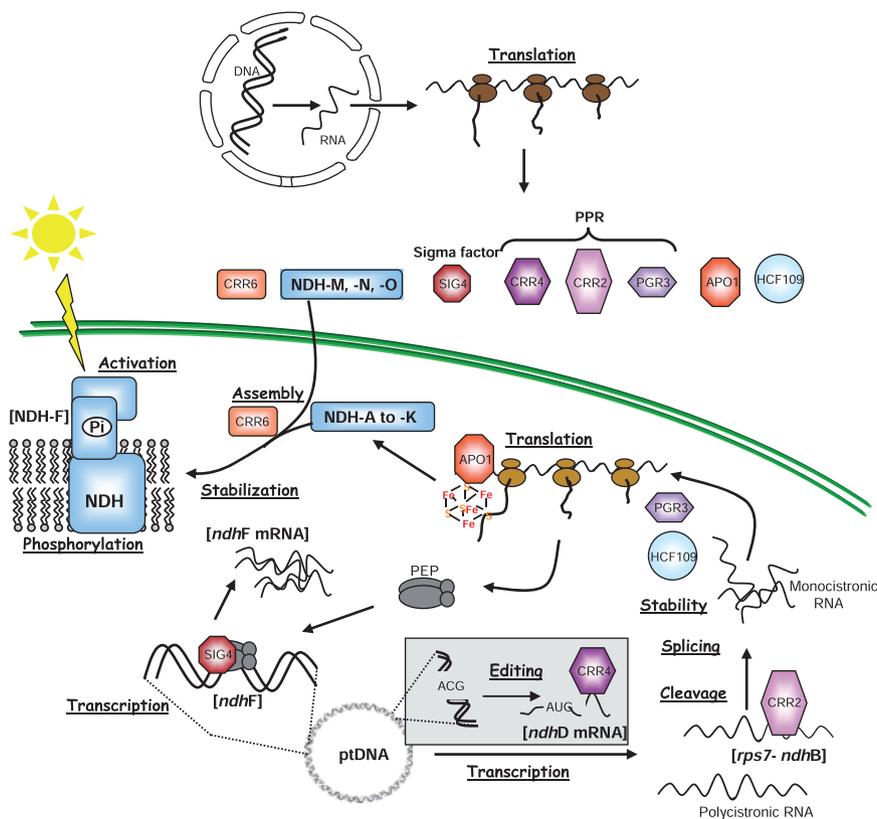


Figure 2. NDH complex biosynthesis.

The different steps involved in the biosynthesis of the nuclear- and plastid-encoded subunits (NDH-M, -N, -O and NDH-A to -K, respectively) are indicated. The nuclear trans-factors that control plastid *ndh* gene expression are shown in the cytosol where they are synthesized and in the chloroplast where they mediate regulation. When known, the specific target of the trans-factor is indicated (between brackets). ptDNA, plastid chromosome; PPR, pentatricopeptide protein; CRR, chlororespiratory reduction; HCF, high chlorophyll fluorescence; PEP, plastid-encoded polymerase; PGR, proton gradient regulation.

et al. 2003; Kotera, Tasaka & Shikanai 2005; Rumeau *et al.* 2005). Different factors including (1) the concerted assembly of nuclear and plastid-encoded subunits of the NDH complex, (2) the difference in gene copy numbers between nucleus- versus chloroplast-encoded subunits and (3) the involvement of the NDH complex in adaptation to environmental changes, may help in understanding the existence of complex, sophisticated and apparently redundant regulatory mechanisms.

Regulation at the transcriptional level has been demonstrated for NDH-F. *ndhF* plastid gene expression has been reported to be triggered by the plastid-encoded RNA polymerase (PEP) under the specific control of SIG4, one of the six nuclear-encoded sigma factors in *Arabidopsis* (Favory *et al.* 2005). *Arabidopsis* mutants, in which the gene encoding SIG4 has been disrupted, specifically lack *ndhF* mRNA and present a strong down-regulation of NDH activity. Other sigma factors have been demonstrated to be induced under multiple stress conditions (high light, low temperature, high salt, high osmotic conditions SIG5; Nagashima *et al.* 2004) or to play stage-specific role in seedling development (SIG6; Loschelder *et al.* 2006). Whether SIG4 expression is modulated by environmental and/or developmental factors known to modulate NDH activity has not been investigated.

An important contribution to elucidating the regulation of NDH biosynthesis has been provided by a genetic approach based on the screening of *Arabidopsis* mutants using chlorophyll fluorescence (Shikanai 2007). Different mutants termed *chlororespiratory reduction* (*crr*) mutants were identified by their lack of transient increase in chlorophyll fluorescence after actinic light illumination consistent with a defect in NDH complex. In this way, CRR4 (Kotera *et al.* 2005), CRR2 (Hashimoto *et al.* 2003) and CRR6 (Munshi, Kobayashi & Shikanai 2006) were identified. CRR4 and CRR2 belong to the PLS subfamily of the PPR (pentatricopeptide repeat) family. CRR4 is specifically responsible for *ndhD* initiation codon editing. RNA editing in plastids is a post-transcriptional process changing C-to-U according to a mechanism that remains to be elucidated. In tobacco, 34 editing sites have been reported on the plastid genome. They are distributed on transcripts of 15 different genes among which *ndhA*, *ndhB*, *ndhD* and *ndhF* are favoured targets as they contain 2, 9, 4 and 1 editing sites, respectively (Chateigner-Boutin & Hanson 2003). In most cases, editing results in amino acid substitution often restoring conserved amino acids (Maier *et al.* 1996; Bock 2000; Tsudzuki, Wakasugi & Sugiura 2001), but it may also create a start or a stop codon. The start codon of *ndhD* transcript must be created by editing. This ACG to AUG conversion is partial and depends on developmental and light conditions, and correlates with the photosynthetic activity of plastids (Hirose & Sugiura 1997; Chateigner-Boutin & Hanson 2003). Whether CRR4 may be responsive of the environmental and development-regulated editing of *ndhD* has not been demonstrated. The role of editing for proper *ndh* gene functioning has also been demonstrated for *ndhA* in which editing appeared to

be necessary to restore recognition sequences that allow intron removal (del Campo, Sabater & Martin 2000).

CRR2 is involved in the intergenic processing of chloroplast mRNA between *rps7* and *ndhB*, which seems to be essential for *ndhB* RNA translation. Most of the chloroplast genes are organized in operons transcribed as polycistronic RNAs that are processed to sets of overlapping RNAs through steps controlled by nuclear-encoded proteins (Barkan & Goldschmidt-Clermont 2000). Polycistronic primary transcripts thus obtained consist of messages for distinct proteins. The NDH complex is poorly represented as compared to both photosystems (Sazanov *et al.* 1998b) or to ribosomal proteins. Nuclear factors such as CRR2 efficiently contribute to balance translation rate of the different genes in the same cluster therefore regulating their expression. A possible regulatory mechanism by multiple endonucleolytic cleavages in the intergenic region has also been reported in the region between *psaC* coding for a PSI subunit and *ndhD* (Hirose & Sugiura 1997; del Campo, Sabater & Martin 2002).

CRR6, a protein with no specific motif may be a chaperone-like peptide for assembly of the NDH complex (Munshi *et al.* 2006).

Other mechanisms have been shown to result in a concerted regulation of the NDH complex and other photosynthetic complexes. By using the high chlorophyll fluorescence (*hcf*) phenotype as selection criterion, many mutants defective in photosynthetic light reactions and electron transfer have been isolated among which *hcf109* is affected in PSI, PSII and the NDH complex (Meurer, Berger & Westhoff 1996). *HCF109* codes for a trans-regulatory component that specifically and concomitantly controls the stability of distinct transcripts. *Arabidopsis pgr3* (*proton gradient regulation*) mutants were obtained using the same selection criterion. PGR3 is a protein containing 27 PPR motifs which may have different functions in conferring RNA stability and allowing translation of different targets, including NDH and *cyt b₆f* complexes.

APO1 (*ACCUMULATION OF PHOTOSYSTEM ONE1*), another *hcf* mutant has significantly reduced levels of FTR and NDH (Amann *et al.* 2004). APO1 is involved in the stable assembly of several [4Fe-4S] cluster-containing complexes of chloroplasts, which argues in favour of the presence of [4Fe-4S] clusters in plant NDH complex which has never been demonstrated.

In addition to these multiple transcriptional and post-transcriptional regulation steps, two post-translational mechanisms involved in NDH activity regulation have been reported. *In vitro*, using isolated thylakoids, it has been demonstrated that NDH activity requires an induction step for maximal rates of activity. Induction can be triggered by a brief illumination or by incubation in a low ionic-strength buffer (Teicher & Scheller 1998). The NDH-F subunit has been shown to be phosphorylated and NDH complex activity that increases in leaf segments treated with H₂O₂ and relative excess light has been closely correlated with the extend of phosphorylation (Lascano *et al.* 2003).

CONCLUSION

A challenge of this last decade has been to identify the molecular actors underlying the chlororespiration and cyclic flow around PSI and to determine the significance of these electron pathways operating in the chloroplast. PTOX and PGR5 have been identified as well as nuclear-encoded subunits of NDH and nuclear trans-factors controlling its biosynthesis. However, other key components such as 'missing' subunits of the NDH electron input module and redox carriers in charge of FQR activity still remain to be identified. Particularly, the role of FNR in these electron transfers needs to be clarified.

These genes and functions appear largely conserved in higher plants. While it has been demonstrated that the escape of genetic material from the chloroplast to the nuclear genome occurs frequently (Stegemann *et al.* 2003), *ndh* has remained in the chloroplast throughout the plant lineage closely associated with the photosynthetic genes, suggesting that the control of *ndh* expression by chloroplast functioning machinery exerted a positive selection. In many plant taxa, *ndh* expression is controlled by RNA editing which is considered a mechanism to rescue genes restoring codons for conserved amino acids that are probably essential for the encoded protein function, thereby demonstrating the need for functional NDH. Although NDH is not ubiquitous among oxygenic photosynthetic organisms, being absent from many green algae and some plant species such as *Pinus thunbergii*, it is probable that NAD(P)H-mediated PQ reduction is conserved and mediated by different enzymes, such as NDH-2 in the green alga *Chlamydomonas reinhardtii* (Mus *et al.* 2005). Moreover, it has been shown that occurrence of a (plasto)quinol oxidase in thylakoids (such as a PTOX homolog or a cyt *b*-type quinol oxidase in many cyanobacteria) is a conserved feature in oxygenic photosynthesis (McDonald & Vanlerberghe 2006).

The physiological significance of the chloroplast electron pathways operating around PSI has been difficult to establish. Although these reactions probably do not play a major role during photosynthesis under optimal conditions, they likely participate to the required flexibility of electron transfer reactions to balance ATP/NADPH requirements, when photosynthesis operates under changing environmental conditions. In this context, it appears likely that FQR would essentially fulfil a bioenergetic role, while NDH would have both bioenergetic and, together with PTOX, regulatory roles. The concerted action of NDH and PTOX would optimize the efficiency of the cyclic pathway by poisoning the redox level of intersystem electron carriers. In stress conditions, other roles of these components might superimpose to this energy balance role. In particular, NDH complex and chlororespiration activities would help avoiding over-reduction of PSI electron acceptors, scavenging ROS and therefore protecting PSI from photoinhibition. However, it must be kept in mind that these chloroplast functions do not operate independently from the cellular context, and in particular, the occurrence of several redox

exchange shuttles between chloroplasts and mitochondria (Hoefnagel, Atkin & Wiskich 1998) allows the mitochondria to participate also in redox and energetic balance of the chloroplast. Regulation of photosynthesis by chlororespiratory functions can then be viewed as a piece of a complex set of regulations and interactions, which all have their specificities and time constants (Cournac *et al.* 2002), and eventually cooperate towards acclimation of photosynthesis to the complex and fluctuating pattern of conditions an organism will be exposed to in natural environment.

Accumulating evidence demonstrates that NDH biosynthesis is controlled at many steps and urges to envision a highly sophisticated regulation mechanism whose components have only been partly characterized. In the future, efforts should be made to elucidate whether some of these mechanisms are effectively involved in the regulation of NDH complex amounts and/or activity in response to environmental changes. It will also be necessary to determine whether *pgr5* expression is also modulated by environmental conditions, and more generally to which extent flexibility in expression of FQR and NDH components cooperate to adjust cyclic electron transfer. Finally, unravelling the roles of chlororespiratory components will probably impose to conduct ecological approaches such as assessing how and in which environmental conditions they contribute to plant fitness.

ACKNOWLEDGMENTS

Drs Bernard Genty and Yuri Munekage are gratefully thanked for communication of unpublished results and together with Dr Michel Havaux, for stimulating discussions.

REFERENCES

- Aluru M.R., Bae H., Wu D. & Rodermel S.R. (2001) The *Arabidopsis immutans* mutation affects plastid differentiation and the morphogenesis of white and green sectors in variegated plants. *Plant Physiology* **127**, 67–77.
- Aluru M.R., Yu F., Fu A.G. & Rodermel S. (2006) Arabidopsis variegation mutants: new insights into chloroplast biogenesis. *Journal of Experimental Botany* **57**, 1871–1881.
- Amann K., Lezhneva L., Wanner G., Herrmann R.G. & Meurer J. (2004) ACCUMULATION OF PHOTOSYSTEM ONE1, a member of a novel gene family, is required for accumulation of [4Fe-4S] cluster-containing chloroplast complexes and antenna proteins. *Plant Cell* **16**, 3084–3097.
- Baena-Gonzalez E., Allahverdiyeva Y., Svab Z., Maliga P., Josse E.M., Kuntz M., Maenpaa P. & Aro E.M. (2003) Deletion of the tobacco plastid *psbA* gene triggers an upregulation of the thylakoid-associated NAD(P)H dehydrogenase complex and the plastid terminal oxidase (PTOX). *The Plant Journal* **35**, 704–716.
- Barkan A. & Goldschmidt-Clermont M. (2000) Participation of nuclear genes in chloroplast gene expression. *Biochimie* **82**, 559–572.
- Barth C. & Krause G.H. (2002) Study of tobacco transformants to assess the role of chloroplastic NAD(P)H dehydrogenase in photoprotection of photosystems I and II. *Planta* **216**, 273–279.
- Battchikova N., Zhang P.P., Rudd S., Ogawa T. & Aro E.M. (2005) Identification of NdhL and Ssl1690 (NdhO) in NDH-1L, and

- NDH-1M complexes of *Synechocystis* sp PCC 6803. *Journal of Biological Chemistry* **280**, 2587–2595.
- Bendall D.S. & Manasse R.S. (1995) Cyclic photophosphorylation and electron transport. *Biochimica et Biophysica Acta* **1229**, 23–38.
- Bennoun P. (1982) Evidence for a respiratory chain in the chloroplast. *Proceedings of the National Academy of Sciences of the USA* **79**, 4352–4356.
- Bock R. (2000) Sense from nonsense: how the genetic information of chloroplasts is altered by RNA editing. *Biochimie* **82**, 549–557.
- Bojko M., Kruk J. & Wieckowski S. (2003) Plastoquinones are effectively reduced by ferredoxin : NADP⁺ oxidoreductase in the presence of sodium cholate micelles. Significance for cyclic electron transport and chlororespiration. *Phytochemistry* **64**, 1055–1060.
- Bondarava N., De Pascalis L., Al-Babili S., Goussias C., Golecki J.R., Beyer P., Bock R. & Krieger-Liszka A. (2003) Evidence that cytochrome b559 mediates the oxidation of reduced plastoquinone in the dark. *Journal of Biological Chemistry* **278**, 13554–13560.
- Breyton C., Nandha B., Johnson G.N., Joliet P. & Finazzi G. (2006) Redox modulation of cyclic electron flow around photosystem I in C3 plants. *Biochemistry* **45**, 13465–13475.
- Bukhov N. & Carpentier R. (2004) Alternative photosystem I-driven electron transport routes: mechanisms and functions. *Photosynthesis Research* **82**, 17–33.
- Bukhov N.G., Samson G. & Carpentier R. (2000) Nonphotosynthetic reduction of the intersystem electron transport chain of chloroplasts following heat stress. Steady-state rate. *Photochemistry and Photobiology* **72**, 351–357.
- Burrows P.A., Sazanov L.A., Svab Z., Maliga P. & Nixon P.J. (1998) Identification of a functional respiratory complex in chloroplasts through analysis of tobacco mutants containing disrupted plastid *ndh* genes. *EMBO Journal* **17**, 868–876.
- del Campo E.M., Sabater B. & Martin M. (2000) Transcripts of the *ndhH-D* operon of barley plastids: possible role of unedited site III in splicing of the *ndhA* intron. *Nucleic Acids Research* **28**, 1092–1098.
- del Campo E.M., Sabater B. & Martin M. (2002) Post-transcriptional control of chloroplast gene expression – accumulation of stable *psaC* mRNA is due to downstream RNA cleavages in the *ndhD* gene. *Journal of Biological Chemistry* **277**, 36457–36464.
- Carol P., Stevenson D., Bisanz C., Breitenbach J., Sandmann G., Mache R., Coupland G. & Kuntz M. (1999) Mutations in the *Arabidopsis* gene *immutans* cause a variegated phenotype by inactivating a chloroplast terminal oxidase associated with phytylene desaturation. *Plant Cell* **11**, 57–68.
- Casano L.M., Zapata J.M., Martin M. & Sabater B. (2000) Chlororespiration and poisoning of cyclic electron transport – plastoquinone as electron transporter between thylakoid NADH dehydrogenase and peroxidase. *Journal of Biological Chemistry* **275**, 942–948.
- Casano L.M., Martin M. & Sabater B. (2001) Hydrogen peroxide mediates the induction of chloroplastic Ndh complex under photooxidative stress in barley. *Plant Physiology* **125**, 1450–1458.
- Chateigner-Boutin A.L. & Hanson M.R. (2003) Developmental co-variation of RNA editing extent of plastid editing sites exhibiting similar cis-elements. *Nucleic Acids Research* **31**, 2586–2594.
- Corneille S., Cournac L., Guedeny G., Havaux M. & Peltier G. (1998) Reduction of the plastoquinone pool by exogenous NADH and NADPH in higher plant chloroplasts – characterization of a NAD(P)H-plastoquinone oxidoreductase activity. *Biochimica et Biophysica Acta* **1363**, 59–69.
- Cournac L., Redding K., Ravenel J., Rumeau D., Josse E.M., Kuntz M. & Peltier G. (2000) Electron flow between photosystem II and oxygen in chloroplasts of photosystem I-deficient algae is mediated by a quinol oxidase involved in chlororespiration. *Journal of Biological Chemistry* **275**, 17256–17262.
- Cournac L., Latouche G., Cerovic Z., Redding K., Ravenel J. & Peltier G. (2002) In vivo interactions between photosynthesis, mitorespiration, and chlororespiration in *Chlamydomonas reinhardtii*. *Plant Physiology* **129**, 1921–1928.
- Darie C.C., De Pascalis L., Mutschler B. & Haehnel W. (2006) Studies of the Ndh complex and photosystem II from mesophyll and bundle sheath chloroplasts of the C-4-type plant *Zea mays*. *Journal of Plant Physiology* **163**, 800–808.
- Endo T., Shikanai T., Takabayashi A., Asada K. & Sato F. (1999) The role of chloroplastic NAD(P)H dehydrogenase in photoprotection. *FEBS Letters* **457**, 5–8.
- Favory J.-J., Kobayashi M., Tanaka K., Peltier G., Kreis M., Valay J.-G. & Lerbs-Mache S. (2005) Specific function of a plastid sigma factor for *ndhF* gene transcription. *Nucleic Acids Research* **33**, 5991–5999.
- Fischer M., Funk E. & Steinmüller K. (1997) The expression of subunits of the mitochondrial complex I-homologous NAD(P)H-plastoquinone-oxidoreductase during plastid development. *Zeitschrift für Naturforschung Section C Journal of Biosciences* **52**, 481–486.
- Fork D.C. & Herbert S.K. (1993) Electron transport and photophosphorylation by photosystem I in vivo in plants and cyanobacteria. *Photosynthesis Research* **36**, 149–168.
- Funk E., Schafer E. & Steinmüller K. (1999) Characterization of the complex I-homologous NAD(P)H-plastoquinone-oxidoreductase (NDH-complex) of maize chloroplasts. *Journal of Plant Physiology* **154**, 16–23.
- Golding A.J., Finazzi G. & Johnson G.N. (2004) Reduction of the thylakoid electron transport chain by stromal reductants – evidence for activation of cyclic electron transport upon dark adaptation or under drought. *Planta* **220**, 356–363.
- Guedeny G., Corneille S., Cuine S. & Peltier G. (1996) Evidence for an association of *ndh B*, *ndh J* gene products and ferredoxin-NADP-reductase as components of a chloroplastic NAD(P)H dehydrogenase complex. *FEBS Letters* **378**, 277–280.
- Guera A., de Nova P.G. & Sabater B. (2000) Identification of the Ndh (NAD(P)H-plastoquinone-oxidoreductase) complex in etioplast membranes of barley: changes during photomorphogenesis of chloroplasts. *Plant and Cell Physiology* **41**, 49–59.
- Guera A., Calatayud A., Sabater B. & Barreno E. (2005) Involvement of the thylakoidal NADH-plastoquinone oxidoreductase complex in the early responses to ozone exposure of barley (*Hordeum vulgare* L.) seedlings. *Journal of Experimental Botany* **56**, 205–218.
- Harbinson J. & Foyer C.H. (1991) Relationships between the efficiencies of photosystems I and II and stromal redox state in CO₂-free air – evidence for cyclic electron flow in vivo. *Plant Physiology* **97**, 41–49.
- Hashimoto M., Endo T., Peltier G., Tasaka M. & Shikanai T. (2003) A nucleus-encoded factor, CRR2, is essential for the expression of chloroplast *ndhB* in *Arabidopsis*. *The Plant Journal* **36**, 541–549.
- Havaux M. (1996) Short-term responses of photosystem I to heat stress. Induction of a PS II-independent electron transport through PS I fed by stromal components. *Photosynthesis Research* **47**, 85–97.
- Havaux M., Greppin H. & Strasser R.J. (1991) Functioning of photosystem I and photosystem II in pea leaves exposed to heat stress in the presence or absence of light. Analysis using *in vivo* fluorescence, absorbance, oxygen and photoacoustic measurements. *Planta* **186**, 88–98.
- Havaux M., Rumeau D. & Ducruet J.M. (2005) Probing the FQR and NDH activities involved in cyclic electron transport around

- photosystem I by the 'afterglow' luminescence. *Biochimica et Biophysica Acta* **1709**, 203–213.
- Heber U. & Walker D. (1992) Concerning a dual function of coupled cyclic electron transport in leaves. *Plant Physiology* **100**, 1621–1626.
- Hirose T. & Sugiura M. (1997) Both RNA editing and RNA cleavage are required for translation of tobacco chloroplast *ndhD* mRNA: a possible regulatory mechanism for the expression of a chloroplast operon consisting of functionally unrelated genes. *The EMBO Journal* **16**, 6804–6811.
- Hoefnagel M.H.N., Atkin O.K. & Wiskich J.T. (1998) Interdependence between chloroplasts and mitochondria in the light and the dark. *Biochimica et Biophysica Acta* **1366**, 235–255.
- Horvath E.M., Peter S.O., Joet T., Rumeau D., Cournac L., Horvath G.V., Kavanagh T.A., Schafer C., Peltier G. & Medgyesy P. (2000) Targeted inactivation of the plastid *ndhB* gene in tobacco results in an enhanced sensitivity of photosynthesis to moderate stomatal closure. *Plant Physiology* **123**, 1337–1349.
- Joet T., Cournac L., Horvath E.M., Medgyesy P. & Peltier G. (2001) Increased sensitivity of photosynthesis to antimycin A induced by inactivation of the chloroplast *ndhB* gene. Evidence for a participation of the NADH-dehydrogenase complex to cyclic electron flow around photosystem I. *Plant Physiology* **125**, 1919–1929.
- Joet T., Cournac L., Peltier G. & Havaux M. (2002a) Cyclic electron flow around photosystem I in C-3 plants. In vivo control by the redox state of chloroplasts and involvement of the NADH-dehydrogenase complex. *Plant Physiology* **128**, 760–769.
- Joet T., Genty B., Josse E.M., Kuntz M., Cournac L. & Peltier G. (2002b) Involvement of a plastid terminal oxidase in plastoquinone oxidation as evidenced by expression of the *Arabidopsis thaliana* enzyme in tobacco. *Journal of Biological Chemistry* **277**, 31623–31630.
- Johnson G.N. (2005) Cyclic electron transport in C3 plants: fact or artefact? *Journal of Experimental Botany* **56**, 407–416.
- Joliot P. & Joliot A. (2005) Quantification of cyclic and linear flows in plants. *Proceedings of the National Academy of Sciences of the USA* **102**, 4913–4918.
- Jordan D.B. & Ogren W. (1984) The CO₂/O₂ specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Planta* **161**, 308–313.
- Khorobrykh S.A. & Ivanov B.N. (2002) Oxygen reduction in a plastoquinone pool of isolated pea thylakoids. *Photosynthesis Research* **71**, 209–219.
- Kofer W., Koop H.U., Wanner G. & Steinmuller K. (1998) Mutagenesis of the genes encoding subunits A, C, H, I, J and K of the plastid NAD(P)H-plastoquinone-oxidoreductase in tobacco by polyethylene glycol-mediated plastome transformation. *Molecular & General Genetics* **258**, 166–173.
- Kotera E., Tasaka M. & Shikanai T. (2005) A pentatricopeptide repeat protein is essential for RNA editing in chloroplasts. **433**, 326–330.
- Kruk J. & Strzalka K. (2001) Redox changes of cytochrome b559 in the presence of plastoquinones. *Journal of Biological Chemistry* **276**, 86–91.
- Kubicki A., Funk E., Westhoff P. & Steinmuller K. (1996) Differential expression of plastome-encoded *ndh* genes in mesophyll and bundle-sheath chloroplasts of the C-4 plant *Sorghum bicolor* indicates that the complex I-homologous NAD(P)H-plastoquinone oxidoreductase is involved in cyclic electron transport. *Planta* **199**, 276–281.
- Lascano H.R., Casano L.M., Martin M. & Sabater B. (2003) The activity of the chloroplastic Ndh complex is regulated by phosphorylation of the NDH-F subunit. *Plant Physiology* **132**, 256–262.
- Lennon A.M., Prommeenate P. & Nixon P.J. (2003) Location, expression and orientation of the putative chlororespiratory enzymes, Ndh and IMMUTANS, in higher-plant plastids. *Planta* **218**, 254–260.
- Lintala M., Allahverdiyeva Y., Kidron H., Piippo M., Battchikova N., Suorsa M., Rintamaki E., Salminen T.A., Aro E.-M. & Mulo P. (2007) Structural and functional characterization of ferredoxin-NADP⁺-oxidoreductase using knock-out mutants of *Arabidopsis*. *The Plant Journal* **49**, 1041–1052.
- Loschelder H., Schweer J., Link B. & Link G. (2006) Dual temporal role of plastid Sigma factor 6 in *Arabidopsis* development. *Plant Physiology* **142**, 642–650.
- Maier R.M., Zeltz P., Kässel H., Bonnard G., Gualberto J.M. & Grienerberger J.M. (1996) RNA editing in plant mitochondria and chloroplasts. *Plant Molecular Biology* **32**, 343–365.
- McDonald A.E. & Vanlerberghe G.C. (2006) Origins, evolutionary history, and taxonomic distribution of alternative oxidase and plastoquinol terminal oxidase. *Comparative Biochemistry and Physiology D-Genomics & Proteomics* **1**, 357–364.
- Meurer J., Berger A. & Westhoff P. (1996) A nuclear mutant of *Arabidopsis* with impaired stability on distinct transcripts of the plastid *psbB*, *psbD/C*, *ndhH*, and *ndhC* operons. *Plant Cell* **8**, 1193–1207.
- Miyake C., Miyata M., Shinzaki Y. & Tomizawa K.-i. (2005) CO₂ response of cyclic electron flow around PSI (CEF-PSI) in tobacco leaves – relative electron fluxes through PSI and PSII determine the magnitude of non-photochemical quenching (NPQ) of Chl fluorescence. *Plant and Cell Physiology* **46**, 629–637.
- Monde R.A., Schuster G. & Stern D.B. (2000) Processing and degradation of chloroplast mRNA. *Biochimie* **82**, 573–582.
- Mubarakshina M., Khorobrykh S. & Ivanov B. (2006) Oxygen reduction in chloroplast thylakoids results in production of hydrogen peroxide inside the membrane. *Biochimica et Biophysica Acta* **1757**, 1496–1503.
- Munekage Y., Hojo M., Meurer J., Endo T., Tasaka M. & Shikanai T. (2002) PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in *Arabidopsis*. *Cell* **110**, 361–371.
- Munekage Y., Hashimoto M., Miyake C., Tomizawa K., Endo T., Tasaka M. & Shikanai T. (2004) Cyclic electron flow around photosystem I is essential for photosynthesis. *Nature* **429**, 579–582.
- Munshi M.K., Kobayashi Y. & Shikanai T. (2005) Identification of a novel protein, CRR7, required for the stabilization of the chloroplast NAD(P)H dehydrogenase complex in *Arabidopsis*. *The Plant Journal* **44**, 1036–1044.
- Munshi M.K., Kobayashi Y. & Shikanai T. (2006) CHLORORESPIRATORY REDUCTION 6 is a novel factor required for accumulation of the chloroplast NAD(P)H dehydrogenase complex in *Arabidopsis*. *Plant Physiology* **141**, 737–744.
- Muraoka R., Okuda K., Kobayashi Y. & Shikanai T. (2006) A eukaryotic factor required for accumulation of the chloroplast NAD(P)H dehydrogenase complex in *Arabidopsis*. *Plant Physiology* **142**, 1683–1689.
- Mus F., Cournac L., Cardellini W., Caruana A. & Peltier G. (2005) Inhibitor studies on non-photochemical plastoquinone reduction and H-2 photoproduction in *Chlamydomonas reinhardtii*. *Biochimica et Biophysica Acta-Bioenergetics* **1708**, 322–332.
- Nagashima A., Hanaoka M., Motohashi R., Seki M., Shinzaki K., Kanamaru K., Takahashi H. & Tanaka K. (2004) DNA microarray analysis of plastid gene expression in an *Arabidopsis* mutant deficient in a plastid transcription factor sigma, SIG2. *Bioscience, Biotechnology and Biochemistry* **68**, 694–704.
- Nixon P.J. (2000) Chlororespiration. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **355**, 1541–1547.

- Niyogi K.K. (2000) Safety valves for photosynthesis. *Current Opinion in Plant Biology* **3**, 455–460.
- Okutani S., Hanke G.T., Satomi Y., Takao T., Kurisu G., Suzuki A. & Hase T. (2005) Three maize leaf ferredoxin:NADPH oxidoreductases vary in subchloroplast location, expression, and interaction with ferredoxin. *Plant Physiology* **139**, 1451–1459.
- Osmond C.B. (1981) Photorespiration and photoinhibition. Some implications for the energetics of photosynthesis. *Biochimica et Biophysica Acta* **639**, 77–98.
- Peltier G. & Cournac L. (2002) Chlororespiration. *Annual Review of Plant Biology* **53**, 523–550.
- Prommeenate P., Lennon A.M., Markert C., Hippler M. & Nixon P.J. (2004) Subunit composition of NDH-1 complexes of *Synechocystis* sp PCC 6803 – identification of two new ndh gene products with nuclear-encoded homologues in the chloroplast Ndh complex. *Journal of Biological Chemistry* **279**, 28165–28173.
- Quiles M.J. (2006) Stimulation of chlororespiration by heat and high light intensity in oat plants. *Plant, Cell & Environment* **29**, 1463–1470.
- Quiles M.J. & Cuello J. (1998) Association of Ferredoxin-NADP oxidoreductase with the chloroplastic pyridine nucleotide dehydrogenase complex in barley leaves. *Plant Physiology* **117**, 235–244.
- Quiles M.J. & Lopez N.I. (2004) Photoinhibition of photosystems I and II induced by exposure to high light intensity during oat plant growth – effects on the chloroplast NADH dehydrogenase complex. *Plant Science* **166**, 815–823.
- Rizhsky L., Hallak-Herr E., Van Breusegem F., Rachmilevitch S., Barr J.E., Rodermel S., Inze D. & Mittler R. (2002) Double antisense plants lacking ascorbate peroxidase and catalase are less sensitive to oxidative stress than single antisense plants lacking ascorbate peroxidase or catalase. *The Plant Journal* **32**, 329–342.
- Rodriguez R.E., Lodeyro A., Poli H.O., *et al.* (2007) Transgenic tobacco plants overexpressing chloroplastic ferredoxin-NADP(H) reductase display normal rates of photosynthesis and increased tolerance to oxidative stress. *Plant Physiology* **143**, 639–649.
- Rosso D., Ivanov A.G., Fu A., *et al.* (2006) IMMUTANS does not act as a stress-induced safety valve in the protection of the photosynthetic apparatus of *Arabidopsis* during steady-state photosynthesis. *Plant Physiology* **142**, 574–585.
- Rumeau D., Becuwe-Linka N., Beyly A., Louwagie M., Garin J. & Peltier G. (2005) New subunits NDH-M, -N, and -O, encoded by nuclear genes, are essential for plastid Ndh complex functioning in higher plants. *Plant Cell* **17**, 219–232.
- Salvucci M.E. & Crafts-Brandner S.J. (2004a) Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. *Physiologia Plantarum* **120**, 179–186.
- Salvucci M.E. & Crafts-Brandner S.J. (2004b) Relationship between the heat tolerance of photosynthesis and the thermal stability of rubisco activase in plants from contrasting thermal environments. *Plant Physiology* **134**, 1460–1470.
- Sazanov L.A., Burrows P.A. & Nixon P.J. (1998a) The chloroplast Ndh complex mediates the dark reduction of the plastoquinone pool in response to heat stress in tobacco leaves. *FEBS Letters* **429**, 115–118.
- Sazanov L.A., Burrows P.A. & Nixon P.J. (1998b) The plastid ndh genes code for an NADH-specific dehydrogenase: isolation of a complex I analogue from pea thylakoid membranes. *Proceedings of the National Academy of Sciences of the USA* **95**, 1319–1324.
- Shikanai T. (2007) Cyclic electron transport around photosystem I: genetic approaches. *Annual Review of Plant Biology* **58**, Epub ahead of print doi:10.1146/annurev.arplant.58.091406.110525.
- Shikanai T., Endo T., Hashimoto T., Yamada Y., Asada K. & Yokota A. (1998) Directed disruption of the tobacco *ndhB* gene impairs cyclic electron flow around photosystem I. *Proceedings of the National Academy of Sciences of the USA* **95**, 9705–9709.
- Sonoike K. (1996) Photoinhibition of photosystem I: its physiological significance in the chilling sensitivity of plants. *Plant and Cell Physiology* **37**, 239–247.
- Stegemann S., Hartmann S., Ruf S. & Bock R. (2003) High-frequency gene transfer from the chloroplast genome to the nucleus. *Proceedings of the National Academy of Sciences of the USA* **100**, 8828–8833.
- Streb P., Josse E.-M., Gallouet E., Baptist F., Kuntz M. & Cornic G. (2005) Evidence for alternative electron sinks to photosynthetic carbon assimilation in the high mountain plant species *Ranunculus glacialis*. *Plant, Cell & Environment* **28**, 1123–1135.
- Sugita M. & Sugiura M. (1996) Regulation of gene expression in chloroplasts of higher plants. *Plant Molecular Biology* **32**, 315–326.
- Takabayashi A., Kishine M., Asada K., Endo T. & Sato F. (2005) Differential use of two cyclic electron flows around photosystem I for driving CO₂-concentration mechanism in C-4 photosynthesis. *Proceedings of the National Academy of Sciences of the USA* **102**, 16898–16903.
- Teicher H.B. & Scheller H.V. (1998) The NAD(P)H dehydrogenase in barley thylakoids is photoactivatable and uses NADPH as well as NADH. *Plant Physiology* **117**, 525–532.
- Tsuzuki T., Wakasugi T. & Sugiura M. (2001) Comparative analysis of RNA editing sites in higher plant chloroplasts. *Journal of Molecular Evolution* **53**, 327–332.
- Wang P., Duan W., Takabayashi A., Endo T., Shikanai T., Ye J.Y. & Mi H.L. (2006) Chloroplastic NAD(P)H dehydrogenase in tobacco leaves functions in alleviation of oxidative damage caused by temperature stress. *Plant Physiology* **141**, 465–474.
- Wu D.Y., Wright D.A., Wetzel C., Voytas D.F. & Rodermel S. (1999) The immutans variegation locus of *Arabidopsis* defines a mitochondrial alternative oxidase homolog that functions during early chloroplast biogenesis. *Plant Cell* **11**, 43–55.
- Yamamoto H., Kato H., Shinzaki Y., *et al.* (2006) Ferredoxin limits cyclic electron flow around PSI (CEF-PSI) in higher plants – stimulation of CEF-PSI enhances non-photochemical quenching of Chl fluorescence in transplastomic tobacco. *Plant and Cell Physiology* **47**, 1355–1371.
- Yamane Y., Shikanai T., Kashino Y., Koike H. & Satoh K. (2000) Reduction of Q(A) in the dark: another cause of fluorescence F_o increases by high temperatures in higher plants. *Photosynthesis Research* **63**, 23–34.
- Zapata J.M., Guera A., Esteban-Carrasco A., Martin M. & Sabater B. (2005) Chloroplasts regulate leaf senescence: delayed senescence in transgenic *ndhF*-defective tobacco. *Cell Death and Differentiation* **12**, 1277–1284.
- Zhang H., Whitelegge J.P. & Cramer W.A. (2001) Ferredoxin:NADP⁺ oxidoreductase is a subunit of the chloroplast cytochrome b₆f complex. *Journal of Biological Chemistry* **276**, 38159–38165.

Received 27 February 2007; received in revised form 30 March 2007; accepted for publication 3 April 2007