Biochimica et Biophysica Acta xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

The onset of NPQ and $\Delta\mu_H^+$ upon illumination of tobacco plants studied through the influence of mitochondrial electron transport

Pierre Cardol [a], Rosine De Paepe [b], Fabrice Franck [c], Giorgio Forti [d], Giovanni Finazzi [e,*,1]

[a] Laboratoire de Génétique des Microorganismes, Département des Sciences la Vie, 27, Bld du rectorat, Université de Liège, B-4000 Liège, Belgium
[b] Laboratoire Mitochondries et Métabolisme, Institut de Biotechnologie des Plantes, UMR 8618 CNRS- Université Paris Sud, 91 405 Orsay, France
[c] Laboratoire de Photobiologie, Département des Sciences la Vie, 27, Bld du rectorat, Université de Liège, B-4000 Liège, Belgium
[d] Istituto di Biofisica del Consiglio Nazionale delle Ricerche, Sezione di Milano. Università degli Studi di Milano, Dipartimento di Biologia, Milano 20133, Italy
[e] UMR 7141 CNRS-UPMC, Institut de Biologie Physico Chimique, 13 rue Pierre et Marie Curie, 75005, Paris, France

ARTICLE INFO

Article history:
Received 23 July 2009
Received in revised form 2 October 2009
Accepted 7 October 2009
Available online xxxx

Keywords:
Non-photochemical quenching
Respiration
Photosynthesis
Cyclic electron flow
Electrochemical proton gradient

ABSTRACT

The relationship between the development of photoprotective mechanisms (non-photochemical quenching, NPQ), the generation of the electrochemical proton gradient in the chloroplast and the capacity to assimilate CO_2 was studied in tobacco dark-adapted leaves at the onset of illumination with low light. These conditions induce the generation of a transient NPQ, which relaxes in the light in parallel with the activation of the Calvin cycle. Wild-type plants were compared with a CMSII mitochondrial mutant, which lacks the respiratory complex I and shows a delayed activation of photosynthesis. In the mutant, a slower onset of photosynthesis was mirrored by a decreased capacity to develop NPQ. This correlates with a reduced efficiency to reroute electrons at the PSI reducing side towards cyclic electron flow around PSI and/or other alternative acceptor pools, and with a smaller ability to generate a proton motive force in the light. Altogether, these data illustrate the tight relationship existing between the capacity to evacuate excess electrons accumulated in the intersystem carriers and the capacity to dissipate excess photons during a dark to light transition. These data also underline the essential role of respiration in modulating the photoprotective response in dark-adapted leaves, by poising the cellular redox state.

1. Introduction

Upon illumination, the in series activity of photosystem I and II provides the chloroplast stroma compartment with reducing molecules (NADPH), which are consumed along with ATP for CO_2 assimilation by the Calvin–Benson–Bassham cycle (hereafter called Calvin cycle). Although it is acknowledged that isolated chloroplasts are capable of performing this process autonomously [1], growing evidences exist for carbon assimilation to be a highly integrated phenomenon *in vivo*. A strong interaction between the respiratory and photosynthetic chains is documented in green algae, where exchanges of reducing equivalents and ATP modulate the redox state of the plastoquinone pool and establish an electrochemical proton gradient across the thylakoid membranes in the dark (reviewed in [2,3]).

In land plants too, it is documented that mitochondria play essential functions during photosynthesis (for review, see [4–6]). They oxidize the malate generated by photosynthetic activity [7], provide ATP to support UDPG formation for sugar synthesis [8,9] and allow the oxidation of glycine generated during photorespiration [10]. In addition, recent studies performed in CMSII, a tobacco mutant lacking the respiratory complex I [11–14], have shown that mitochondria are involved in the control of the cellular redox homeostasis [15] in the integration of carbon/nitrogen metabolism [16] and in acclimation to high growth irradiance [17]. Finally, oxidative phosphorylation in mitochondria has been suggested to modulate the onset of CO_2 assimilation in a dark-light transition, as indicated by the delayed activation of the Calvin cycle in CSMII plants [18].

The dark to light transition is a critical phase for photosynthesis, because light absorption and photochemistry attain their steady state efficiency almost instantaneously, whereas achievement of maximum CO_2 assimilatory capacity takes several minutes. The consequent unbalance between the rates of electron injection into the chain–driven by reaction centre photochemistry–and withdrawal–due to carbon assimilation–leads to over-reduction of the soluble electron carriers also in limiting light, opening the way to possible photodamage. Plants are capable of responding to such a stress through a

Abbreviations: CEF, cyclic electron flow; ECS, electrochromic shift; LHCII, light harvesting complex II; NPQ, non-photochemical quenching; PS, photosystem; Vx, violaxanthin; Zx, zeaxanthin

* Corresponding author. Laboratoire de Physiologie Cellulaire Végétale, UMR5168 CNRS, CEA, INRA, Université Joseph Fourier 14 rue des Martyrs, Grenoble, France. Tel.: +33 04 38 78 41 84; fax: +33 04 38 78 50 91.
E-mail address: giovanni.finazzi@cea.fr (G. Finazzi).
[1] Present address: UMR 5168 «Laboratoire de Physiologie Cellulaire Végétale (LPCV)»CNRS (UMR5168) / UJF / INRA (UMR1200) / CEA, Institut de Recherches en Technologies et Sciences pour le Vivant (iRTSV), 17 rue des Martyrs, 38054 Grenoble cedex 9, France.

0005-2728/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.bbabio.2009.10.002

variety of mechanisms, which involve changes in the electron flow capacity as well as in the light absorption efficiency. In steady state, electron transfer mainly stems from linear flow, which involves both PSII and PSI activity, and leads to CO_2 assimilation. Conversely, a significant fraction of the photogenerated electrons can be directed away from CO_2 at the onset of illumination, when the Calvin cycle is inactive (see e.g. [19–21] for a more detailed discussion). In principle, this role is fulfilled by different pathways, which mostly happen at the PSI reducing side: the reduction of molecular oxygen [22], the transport of reducing equivalents between the chloroplast and the mitochondria via the malate or the triose phosphate transporters (reviewed in [23]) and the recycling of electron around PSI via cyclic electron flow (CEF, [21]).

Plants also respond to over-reduction of the electron flow chain by a series of mechanisms involving PSII, which involve regulation of light utilization, and are collectively referred to as non-photochemical quenching mechanisms (NPQ, reviewed e.g. in [24–26]). According to the definition provided by Horton and colleagues [24], this term encompasses at least three processes: (i) (qI), a slowly-reversible damage to PSII reaction centers [27], see however [28] (ii) (qT), a change in the relative antenna sizes of PSII and PSI, due to the reversible phosphorylation and migration of antenna-proteins (LHCII) (e.g. [29]), and (iii) (qE), a quenching associated with the acidification of the lumenal pH [30]. In plants, qE is the dominant form of NPQ (e. g. [31,32]). It is generally thought to be associated with an increase in thermal dissipation within the light harvesting apparatus [33], which requires the deepoxidation of xanthophyll cycle pigment violaxanthin (Vx) into zeaxanthin (Zx) [34], and the presence of the PSII subunit PsbS [35,36]. Zx may act as an allosteric effector of NPQ, modulating a pH/PsbS driven conformational change that would lead to quenching interactions between pigments [24,28]. Alternatively, Zx could directly participate in the quenching process, by forming a radical pair with a special Chl a, again by a mechanism that would depend on the acidification of the luminal pH and on PsbS [32].

In addition to the Zx-driven processes, the presence of a quenching poorly related to zeaxanthin generation is documented [37–39]. This quenching can be observed in leaves completely devoid of this xanthophylls cycle carotenoid [37,38] but is enhanced upon zeaxanthin accumulation [39]. The nature of the quenching is still controversial: it could involve quenching at the reaction centre [37], or reflect a modulation on the light harvesting capacity of PSII by its small subunit PsbS [38,39]. In dark-adapted plants, the zeaxanthin independent quenching is prominent upon exposure to low light, and largely disappears upon attainment of steady state photosynthesis [37,39]. Quenching onset and disappearance are interpreted as to reflect changes in the activity of the Calvin cycle. Indeed, a modified rate of CO_2 assimilation also affects the chloroplast ATP turnover, by modulating the ATP demand. This in turn leads to changes in the size of the trans-thylakoid ΔpH and eventually of the NPQ response. The possible link between CO_2 assimilation and the extent of the NPQ in both pre steady state [37,39,40] and steady state conditions (e.g. [41–43]) has been already considered in the past. Furthermore, previous studies have documented that fitness is reduced in mutants bearing qE deficiencies when exposed to a fluctuating light regime [44]. This underlines that acclimation during the dark–light phase is a critical step for efficient photosynthesis.

Still, very few attempts have been performed so far to establish a relationship between changes in the electrochemical proton gradient ($\Delta\mu_H{}^+$), the capacity to reroute electron flow out of the CO_2 assimilation and the NPQ response during a dark to light transition *in vivo* (see e.g. [45,46], for further discussion). These works have pointed out that electron diversion toward redox sinks other than CO_2, as well as modulation of the ATPsynthase-ATPase activity, can deeply affect the size as well as the composition of the electrochemical proton gradient established in the light, and therefore the photoprotective response. In this work we have focused on the role of mitochondrial activity in photoprotection, by comparing changes in NPQ, $\Delta\mu_H{}^+$and cyclic electron flow around PSI in wild-type and CMSII plants of *Nicotiana sylvestris*. By measuring relevant fluorescence and spectroscopic parameters, we confirm the existence of a link between onset of photosynthesis, activation of alternative electron pathways at the reducing side of PSI and onset of photoprotection, and reveal the fundamental role of mitochondrial activity in modulating the interplay between these three parameters during the early steps of light acclimation.

2. Materials and methods

2.1. Growth conditions

N. sylvestris WT and CMSII plants were grown in soil in a greenhouse under natural lighting at a day/night temperature of 23.5 °C/17.5 °C and a day/night 60%/50% relative humidity. Artificial lighting (Philips SON-7 AGRO 400 W lamps) was supplemented to ensure a minimum lighting of 300 μmol of photons m^{-2} s^{-1} at the leaf surface. Plants were irrigated with nutrient solution (Hydrokani C_2, Hydro Agri Spécialités, France). They were studied at a similar stage of development, i.e. about 2 months and 3 months old for the WT and CMSII, respectively, to compensate for the reduced growth rate of the mutant [15]. Leaves were dark adapted for at least 5 h prior to measurements.

2.2. Spectroscopic measurements

Spectroscopic measurements were performed on intact leaves with a flash spectrophotometer (JTS 10, Biologic France). P_{700} oxidation kinetics was assessed at 820–870 nm, as previously described [47]. Far-red illumination was provided by a LED peaking at 720 nm, filtered through three Wratten filters 55 that block wavelengths shorter than 700 nm. A signal associated with fluorescence emission (normally <5%) was subtracted from the kinetics. When needed, the maximum extent of $P_{700}{}^+$ was estimated by imposing a saturating flash of white light on top of the far red, as described in [48]. Actinic light was provided by a green LED peaking at 520 nm.

Membrane potential changes (ECS) were measured under the same actinic illumination used for measurements of the P_{700} redox changes. They were detected at 520–545 nm, using a white LED source (Luxeon, Lumileds) filtered through appropriate interference filters. This procedure allows deconvoluting the ECS signal from redox changes associated with the cytochrome b_6f complex. The photodiodes were protected from actinic light by a Schott BG 39 filter. Pre-illumination was provided by green LEDs with a peak emission at 520 nm (FWHM 20 nm).

In the case of both P_{700} and membrane potential changes, continuous actinic light was switched off transiently while measuring appropriate wavelengths. Furthermore, leaves were exposed to dark-light cycles to allow measuring absorption changes at different wavelengths (as required to correctly evaluate the two parameters) in samples keeping a low capacity of CO_2 assimilation. Briefly, attached dark-adapted leaves were exposed to actinic light, and kinetics was followed at a given wavelength for 10 min. Leaves were then let recover in the dark for 40 min, to allow full inactivation of the Calvin cycle (e.g. [48]) and zeaxanthin epoxidation [49]. This procedure was followed until all the wavelengths of interest were measured. To ensure reproducibility of the data, kinetics was measured twice at every wavelength, first from the lowest to the highest and then in the opposite direction. At least three different leaves were measured in each condition, unless different stated in the figure captions. At the beginning and at the end of every experiment, fluorescence parameters were measured on the same leaf, to verify its physiological status, and test the experimental variability.

To avoid excessive exposure of leaves to light and dark cycles, we limited our analysis to the minimum number of wavelengths required

Please cite this article as: P. Cardol, et al., The onset of NPQ and $\Delta\mu_H^+$ upon illumination of tobacco plants studied through the influence of mitochondrial electron transport, Biochim. Biophys. Acta (2009), doi:10.1016/j.bbabio.2009.10.002

Table 1
NPQ and deepoxidation state of WT and CMSII leaves in limiting light conditions.

Conditions	WT		CMSII	
	NPQ	DPS	NPQ	DPS
150 μmol of photons m^{-2} s^{-1} (45")	0.75 ± 0.12	0.027 ± 0.007	0.52 ± 0.09	0.027 ± 0.014
150 μ μmol of photons m^{-2} s^{-1} (5′)	0.15 ± 0.09	0.079 ± 0.010	0.13 ± 0.08	0.056 ± 0.025

NPQ was measured as $(F_m - F_{m'})/F_m$, where F_m is the maximum fluorescence yield measured upon illumination with a short (200 ms) saturating flash in dark-adapted leaves, while $F_{m'}$ is the maximum fluorescence yield measured in leaves subjected to actinic illumination [94]. Carotenoid content was assessed in leaf cell extracts as indicated in methods. The deepoxidation state (DPS) was calculated as (Zx + 0.5Ax)/(Zx + Ax + Vx).

for proper estimation of the different optical parameters. Therefore, since no sustained zeaxanthin synthesis was detected at the beginning of illumination (Table 1), spectroscopic changes associated with the generation of a transmembrane potential were not deconvoluted from the zeaxanthin red shift signal (ΔA_{535}, [50]). However, no major modification of the ECS kinetics profiles was seen in experiments where such deconvolution was taken into account by measuring appropriate wavelengths (505 and 535 nm, e.g. [51], not shown).

2.3. Fluorescence measurements

Fluorescence was measured with the same apparatus used for spectroscopy, using green light for both pre-illumination and leaf excitation. Estimation of the size of the PSI and PSII acceptor pools in Fig. 5 was obtained from plots of the area bound above the second fluorescence induction curve as a function of the dark time between the first and the second light pulses. The biphasic kinetics obtained was then fitted with a sum of two exponential functions to estimate their relative amplitude and decay rates.

2.4. CO_2 exchange measurements

To assess the CO_2 assimilation capacity, leaves from mutant and WT plants were clamped into an infra-red gas analyzer chamber (Ciras-1; PP-Systems, Hitchin, UK). Measurements were performed supplying external CO_2 at 360 μl l^{-1}. All experiments were conducted at 22 °C and 50% relative humidity. Irradiance was provided by a xenon lamp, filtered with a Wratten 55 filter, the intensity of which was adjusted by neutral density sheeting. Respiratory CO_2 release was monitored for 20 min in the dark, and the time course of CO_2 uptake was followed upon light exposure.

2.5. Pigment analysis

Pigments were extracted from leaves in methanol 90% and debris was removed by centrifugation at 10,000×*g* for 15 min. 50 μl of pigment extract was subjected to reverse-phase HPLC analysis using a commercial equipment (Waters, Milford, MA) [52]. A Nova Pak C18, 60A column (150-mm length, 4-μm pore size) was used for separation.

2.6. Determination of the cellular ATP content

A sample of the leaf used to measure fluorescence was immediately frozen after illumination. Samples were extracted in 1.5% TCA, centrifuged at 15,000 rpm, and a volume of the supernatant was diluted in 0.5^{-}M Tris-Acetate pH 7.75 (1/300, v/v). Determination of ATP content was made using the Enliten ATP assay (Promega), according to the manufacturer's instructions.

3. Results and discussion

3.1. Onset and relaxation of reversible NPQ are delayed in a tobacco mutant lacking the respiratory complex I

The CMSII mutant shows a significant slowing down of CO_2 assimilation at the onset of illumination, which is evident in high light [18], but also under the limiting light conditions employed in this work (Fig 1A, inset). We thus expect its capacity to develop a non-photochemical quenching during a dark to light transition to be also modified to some extent. As shown in Fig. 1A (main panel) while a NPQ response could still be developed in CMSII, the extent of the quenching was reduced. Both the onset and the relaxation kinetics were slower than in the WT (Fig. 1B). In both genotypes, the quenching was completely abolished by addition of the H^+/K^+ exchanger nigericin (not shown), which relaxes the light-induced ΔpH. This confirms that NPQ is triggered by acidification of the thylakoid lumen, and therefore represents a typical qE response (e.g. [24,30]). In agreement with our previous findings in barley [37], the fluorescence quenching observed in these conditions was poorly related to the presence of zeaxanthin, the synthesis of which was barely detectable after 45 s of exposure at 150 μmol photons m^{-2} s^{-1} (Table 1), i.e. when the quenching was maximum (Fig. 1B). Consistent with previous studies, we do not observe any specific photosynthetic phenotype in CMSII in steady state conditions, in low (Fig. 1 and Table 1) as well as in high light (not shown, see however [18]). Therefore, we consider that this mutant was a very good experimental system to study acclimation of photosynthesis during the first minutes of light exposure, where the relationship between quenching onset and disappearance and photosynthesis is most evident [37,39,40] and where we expect a maximum limitation of CO_2 assimilation by ATP availability [4].

To confirm that the reduced NPQ response observed at low light was linked to changes in the respiratory activity, leaves were infiltrated with a solution containing either myxothiazol (a known and specific inhibitor of the respiratory chain at the level of complex III -cytochrome bc_1- [53]) or the same volume of the solvent (ethanol) without the inhibitor ("control"). Sorbitol (150 mM) was added to all samples, to avoid a substantial modification of the osmotic pressure within the leaf upon infiltration. As shown in Fig. 2, incubation of WT leaves for 4 h with myxothiazol induced a modification in the NPQ onset and relaxation (Fig. 2A), the kinetics of which closely resembles the ones observed in the CMSII leaves (Fig. 2B). Addition of myxothiazol to the mutant increased the effect on NPQ onset (Fig. 2B), suggesting that concomitant inhibition of complex III (by myxothiazol) and of complex I (by the CMSII mutation) further decreases the capacity of tobacco leaves to develop a NPQ response.

3.2. Relationship between the light-induced $\Delta\mu_H^+$ and changes in the transient fluorescence quenching

In order to better understand the relationship between non-photochemical quenching and carbon assimilation, we compared the efficiency of photosynthesis (evaluated from the ΦPSII parameter, [54], Fig. 1C) and the NPQ response at the beginning of illumination (Fig. 1B). A complex relationship between these two parameters was found in both the WT and the CMSII. Photosynthesis and NPQ increase during the first seconds of illumination, following a nonlinear relationship (Fig. 1D, light grey panel). The much steeper increase of NPQ observed in WT leaves suggests a higher capacity to generate a quenched state by electron flow in this strain. After this initial phase, the increase of photosynthesis was accompanied by a decline in NPQ, and an almost linear relationship between electron flow onset and

Please cite this article as: P. Cardol, et al., The onset of NPQ and $\Delta\mu_H^+$ upon illumination of tobacco plants studied through the influence of mitochondrial electron transport, Biochim. Biophys. Acta (2009), doi:10.1016/j.bbabio.2009.10.002

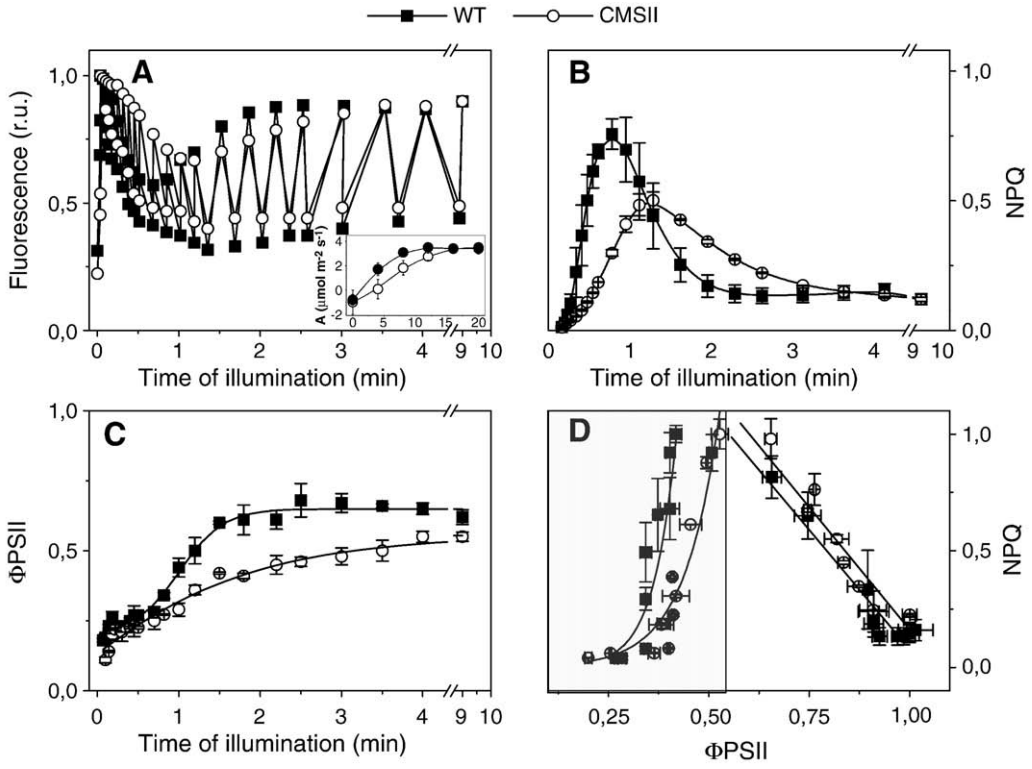

Fig. 1. (A) Fluorescence changes and CO_2 assimilation in leaves subjected to illumination with low light (150 μmoles of photons m^{-2} s^{-1}). Main panel: fluorescence changes (representative traces). The maximum quantum yield of PSII in the dark (F_v/F_m, [93]), was 0.81 ± 0.03 and 0.8 ± 0.02 (average of 6 independent measurements) in CMSII and WT leaves, respectively. Inset: CO_2 assimilation rates. Leaves were clamped into an infra-red gas analyzer chamber, and CO_2 assimilation was followed upon illumination. Standard errors refer to three independent experiments. Open symbols (CMSII); closed symbols: WT. (B) NPQ profile, calculated form traces as in panel A using the $(F_m - F_{m'})/F_m$ formula [94], where F_m is the maximum fluorescence yield measured upon illumination with a short (200 ms) saturating flash in dark-adapted leaves, and $F_{m'}$ is the maximum fluorescence yield measured in leaves subjected to actinic illumination. (C) Changes in the photosynthetic electron flow, expressed by the ΦPSII parameter $(F_{m'} - F_{ss})/F_{m'}$ [54], where F_{ss} is the fluorescence level induced by the actinic illumination. (D) Relationship between the NPQ relaxation in the light and the increase in the photosynthetic assimilatory capacity. NPQ and ΦPSII were normalized to 1 in both WT and CMSII strains to allow for a better comparison. Standard errors refer to three independent experiments.

NPQ decrease could be observed in both genotypes. This confirms that the relaxation of the transient NPQ in the light is mostly due to activation of CO_2 assimilation, as previously suggested (e.g. [37,40]).

We reasoned that the onset of the Calvin cycle should lead to a double effect on the photosynthetic apparatus: on the one side, it should promote the oxidation of the electron carriers, due to enhanced electron flux to the terminal sink. On the other side, enhanced CO_2 assimilation should be accompanied by a decrease of the $\Delta\mu_H{}^+$ during illumination, owing to increased ATP consumption. As NPQ onset and relaxation are linked to changes in the trans-thylakoid proton motive force (e.g. [30,55]), it is likely that the slower relaxation of NPQ in the light observed in CMSII leaves may reflect a slower relaxation of the $\Delta\mu_H{}^+$, due to the delayed activation of the Calvin cycle in this mutant.

To test this hypothesis, we measured the size of the proton motive force generated in the WT and the mutant genotypes by assessing the overall size of the electrochromic signal (ECS). In land plants, algae and photosynthetic bacteria, the light-induced building of the transmembrane electric field in thylakoids modifies the spectroscopic properties of some chromophores, owing to the Stark

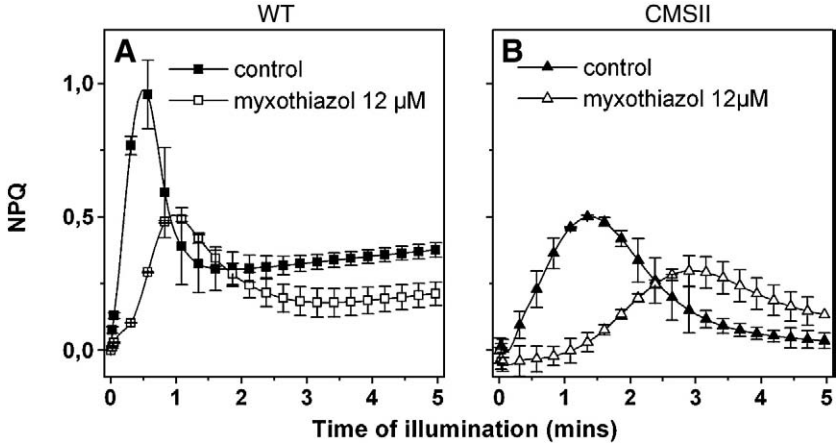

Fig. 2. Effect of myxothiazol on the transient fluorescence quenching in WT (A) and CMSII (B) leaves. Dark-adapted leaves were treated with a solution containing sorbitol 150 mM (to avoid osmotic effects) in the absence (control, closed symbols) and presence of myxothiazol (12 μM, open symbols) by a mild vacuum infiltration. Then fluorescence changes were evaluated as in Fig. 1B. Standard errors refer to three independent experiments.

Please cite this article as: P. Cardol, et al., The onset of NPQ and $\Delta\mu_H^+$ upon illumination of tobacco plants studied through the influence of mitochondrial electron transport, Biochim. Biophys. Acta (2009), doi:10.1016/j.bbabio.2009.10.002

effect [56]. This results in a shift of their absorption spectrum, the amplitude of which is linearly related to the size of the photo-generated field [56]. When illumination is interrupted, relaxation of the $\Delta\mu_H^+$ takes place in the ten of seconds' time range. Due to the different relaxation rates of the electric ($\Delta\Psi$) and pH (ΔpH) components of the $\Delta\mu_H^+$, recent attempts have been made to estimate their relative amplitude based on the transient inversion of the ECS that is observed at the end of illumination (reviewed in [45]). However, this procedure, which has proven reliable in steady state conditions, could not be employed in our conditions (pre-steady state illumination), due to the difficulty in correctly assessing the extent of the inversion of the ECS signal (not shown). Therefore, we limited our analysis to the sole estimation of the $\Delta\mu_H^+$, based on the total amplitude of the ECS signal, without trying to further discriminate between its ΔpH and the $\Delta\Psi$ components. Since our experimental setup did not allow measuring several wavelengths simultaneously, light dark cycles were employed, to allow correct evaluation of the optical changes in the same leaves, and to ensure at the same time full deactivation of photosynthesis in the dark time between two consecutive experiments (see methods). Results are presented in Fig. 3, which shows the time course of changes in the $\Delta\mu_H^+$ (open symbols) and NPQ (closed symbols) in WT (panel A) and CMSII (panel B) leaves during a dark to light transition.

In both genotypes, illumination led to a transient increase of the $\Delta\mu_H^+$, as evidenced by the increased size of the ECS signal. This was followed by a slower relaxation, which was in the same range as the time required for activation of photosynthesis. In both the WT and CMSII, a parallel could be established between changes in fluorescence quenching on the one hand, and changes in the size of the proton motive force on the other one, suggesting a possible cause-effect relationship. Indeed, the slower decay of NPQ in the light observed in CMSII (panel B) was mirrored by a slower relaxation of the $\Delta\mu_H^+$during illumination, when compared to the WT (panel A). Still, complete relaxation of NPQ occurred before full $\Delta\mu_H^+$ decline in both genotyped, as indicated by the strong deviation of the NPQ/ECS profile during the decay of the transmembrane proton motive force (open triangles) from a straight line (dashed lines) (Fig. 3, panels C and D). In principle, this lack or correlation could merely reflect the complex relationship between the NPQ and the different components of the $\Delta\mu_H^+$. While a tight relationship exists between NPQ relaxation and the dissipation of the ΔpH, this component only contributes 1/2, 2/3 of the overall $\Delta\mu_H^+$ in the light (e.g. [45]). It is possible therefore that the different kinetics of NPQ and $\Delta\mu_H^+$ decay may reflect the different kinetics of ΔpH and $\Delta\Psi$ relaxation in the dark, as already discussed (e.g. [57], see however the discussion below for alternative interpretations).

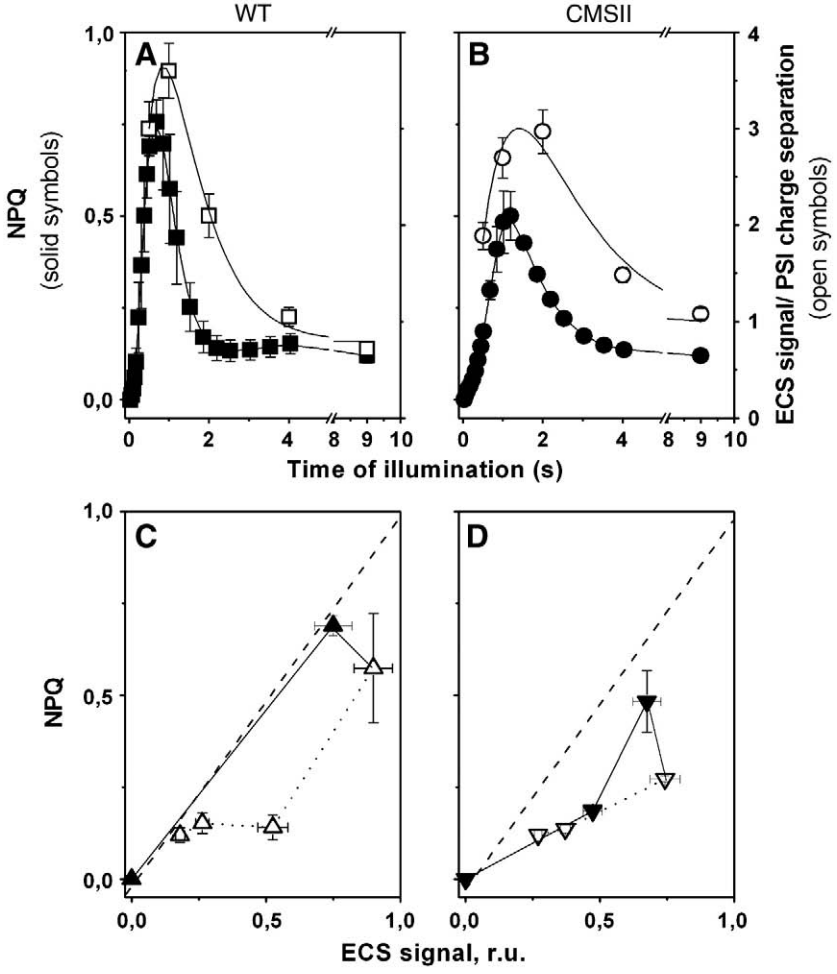

Fig. 3. Time course of NPQ and of the proton motive force during illumination with low light in WT and CMSII leaves. (A and B) Solid symbols (squares, circles): NPQ changes. Open symbols (squares, circles): light induced $\Delta\mu_H^+$, estimated by the size of the ECS signal (520–546 nm) at different times of illumination. Same illumination conditions as in Fig. 1 (150 μmol photons m^{-2} s^{-1}). ECS data were normalized to the signal corresponding to 1 charge separation per photosystem, i.e. the absorption changes induced by a saturating laser flash in the presence of DCMU (20 μM) and hydroxylamine (2 mM) to inhibit PSII activity. (C and D) Relationship between NPQ and ECS changes. Solid triangles (solid line): NPQ rise phase (dotted line). Open triangles: NPQ relaxation phase (solid line). The dashed line represents the expected relationship between the two parameters in case of a concomitant relaxation of NPQ and of the ECS. ECS was normalized to 1 in both WT and CMSII strains to allow for a better comparison. Standard errors refer to three independent experiments.

Please cite this article as: P. Cardol, et al., The onset of NPQ and $\Delta\mu_H^+$ upon illumination of tobacco plants studied through the influence of mitochondrial electron transport, Biochim. Biophys. Acta (2009), doi:10.1016/j.bbabio.2009.10.002

3.3. The transition from cyclic to linear electron flow is delayed in CMSII plants

In steady state, electron transfer mainly stems from linear electron flow, which involves both PSII and PSI activity, and leads to CO_2 assimilation. Conversely, a significant fraction of the photogenerated electrons can be directed away from CO_2 at the onset of illumination, when the Calvin cycle is inactive ([19–21], see also Introduction). In particular CEF activity has been proposed to be dominant during the induction of photosynthesis (e.g. [58]), i.e. in the conditions where we observe the onset of the transient NPQ. The high efficiency of cyclic flow is interpreted as the consequence of a redox modulation of the relative efficiencies of the linear and cyclic processes (see [48] for a further discussion). Under steady-state photosynthesis, when enzymes of the Calvin cycle are active, linear flow is expected to out-compete the cyclic process, as carbon fixation forms an efficient sink for electrons from PSI. Conversely, recycling of electrons around PSI is predominant when linear flow is limited by the low rate of NADPH oxidation (reviewed in [20,21]).

Two major roles for CEF have been suggested in vascular plants: (i) to enhance ATP generation to maintain an appropriate ATP/NADPH ratio in the chloroplast (i.e. [59]), and (ii) to down-regulate PSII via generation of NPQ [60]. Indeed, CEF fuels electron transport under conditions where CO_2 assimilation is low. Therefore it contributes to the building of a $\Delta\mu_H^+$, which is essentially not consumed for ATP synthesis. Because the NPQ response in the WT and the CMSII mutant is at least qualitatively related to changes in lumen acidification (Fig. 3), we decided to explore the relationship between transient NPQ and CEF. The rationale was to verify whether the slower activation of the Calvin cycle observed in CMSII–which should keep the soluble PSI acceptors reduced for a longer period during light acclimation ([18], see also [61])–may lead to a longer lifetime of cyclic flow, owing to the redox regulation mechanism mentioned above. To quantify CEF in WT and CMSII leaves, we followed the kinetics of P_{700} oxidation upon illumination with far red, i.e. under conditions where PSI is preferentially excited. In the WT, slow P_{700} oxidation rates were observed in dark-adapted conditions, while rather fast P_{700} oxidation appeared upon exposure to 150 μmoles photons m^{-2} s^{-1} for 10 min (Fig. 4A), in agreement with previous findings in spinach [62] and Arabidopsis [63,64]. The fast rate of P_{700} oxidation has previously been interpreted as representing the reduction of an efficient acceptor pool (probably NADP, [65]) while the slower component is considered as a signature of CEF, representing the gradual leak of electrons from the cyclic pathway to a stromal electron acceptor [48,62]. Alternatively, the slow phase of P_{700} oxidation may also reflect a mere inhibition of PSI activity due to reduction of its soluble electron acceptor pool. To discriminate between these two hypotheses, we measured the generation of the ECS signal in parallel to the oxidation rate of P_{700}. We followed this rationale: PSI turnover in the cyclic mode results in charge separation, and therefore promotes the generation of a transmembrane potential, which can be revealed by measuring the ECS signal. Conversely, a mere inhibition of PSI turnover should prevent the generation of the transmembrane potential, leading to a diminished ECS signal. As expected in the case of significant CEF activity during the dark to light transition, a sustained ECS generation was observed in both the WT and the CMSII mutant during the slow phase of P_{700} oxidation, when tested under the conditions of Fig. 4A (not shown). In principle, other alternative electron flow phenomena downstream of PSII (e.g. the PTOX mediated, PSII-driven reduction of molecular oxygen) could contribute to ECS generation in leaves where CO_2 assimilation is largely inactive. Indeed, this enzyme oxidizes the plastoquinone pool, using molecular oxygen as an electron acceptor [66]. However we tend to exclude their involvement in the ECS kinetics measured here, because the turnover rates measured under these conditions (10–20 s^{-1}, not shown), are by far faster than the PTOX activity measured under the same conditions (~0.7 s^{-1}, Fig. 5C, see also [66]).

In dark-adapted leaves, no significant differences were seen between the WT and CMSII (Fig. 4A and B), suggesting a similar capacity to perform CEF in the two genotypes. In both lines, the rates of P_{700} oxidation progressively increased upon exposure to light for 10 min (Fig. 4A and B), indicating the progressive substitution of CEF by linear flow [48,62,63]). However, the transition from cyclic to linear flow was significantly slower in the mutant. In steady state, similar rates of P_{700} oxidation were again measured in both WT and CMSII leaves, indicating that the rate of linear flow was similar in the two genotypes. This confirms that mitochondrial respiration does not influence the rate of electron flow in the chloroplast in steady state conditions (e.g. [18]). The half time of P_{700} oxidation measured in traces as in panels A and B was used to evaluate the fraction of P_{700} engaged in cyclic flow. Indeed, this parameter is proportional to the number of PSI turnovers taking place during far-red illumination. In linear flow conditions, the number of PSI turnovers is equal to the number of charges stored in PSI donors at time zero. Conversely, occurrence of cyclic flow induces a slowdown of P_{700} oxidation, due to multiple turnovers. It follows that if t_1 and t_2 are the half times for P_{700} oxidation in the two conditions, the probability for an electron generated on the PSI acceptor side to be oxidized via the linear pathway at any time during the dark to light transition is given by t_1/t_2, while the probability to be oxidized via the cyclic pathway is $(t_2-t_1)/t_2$. Based on this analysis, we confirmed that a large fraction (up to ~80 %) of the electrons formed on the stromal side of PSI were recycled in cyclic flow in fully dark adapted conditions. As expected, this fraction largely decreased during the light transition, (Fig. 4C and D) rapidly attaining a value of ~10–15% in steady state conditions, in agreement with previous suggestions [21,67].

As in the case of the $\Delta\mu_H^+$, we observe a parallel between changes in NPQ and CEF. However, quenching relaxation preceded the activation of linear flow, as indicated by the deviation of the NPQ/CEF profile during the decay of the transmembrane proton motive force from a straight line (panels E and F, dashed lines). As in the case of the NPQ/$\Delta\mu_H^+$ relationship, the effect was more pronounced in WT than in CMSII leaves. Once again, the simplest explanation for this discrepancy would be that CEF is able to generate not only a ΔpH, but also a $\Delta\Psi$, which is however not involved in NPQ generation (see also discussion below).

From these sets of data two conclusions can be drawn: (i) we confirm that cyclic flow is directly modulated by the leaf capacity to assimilate CO_2, as shown by the kinetics of transition from CEF to linear flow. Indeed, the slower activation of CO_2 assimilation in CMSII, as shown by the slower increase in the ΦPSII parameter (Fig. 1C; see also [18]), is accompanied by a longer lifetime of cyclic flow. Moreover, (ii) we observe that the longer lifetime of cyclic flow observed in CMSII correlates with a longer duration of the transient NPQ in this genotype, likely because of the stabilization of the $\Delta\mu_H^+$ in the light.

3.4. The size of the soluble pool of PSI electron acceptors is reduced in CMSII

The changes in the rate of cyclic flow described above can, in principle, account for the delayed relaxation of the $\Delta\mu_H^+$ and of the NPQ shown in Fig. 3. However, they do not explain the reduced capacity to generate a NPQ that is observed in the mutant during the first 30–40 s of illumination (Fig. 1D, grey panel), where the capacity of leaves to perform cyclic flow, as estimated by our spectroscopic approach, is the same in CMSII and WT leaves (Fig. 4A and B).

This suggests that besides CEF, other processes may affect the capacity of mutant leaves to trigger NPQ onset. As stated above, alternative electron flow processes may actively contribute to the

Please cite this article as: P. Cardol, et al., The onset of NPQ and $\Delta\mu_H^+$ upon illumination of tobacco plants studied through the influence of mitochondrial electron transport, Biochim. Biophys. Acta (2009), doi:10.1016/j.bbabio.2009.10.002

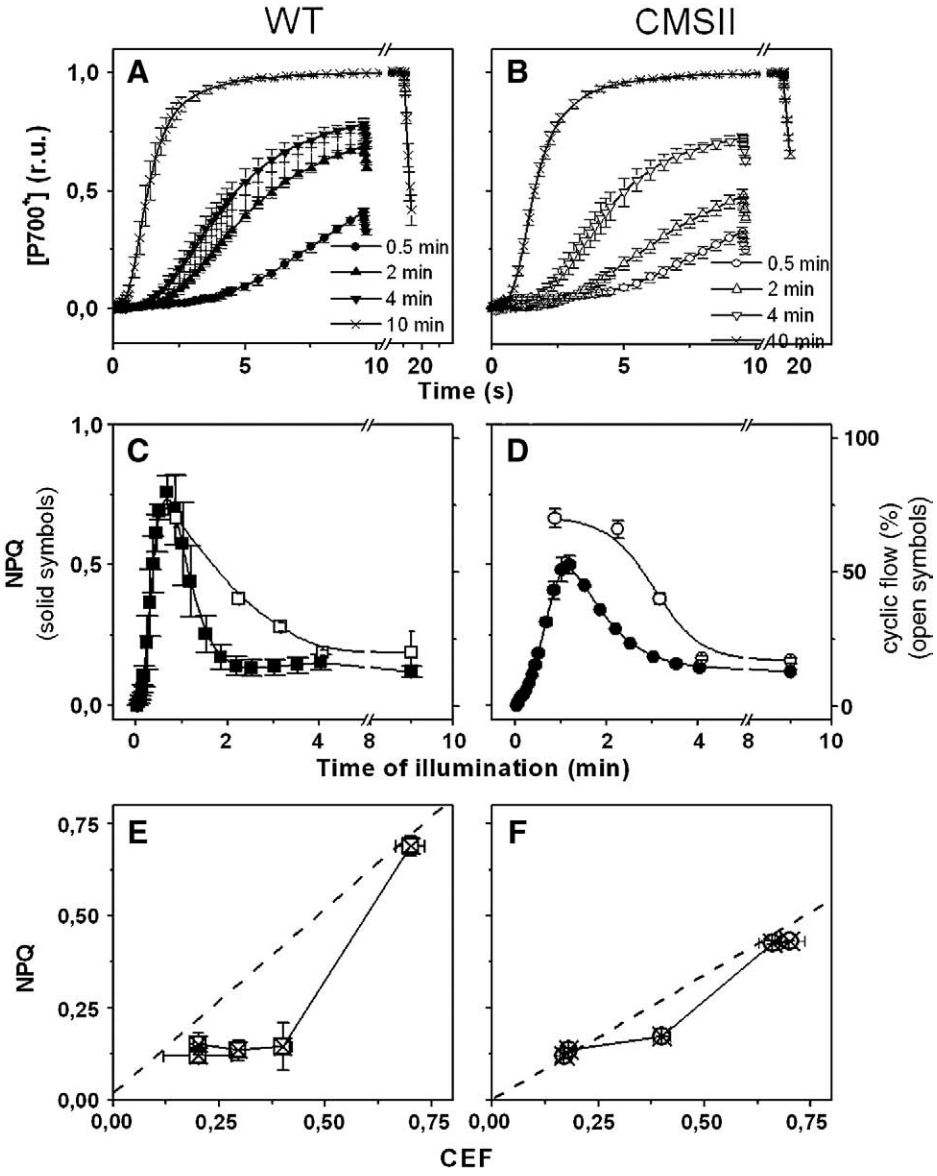

Fig. 4. Relationship between NPQ onset and changes in cyclic electron flow around PSI in WT and CMSII leaves. (A, B) Absorption changes measured at 810–870 nm after different times of green-light illumination. Dark-adapted leaves were subjected to the same light intensity used to induce NPQ generation (150 μmol photons m^{-2} s^{-1}, see Fig. 1). Light was switched off at different times, and P_{700} oxidation was recorded after a short dark adaptation (200 ms) upon exposure to far red light. P_{700} absorption changes are expressed as relative values. (C, D) Relationship between NPQ relaxation in the light, and changes in the extent of cyclic flow. Cyclic flow was estimated as described in the text. (E, F) Relationship between NPQ and cyclic flow during the relaxation phase. The dashed lines represent the expected relationship between the two parameters in case of a concomitant relaxation of CEF and NPQ. Standard errors refer to three independent experiments. Note that very similar relationships were found when changes in cyclic electron flow were computed by measuring the area above the P_{700} oxidation kinetics (i.e. the overall number of P_{700} turnover, [62]) instead of the half time of P_{700} oxidation (not shown).

generation of a light driven $\Delta\mu_H^+$ and therefore to the onset of NPQ, by diverting electrons away from the Calvin cycle, when the latter is not fully active. Among the two possible choices (the Mehler reaction and the exchange of redox equivalents between the chloroplast and the mitochondria), the latter is probably a better candidate. Indeed, both processes are capable of alleviating electron pressure on PSII when the CO_2 assimilation capacity is low (see e.g. [68]), and are expected contribute to lumen acidification in synergy with CEF. However, while a reduced efficiency of redox export from the chloroplast to the mitochondrion [69] could easily account for the reduced capacity of CMSII to evacuate reducing equivalents generated in the light (as discussed in [18]), removal of mitochondrial complex I is in principle not expected to affect directly the Mehler reaction.

In order to estimate a possible modification in the capacity to export redox equivalents out of the chloroplast in CMSII, we assess the total size of the PSI acceptors pool by a fluorescence approach. We followed the regeneration of the variable fluorescence in leaves that were subjected to a short illumination at time 0, and then illuminated with a second pulse after a variable time interval Δt. The rationale for this approach was the following: during the first illumination all the soluble electron carriers are reduced because the Calvin cycle represents a major bottleneck for electron flow [58] see also [70]). Eventually, reduction of the primary PSII quinone acceptor Q_A takes place, promoting an increase of room temperature fluorescence (which is driven by PSII) to the maximum level. Then, oxidation of the acceptors occurs in the dark time between the two consecutive illuminations, due to the activity of the chlororespiratory enzyme PTOX and to the redox re-equilibration between PSI acceptors in the stroma and the mitochondria [71]. This allows a partial oxidation of Q_A, i.e. a fluorescence decrease. A subsequent illumination induces a fluorescence rise (Fig. 5A and B) that reveals the concentration of the carriers reoxidized in the dark (e.g. [71]). This parameter can be

Please cite this article as: P. Cardol, et al., The onset of NPQ and $\Delta\mu_H^+$ upon illumination of tobacco plants studied through the influence of mitochondrial electron transport, Biochim. Biophys. Acta (2009), doi:10.1016/j.bbabio.2009.10.002

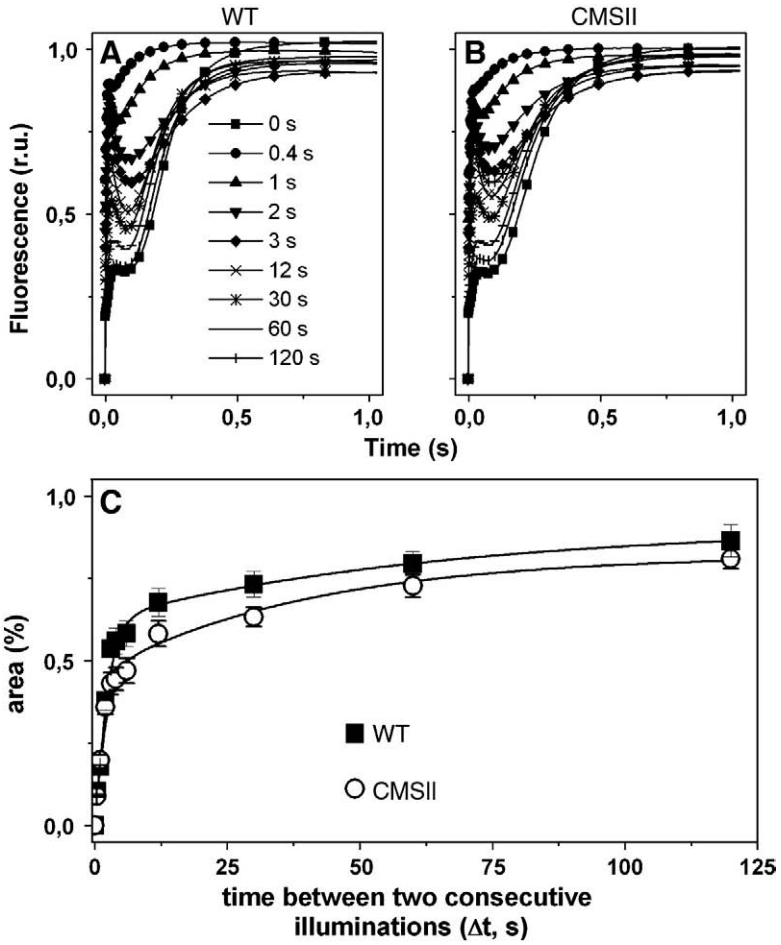

Fig. 5. Fluorescence transients measured in WT (A) and CMSII (B) dark-adapted leaves ($t=0$) after a variable dark time Δt after a 1-s of preillumination with 150 μmol photons m^{-2} s^{-1} (C). Recovery of the bound area above the fluorescence induction curve as a function of the dark period after 1 s illumination. The recovered area has been normalized to the area measured after 5 min of dark. Standard errors refer to three independent experiments. Closed squares: WT. Open circles: CMSII.

quantified by the bound area above the second fluorescence induction curve. A plot of the area as a function of the dark time between the first and the second light pulses (Fig 5C) shows a biphasic kinetic in both WT and CMSII. Following previous suggestions (see [58] for a further discussion), we ascribe the fast phase ($t_{1/2}$ ~ 0.6 s) to the oxidation of the PSI acceptors and the slow phase ($t_{1/2}$ ~ 100 s) to the oxidation of the PQ pool. Fitting the kinetics of Fig. 5C with a double exponential indicates that although the rate of regeneration of the soluble acceptors was similar in the two genotypes, the amplitude of the fast phase is reduced by ~20–30% in CMSII, suggesting a decrease in the concentration of PSI acceptors in this genotype. This possibly is consistent with the diminished capacity of mitochondria to oxidize chloroplast-generated reductants, as proposed in [18].

3.5. Relationship between the transient NPQ and the cellular ATP content

As an alternative interpretation, we considered the possibility that the slower rate of NPQ onset in CMSII may reflect the requirement for a longer time of illumination to attain the ΔpH threshold required for quenching onset in this strain. The rationale behind this idea is that both the dark and light induced $\Delta\mu_H{}^+$can sustain NPQ generation [72]. Thus, quenching is switched on only when the sum of the electrochemical proton gradient established in the dark (due to slow ATP hydrolysis activity of the ATPase-ATP synthase enzyme, [73,74]), and the light induced proton motive force overcome the threshold level of NPQ onset (e.g. [75]). Respiratory defects could diminish the overall cellular ATP content in plants, as already evidenced in green algae [52,76,77], and therefore decrease the size of the chloroplastic $\Delta\mu_H{}^+$ present in the dark, which is in equilibrium with the ATP/ADP + Pi cellular pools [78] Because of the decreased size of the dark $\Delta\mu_H{}^+$, a larger light-induced ΔpH (i.e. a larger number of turnovers or, in other words, a longer illumination time) would be required in the mutant to attain the same NPQ as in the WT.

To test this hypothesis, we first evaluated the overall cellular ATP pool in the two genotypes. Leaves were taken in the dark, after transient illumination or in steady state conditions, and their ATP content was estimated enzymatically in cellular extracts. Table 2 indicates that the ATP content was very similar in dark-adapted CMSII and WT samples. Transient exposure to light induced an ATP increase in both genotypes (higher in CMSII), which we interpret in terms of ATP generation by photosynthesis (mostly CEF), without sustained consumption to fuel CO_2 assimilation. Consistent with this view, activation of photosynthesis upon prolonged illumination decreased the ATP content in both genotypes. Based on these findings, we conclude: (i) that no significant changes in the cellular ATP content are seen in CMSII and wild type leaves, in agreement with previous

Table 2
Cellular ATP content in WT and CMSII leaves in the dark (2 h), upon short illumination with 150 μmol m^{-2} s^{-1} and during steady state illumination (8 h).

ATP content pM/mg FW	WT	CMSII
Dark	29.8 ± 3.2	32.0 ± 5.8
Dark light transition (1 min)	39.2 ± 22.0	56.4 ± 18.0
Light	18.6 ± 3.6	30.7 ± 10.0

Cellular ATP content was assessed in leaf cell extracts as indicated in methods. Means ± SE are shown and refer to 6–12 independent measurements.

Please cite this article as: P. Cardol, et al., The onset of NPQ and $\Delta\mu_H^+$ upon illumination of tobacco plants studied through the influence of mitochondrial electron transport, Biochim. Biophys. Acta (2009), doi:10.1016/j.bbabio.2009.10.002

findings [79]. This unexpected effect of the CMSII mutation on the cellular energy charge can be interpreted, at last partially, by the enhanced activity of the mitochondrial cytochrome electron flow pathway, as previously described [80,81]. In addition, (ii) we also conclude that no apparent relationship can be established between the capacity to develop a transient NPQ and the overall cellular ATP content.

On the other hand, it is known that the chloroplast ATP pool represents between 30% and 50% of the overall cellular amount in plant cells ([82,83] see however [84]). Therefore, we decide to resort to an alternative approach to measure possible changes in the chloroplast ATP content in intact leaves. This is based on the spectroscopic approach recently established by Joliot and Joliot [74]. It consists in estimating the $\Delta\mu_H{}^+$ established in the dark by assessing the maximum ECS signal that is induced by a short saturating illumination (8 ms). Based on the assumption that the $\Delta\mu_H{}^+$ storage capacity of the thylakoids is constant, the sum of the dark- plus the light-induced proton motive force should also be constant. Therefore, any change in the amplitude of the light induced ECS, which reflects the amplitude of the light-generated $\Delta\mu_H{}^+$ (and can be easily measured), would mirror an opposite change in the size of the $\Delta\mu_H{}^+$ present in the dark, which is the parameter we are interested in.

As shown in Fig. 6, illumination generates an ECS signal (i.e. a $\Delta\mu_H{}^+$) in both WT (closed symbols) and CMSII (open symbols) leaves. The light-induced ECS signal was larger in CMSII than in the WT (Fig. 6A). After illumination the ECS signal relaxed to the dark values according to biphasic kinetics. The first phase completed in less than 100 ms has been ascribed to the proton transfer through the ATP synthase, i.e. to ATP synthesis (e.g. [85]). Conversely, the second phase (completed in ~1 s) is related to ions leaks through the membrane, due to inactivation of this complex. The break between the two phases has been interpreted as reflecting the switch of the ATP synthase from an active to an inactive state [85]. To verify if the membrane permeability was the same in the two genotypes, a prerequisite for the evaluation of the light and dark built $\Delta\mu_H^+$, we normalize the ECS kinetics in the two genotypes to the maximum value (Fig. 6B). In this case, both the amplitude and the kinetics of the first phase turned out to be very similar in the WT and in CMSII, suggesting that the chloroplastic ATP synthesis capacity was not modified by the mutation. After normalization, the rate of the slow phase also became similar in both genotypes, as expected if the ion permeability was the same in the two genotypes. Furthermore, their photochemical capacity did not change, as shown by the identical rates of the ECS increase measured in WT and CMSII leaves at the onset of illumination (Fig. 6A). Therefore, the following conclusions can be drawn: (i) the maximum $\Delta\mu_H{}^+$ storage capacity of WT and mutant thylakoids is the same (and therefore their ECS signals can be directly compared). Moreover, (ii) the larger ECS built upon illumination in CMSII (dashed arrows) reflects a decreased size of the electrochemical proton gradient present in the dark (dots). This conclusion is further supported by the finding that the amplitude of the slow phase of the ECS is larger in the mutant, indicating that the $\Delta\mu_H{}^+$ achieved at equilibrium is lower than the one achieved in the WT. Since this $\Delta\mu_H{}^+$ is determined by the stromal ATP/ADP + Pi pools (see above), we conclude that the chloroplast ATP/ADP ratio in the dark is reduced in CMSII.

4. Conclusion

Our data confirm that both the onset and the relaxation of the transient fluorescence quenching observed upon exposure of dark-adapted plants to a few minutes of light are related to changes in the proton motive force in the thylakoid lumen, in agreement with previous suggestions (see e.g. [37,40]). However, our analysis of the relationship between NPQ and the proton motive force shows that quenching relaxation in the light is faster than the decay of the $\Delta\mu_H{}^+$. This apparent contradiction could merely reflect the well established notion that NPQ relaxation follows the relation of the ΔpH, while the $\Delta\mu_H{}^+$ established during illumination comprises a significant contribution of the electric component, the ΔΨ, the relaxation of which could not follow the same kinetics as the that of the ΔpH (e.g. [57]). On the other hand, the almost linear relationship between the relaxation of NPQ and the increase of the ΦPSII parameter, which is representative of the redox state of electron carriers localized downstream of PSII (Fig. 1D, right) suggests that NPQ could also be under the control of the redox state of the electron flow chain. The existence of such a direct redox modulation of NPQ, via changes in the PSII primary electron acceptor Q_A, has been already proposed in the past [86]. However, an indirect effect can also be conceived, due to the existence of a tight relationship between the redox state of the electron flow chain, the occurrence of cyclic flow around PSI and the generation of the $\Delta\mu_H{}^+$.

Altogether, the following series of events can be proposed to account for the kinetics of NPQ onset and relaxation during the onset of light acclimation: upon exposure of dark-adapted leaves to light, the rate of electron flow is expected to rapidly overcome the capacity of CO_2 assimilation, owing to the almost inactive state of the Calvin cycle. Moreover, kinetic limitation by FNR turnover (e.g. [87]) could also affect the overall carbon assimilation capacity. This should lead to

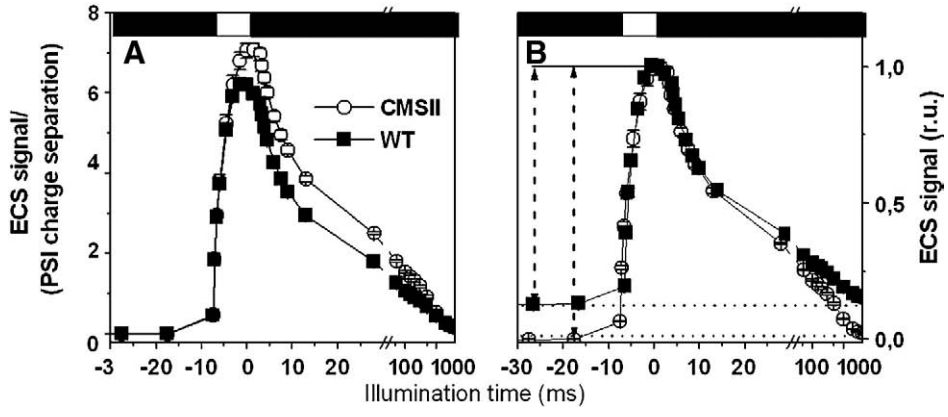

Fig. 6. Light-induced membrane potential changes in WT and CMSII leaves. (A) Membrane potential increase measured during a saturating light-pulse (5000 μmol of photons $m^{-2}\,s^{-1}$, 8 ms duration) given to dark-adapted leaves. ECS data were normalized to the signal corresponding to 1 charge separation per photosystem, i.e. the absorption changes induced by a saturating laser flash in the presence of DCMU (20 μM) and Hydroxylamine (2 mM) to inhibit PSII activity. Closed symbols: WT; open symbols: CMSII. (B) Data normalized to the maximum ECS amplitude. The duration of the pulse was such that the maximum membrane potential level was reached, and the time at which the light was switched off was taken as time zero. Continuous line: maximum membrane potential level. Dashed arrows: light induced ECS increase. Dotted lines: dark level. Standard errors refer to four independent experiments. White box: light on. Black box: light off.

Please cite this article as: P. Cardol, et al., The onset of NPQ and $\Delta\mu_H^+$ upon illumination of tobacco plants studied through the influence of mitochondrial electron transport, Biochim. Biophys. Acta (2009), doi:10.1016/j.bbabio.2009.10.002

over-reduction of the electron carriers and activation of cyclic flow around PSI (e.g. [21,48,59]). Proton release in the lumen, alimented by cyclic flow, would not be counterbalanced by sustained H^+ consumption for ATP turnover in the CO_2 assimilation processes. Therefore NPQ would rise, despite the rather moderate light intensity employed. Longer light exposure is expected to stimulate the Calvin cycle and therefore promote a decrease in the $\Delta\mu_H{}^+$ (as observed, Fig. 3) via the enhanced ATP consumption for CO_2 assimilation. At the same time, activation of the Calvin cycle could also induce a partial reoxidation of PSI acceptors, allowing linear flow to out-compete cyclic flow (CEF). Thus, both the direct ($\Delta\mu_H{}^+$consumption for ATP synthesis) and indirect ($\Delta\mu_H{}^+$ decrease owing to reduced CEF activity) effects on photosynthesis would result in a net NPQ decrease, in agreement with the data of Fig. 1. In the frame of this hypothesis, the slower activation of photosynthesis in CMSII upon illumination (Fig. 1C; [18]) would explain the delayed lifetime of NPQ, because the longer lifetime of CEF (Fig. 4) would maintain a higher $\Delta\mu_H{}^+$ during the first minutes of light acclimation (Fig. 3).

This series of events should provide the leaf with a very simple and efficient feedback loop capable of developing a fast photoprotective response for PSII. Furthermore, the development of such a form of NPQ, which is poorly related to the deepoxidation of Vx to Zx (Table 1), may represent an advantage under the low light conditions explored here, in which quenching must only develop during the first phase of illumination, i.e. when CO_2 assimilation is low. Lack of significant Zx synthesis allows a fast and complete relaxation of the quenching upon activation of the CO_2 assimilatory cycle [39], i.e. as soon as the $\Delta\mu_H{}^+$ is decreased. Conversely, accumulation of this carotenoid, which decreases the ΔpH threshold required for the establishment of fluorescence quenching [88] is likely to stabilize the quenching beyond the transient phase. Owing to its rather long lifetime (reviewed in [25]), presence of Zx in low light would therefore maintain quenching at steady state even upon the consumption of the $\Delta\mu_H{}^+$ by enhanced CO_2 assimilation, as already been reported in the *npq2* mutant of Arabidopsis, which constitutively accumulates Zx in the dark [28,39]. By reducing the number of photons available for photosynthesis at low light, the presence of a Zx-mediated steady state quenching state should reduce the overall photosynthetic performances and therefore affect biomass production, as previously shown in *npq2* [28,39,89]).

While most of the data presented in this work can be rationalized within the model described above, the delayed NPQ generation in CMSII at the onset of illumination (Fig. 1D, grey panel) cannot be explained in terms of changes in the lumen acidification owing to different cyclic flow efficiency. Indeed, CEF is similar in both genotypes at the onset of illumination. The non-linear relationship between linear electron flow (ΦPSII) and NPQ observed at this stage of illumination is reminiscent of previous data obtained in steady-state conditions of illumination in tobacco leaves, when exposed to changing CO_2 concentration conditions [51,90,91]. These results have been previously explained in terms of changes in the proton conductivity of the chloroplast ATPase complex, owing to a possible reduction of the ADP + Pi/ATP ratio by CO_2 limitation. Changes in H^+ flux at the level of the ATPase-ATPsynthase have been ascribed either to a direct effect on ATP synthesis, or to an indirect allosteric control on this enzyme. Independently of the mechanism, reduced ATP-synthase activity should lead to an enhanced ΔpH, and therefore to a larger NPQ (see e.g. [91] for a further discussion). This hypothesis could therefore account for the different relationship between the ΦPSII and the NPQ, which is seen at the beginning of illumination in the WT and CMSII (Fig. 1A, grey panel). However, this possibility is not consistent with our estimates of the total ATP content in CMSII in the light (Table 1), which is the same (or even higher) in both transient and steady state conditions.

Therefore, we consider another hypothesis, where the decreased rate of $\Delta\mu_H{}^+$ generation at the onset of illumination would stem from a slower consumption of reducing equivalents generated during illumination ([18], see also Fig. 5). Alternatively, it can be conceived that in the mutant, part of the ATP generated in the light may be consumed to fuel metabolic processes (e.g. protein synthesis) otherwise fuelled by respiration. In both cases (a reduced cellular ATP content, or the slower rate of electron flow, i.e. a slower capacity to generate a proton motive force in the light) a delayed NPQ onset can be conceived. These effects in the light, plus the reduced size of the $\Delta\mu_H{}^+$ established in the dark in CMSII (owing to reduced ATP content) would fully account for both the reduced size, and the reduced rate of NPQ generation in the mutant. Moreover, this hypothesis would be consistent with previous observations of higher malate and NADPH concentrations in the mutant [16]. The finding that the activity of the stromal NADP-malate dehydrogenase remains high throughout the induction period of photosynthesis [18] is also consistent with this hypothesis. While the NADP-malate dehydrogenase in the WT showed an initial rise and a subsequent fall during the induction of carbon assimilation, in parallel with leaf NADPH/NADP [92], this activity was rapidly induced in CMSII and remained high even when the rate of net CO_2 fixation approached that of the WT, suggesting an increased reduction state of the stroma because of insufficient electron sinks outside the chloroplast [92].

Finally, this hypothesis would also provide a rationale for the decreased NPQ response that is observed in myxothiazol treated CMSII leaves (Fig. 2) where both the respiratory complexes I and III are blocked. In myxothiazol-treated CMSII leaves the only pathways for electron flow operating would be thus from alternative NAD(P)H deshydogenases and complex II to the alternative oxidase(s). This would further reduce their capacity to oxidize reducing equivalents generated by the chloroplast (reviewed in [24]), and therefore diminish the capacity of this organelle to build a NPQ response.

In conclusion, our comparative analysis of the photosynthetic induction in the WT and the CMSII mutant of *N. sylvestris* allows to address two major aspects of the regulation of light acclimation in plants: (i) the role of the transthylakoidal electrochemical potential and of cyclic electron flow in regulating the transient NPQ, which develops at the onset of photosynthetic activity and, (ii) the role of respiration in balancing light utilization and carbon assimilation, by controlling the cellular redox poise.

Acknowledgements

M. Radoux is thanked for technical assistance. Thanks are due to Pierre Joliot for his contribution during the early phase of this study, and to Giles Johnson (University of Manchester) for use of instruments to measure CO_2 assimilation. Research was supported by the Belgian "Fonds National de la Recherche Scientifique" (FNRS) (F.4735.06) to PC, the Paris-Sud XI University, and the French "Centre National de la Recherche Scientifique" (CNRS) to GF and RDP. PC and FF are research associate and senior research associate of the FNRS, respectively.

References

[1] R.G. Jensen, J.A. Bassham, Photosynthesis by isolated chloroplasts, Proc. Natl. Acad. Sci. U. S. A. 56 (1966) 1095–1101.
[2] P. Bennoun, Chlororespiration: sixteen years later, in: J.-D. Rochaix, M. Goldschmidt-Clermont, S. Merchant (Eds.), The Molecular Biology of Chloroplast and Mitochondria in Chlamydomonas, Kluwer Academic Publishers. Dordrecht, The Netherlands, 1998, pp. 529–537.
[3] G. Peltier, L. Cournac, Chlororespiration, Annu. Rev. Plant Biol. 53 (2002) 523–550.
[4] S. Krömer, Respiration during photosynthesis, Annu. Rev. Plant. Physiol. Plant. Mol. Biol. 46 (1995) 45–70.
[5] M.H.N. Hoefnagel, O.K. Atkin, J.T. Wiskich, Interdependence between chloroplasts and mitochondria in the light and the dark, Biochim. Biophys. Acta 1366 (1998) 235–255.
[6] P. Gardeström, A.U. Igamberdiev, A.S. Raghavendra, Mitochondrial functions in the light and significance to carbon-nitrogen interactions, in: C.H. Foyer, G. Noctor (Eds.), Photosynthetic nitrogen assimilation and associated carbon and

Please cite this article as: P. Cardol, et al., The onset of NPQ and $\Delta\mu_H^+$ upon illumination of tobacco plants studied through the influence of mitochondrial electron transport, Biochim. Biophys. Acta (2009), doi:10.1016/j.bbabio.2009.10.002

respiratory metabolism. Advances in Photosynthesis, vol. 12, Kluwer Academic Press, Dordrecht, the Netherlands, 2002, pp. 151–172.
[7] L.E. Fridlyand, J.E. Backhausen, R. Scheibe, Flux control of the malate valve in leaf cells, Arch. Biochem. Biophys. 349 (1998) 290–298.
[8] S. Krömer, M. Stitt, H.W. Heldt, Mitochondrial oxidative phosphorylation participating in photosynthetic metabolism of a leaf cell, FEBS Lett. 226 (1988) 352–356.
[9] S. Krömer, G. Malmberg, P. Gardeström, Mitochondrial contribution to hotosynthetic metabolism. A study with barley (*Hordeum vulgare* L.) leaf protoplasts at different light intensities and CO_2 concentrations, Plant Physiol. 102 (1993) 947–955.
[10] R. Douce, M. Neuburger, The uniqueness of plant mitochondria, Annu. Rev. Plant Physiol. Plant Mol. Biol. 40 (1989) 371–414.
[11] S. Gutierres, M. Sabar, C. Lelandais, P. Chétrit, P. Diolez, H. Degand, M. Boutry, F. Vedel, Y. de Kouchkovsky, R. De Paepe, Lack of mitochondrial and nuclear encoded subunits of complex I and alteration of respiratory chain in *Nicotiana sylvestris* mitochondrial deletion mutants, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 3436–3441.
[12] M. Sabar, R. De Paepe, Y. de Kouchkovsky, Complex I impairment, respiratory compensations and photosynthetic decrease in nuclear and mitochondrial male sterile mutants of *Nicotiana sylvestris*, Plant Physiol. 124 (2000) 1239–1250.
[13] G. Noctor, C. Dutilleul, R. De Paepe, C.H. Foyer, Use of mitochondrial electron transport mutants to evaluate the effects of redox state on photosynthesis, stress tolerance and the integration of carbon/nitrogen metabolism, J. Exp. Bot. 55 (2004) 49–57.
[14] G. Noctor, R. De Paepe, C.H. Foyer, Mitochondrial redox biology and homeostasis in plants, Trends Plant Sci. 12 (2007) 125–134.
[15] C. Dutilleul, M. Garmier, G. Noctor, C. Mathieu, P. Chétrit, C.H. Foyer, R. de Paepe, Leaf mitochondria modulate whole cell redox homeostasis, set antioxidant capacity, and determine stress resistance through altered signaling and diurnal regulation, Plant Cell 15 (2003) 1212–1226.
[16] C. Dutilleul, C. Lelarge, J.L. Prioul, R. De Paepe, C.H. Foyer, G. Noctor, Mitochondria-driven changes in leaf NAD status exert a crucial influence on the control of nitrate assimilation and the integration of carbon and nitrogen metabolism, Plant Physiol. 139 (2005) 64–78.
[17] P. Priault, C. Fresneau, G. Noctor, R. De Paepe, G. Cornic, P. Streb, The mitochondrial CMSII mutation of *Nicotiana sylvestris* impairs adjustment of photosynthetic carbon assimilation to higher growth irradiance, J. Exp. Bot. 57 (2006) 2075–2085.
[18] C. Dutilleul, S. Driscoll, G. Cornic, R. De Paepe, C.H. Foyer, G. Noctor, Functional mitochondrial complexI , is required by tobacco leaves for optimal photosynthetic performance in photorespiratory conditions and during transients, Plant Physiol. 131 (2003) 264–275.
[19] D.R. Ort, N.R. Baker, A photoprotective role for O_2 as an alternative electron sink in photosynthesis? Curr. Opin. Plant Biol. 5 (2002) 193–198.
[20] G.N. Johnson, Cyclic electron transport in C3 plants: fact or artefact? J. Exp. Bot. 56 (2005) 407–416.
[21] P. Joliot, A. Joliot, Cyclic electron flow in C3 plants, Biochim. Biophys. Acta 1757 (2006) 362–368.
[22] A.M. Mehler, Studies on reactions of illuminated chloroplasts. I. Mechanism of the reduction of oxygen and other Hill reagents, Arch. Biochem. Biophys. 33 (1951) 65–77.
[23] K. Noguchi, K. Yoshida, Interaction between photosynthesis and respiration in illuminated leaves, Mitochondrion 8 (2008) 87–99.
[24] P. Horton, A.V. Ruban, R.G. Walters, Regulation of light harvesting in green plants, Annu. Rev. Plant. Physiol. Plant Mol. Biol. 47 (1996) 655–684.
[25] B. Demmig-Adams, A.M. Gilmore, W.W. Adams, Carotenoids 3: *in vivo* function of carotenoids in higher plants, Faseb J. 10 (1996) 403–412.
[26] K.K. Niyogi, PHOTOPROTECTION REVISITED: genetic and molecular approaches, Annu. Rev. Plant Physiol. Plant Mol. Biol. 50 (1999) 333–359.
[27] E.M. Aro, I. Virgin, B. Andersson, Photoinhibition of photosystem II. Inactivation, protein damage and turnover, Biochim. Biophys. Acta 1143 (1993) 113–134.
[28] L. Dall'Osto, S. Caffarri, R. Bassi, A mechanism of nonphotochemical energy dissipation, independent from PsbS, revealed by a conformational change in the antenna protein CP26, Plant Cell 17 (2005) 1217–1232.
[29] J.F. Allen, Protein phosphorylation in regulation of photosynthesis, Biochim. Biophys. Acta 1098 (1992) 275–335.
[30] C.A. Wraight, A.R. Crofts, Energy-dependent quenching of chlorophyll alpha fluorescence in isolated chloroplasts, Eur. J. Biochem. 17 (1970) 319–327.
[31] I. Baroli, K.K. Niyogi, Molecular genetics of xanthophyll-dependent photoprotection in green algae and plants, Philos. Trans. R. Soc. Lond. B Biol. Sci. 355 (2000) 1385–1394.
[32] N.E. Holt, G.R. Fleming, K.K. Niyogi, Toward an understanding of the mechanism of nonphotochemical quenching in green plants, Biochemistry 43 (2004) 8281–8289.
[33] B. Demmig-Adams, W.W. Adams, U. Heber, S. Neimanis, K. Winter, A. Krüger, F.C. Czygan, W. Bilger, O. Björkman, Inhibition of zeaxanthin formation and of rapid changes in radiationless energy dissipation by dithiothreitol in spinach leaves and chloroplasts, Plant Physiol. 92 (1990) 293–301.
[34] H.Y. Yamamoto, T.O. Nakayama, C.O. Chichester, Studies on the light and dark interconversions of leaf xanthophylls, Arch. Biochem. Biophys. 97 (1962) 168–173.
[35] X.P. Li, O. Björkman, C. Shih, A.R. Grossman, M. Rosenquist, M.S. Jansson, K.K. Niyogi, A pigment-binding protein essential for regulation of photosynthetic light harvesting, Nature 403 (2000) 391–395.
[36] X.P. Li, A.M. Gilmore, S. Caffarri, R. Bassi, T. Golan, D. Kramer, K.K Niyogi, Regulation of photosynthetic light harvesting involves intrathylakoid lumen pH sensing by the PsbS protein, J. Biol. Chem. 279 (2004) 22866–22874.
[37] G. Finazzi, G.N. Johnson, L. Dall'Osto, P. Joliot, F.A. Wollman, R. Bassi, A zeaxanthin-independent nonphotochemical quenching mechanism localized in the photosystem II core complex, Proc. Natl. Acad. Sci U. S. A. 101 (2004) 12375–12380.
[38] S. Crouchman, A.V. Ruban, P. Horton, PsbS enhances nonphotochemical fluorescence quenching in the absence of zeaxanthin, FEBS Lett. 580 (2006) 2053–2058.
[39] L. Kalituho, K.C. Beran, P. Jahns, The transiently generated nonphotochemical quenching of excitation energy in Arabidopsis leaves is modulated by zeaxanthin, Plant Physiol. 143 (2007) 1861–1870.
[40] Y. Munekage, M. Hojo, J. Meurer, T. Endo, M. Tasaka, T. Shikanai, PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in Arabidopsis, Cell 110 (2002) 361–371.
[41] A.J. Golding, G.N. Johnson, Down regulation of linear and activation of cyclic electron transport during drought, Planta 218 (2003) 107–114.
[42] C. Miyake, M. Miyata, Y. Shinzaki, K. Tomizawa, CO_2 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 Cell Physiol. 46 (2005) 629–637.
[43] J. Melkonian, D.W. Wolfe, T.G. Owens, Effects of elevated carbon dioxide on gas exchange and photochemical and nonphotochemical quenching at low temperature in tobacco plants varying in Rubisco activity, Photosynth. Res. 83 (2005) 63–74.
[44] C. Külheim, J. Agren, S. Jansson, Rapid regulation of light harvesting and plant fitness in the field, Science 297 (2002) 91–93.
[45] D.M. Kramer, J.A. Cruz, A. Kanazawa, Balancing the central roles of the thylakoid proton gradient, Trends Plant Sci. 8 (2003) 27–32.
[46] K. Takizawa, J.A. Cruz, A. Kanazawa, D.M. Kramer, The thylakoid proton motive force *in vivo*. Quantitative, non-invasive probes, energetics, and regulatory consequences of light-induced pmf, Biochim. Biophys. Acta 1767 (2007) 1233–1244.
[47] P. Joliot, D. Béal, A. Joliot, Cyclic electron flow under saturating excitation of dark-adapted Arabidopsis leaves, Biochim. Biophys. Acta 1656 (2004) 166–176.
[48] C. Breyton, B. Nandha, G.N. Johnson, P. Joliot, G. Finazzi, Redox modulation of cyclic electron flow around photosystem I in C3 plants, Biochemistry 45 (2006) 13465–13475.
[49] D. Siefermann, H.Y. Yamamoto, Properties of NADPH and oxygen-dependent zeaxanthin epoxidation in isolated chloroplasts: a transmembrane model for the violaxanthin cycle, Arch. Biochem. Biophys. 171 (1975) 70–77.
[50] U. Heber, Conformational changes of chloroplasts induced by illumination of leaves in vivo, Biochim. Biophys. Acta 180 (1969) 302–319.
[51] T.J. Avenson, J.A. Cruz, D.M. Kramer, Modulation of energy-dependent quenching of excitons in antennae of higher plants, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 5530–5535.
[52] P. Cardol, G. Gloire, M. Havaux, C. Remacle, R. Matagne, F. Franck, Photosynthesis and state transitions in mitochondrial mutants of *Chlamydomonas reinhardtii* affected in respiration, Plant Physiol. 133 (2003) 2010–2020.
[53] G. Von Jagow, T.A. Link, Use of specific inhibitors on mitochondrial bc1 complex, Methods Enzymol. 126 (1986) 253–271.
[54] B. Genty, J. Harbinson, J.-M. Briantais, N.R. Baker, The relationship between non-photochemical quenching of chlorophyll fluorescence and the rate of photosystem 2 photochemistry in leaves, Photosynth. Res. 25 (1990) 249–257.
[55] J.M Briantais, C. Vernotte, M. Picaud, G.H. Krause, A quantitative study of the slow decline of chlorophyll a fluorescence in isolated chloroplasts, Biochim. Biophys. Acta 548 (1979) 128–138.
[56] H.T. Witt, Energy conversion in the functional membrane of photosynthesis. Analysis by light pulse and electric pulse methods. The central role of the electric field, Biochim. Biophys. Acta 505 (1979) 355–427.
[57] J.A. Cruz, C.A. Sacksteder, A. Kanazawa, D.M. Kramer, Contribution of electric field (Δ_Ψ) to steady-state transthylakoid proton motive force (pmf) in vitro and in vivo. Control of pmf parsing into Δ_Ψ and ΔpH by ionic strength, Biochemistry 40 (2001) 1226–1237.
[58] P. Joliot, A. Joliot, Cyclic electron transfer in plant leaf, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 10209–10214.
[59] J.F. Allen, Photosynthesis of ATP-electrons, proton pumps, rotors, and poise,, Cell 110 (2002) 273–276.
[60] U. Heber, D. Walker, Concerning a dual function of coupled cyclic electron transport in leaves, Plant Physiol. 100 (1992) 1621–1626.
[61] Y.J. Liu, F.E. Norberg, A. Szilágyi, R. De Paepe, H.E. Akerlund, A.G. Rasmusson, The mitochondrial external NADPH dehydrogenase modulates the leaf NADPH/$NADP^+$ ratio in transgenic *Nicotiana sylvestris*, Plant Cell Physiol. 49 (2008) 251–263.
[62] P. Joliot, A. Joliot, Quantification of cyclic and linear flows in plants, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 4913–4918.
[63] B. Nandha, G. Finazzi, P. Joliot, S. Hald, G.N. Johnson, The role of PGR5 in the redox poising of photosynthetic electron transport, Biochim. Biophys. Acta 1767 (2007) 1252–1259.
[64] G. DalCorso, P. Pesaresi, S. Masiero, E. Aseeva, D. Schünemann, G. Finazzi, P. Joliot, R. Barbato, D. Leister, A complex containing PGRL1 and PGR5 is involved in the switch between linear and cyclic electron flow in Arabidopsis, Cell 132 (2008) 273–285.
[65] K. Siebke, A. Laisk, S. Neimanis, U. Heber, Evidence that NADP-dependent glyceraldehydephosphate dehydrogenase, but not ferredoxin-NADP reductase, controls electron flow to phosphoglycerate in the dark–light transition, Planta 185 (1991) 337–343.

Please cite this article as: P. Cardol, et al., The onset of NPQ and $\Delta\mu_H^+$ upon illumination of tobacco plants studied through the influence of mitochondrial electron transport, Biochim. Biophys. Acta (2009), doi:10.1016/j.bbabio.2009.10.002

[66] T. Joët, B. Genty, E.M. Josse, M. Kuntz, L. Cournac, G. Peltier, Involvement of a plastid terminal oxidase in plastoquinone oxidation as evidenced by expression of the *Arabidopsis thaliana* enzyme in tobacco, J. Biol. Chem. 277 (2002) 31623–31630.
[67] T.J. Avenson, J.A. Cruz, A. Kanazawa, D.M. Kramer, Regulating the proton budget of higher plant photosynthesis, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 9709–9713.
[68] A. Laisk, H. Eichelmann, V. Oja, E. Talts, R. Scheibe, Rates and roles of cyclic and alternative electron flow in potato leaves, Plant Cell Physiol. 48 (2007) 1575–1588.
[69] R. Scheibe, R, NADP-malate dehydrogenase in C3 plants: regulation and role of a light-activated enzyme, Physiol. Plant. 71 (1987) 393–400.
[70] G. Schansker, S.Z. Tóth, R.J. Strasser, Methylviologen and dibromothymoquinone treatments of pea leaves reveal the role of photosystem I in the Chl a fluorescence rise OJIP, Biochim. Biophys. Acta 1706 (2005) 250–261.
[71] P. Bennoun, Evidence for a respiratory chain in the chloroplast, Proc. Natl. Acad. Sci. U. S. A. 79 (1982) 4352–4356.
[72] A.M. Gilmore, H.Y. Yamamoto, Dark induction of zeaxanthin-dependent non-photochemical fluorescence quenching mediated by ATP, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 1899–1903.
[73] B. Diner, P. Joliot, Effect of the transmembrane electric field on the photochemical and quenching properties of photosystem II in vivo, Biochim. Biophys. Acta 423 (1976) 479–498.
[74] P. Joliot, A. Joliot, Quantification of the electrochemical proton gradient and activation of ATP synthase in leaves, Biochim. Biophys. Acta 1777 (2008) 676–683.
[75] E.E. Pfundel, M. Renganathan, A.M. Gilmore, H.Y. Yamamoto, R.A. Dilley, Intrathylakoid pH in isolated pea chloroplasts as probed by violaxanthin deepoxidation, Plant. Physiol. 106 (1994) 1647–1658.
[76] L. Bulté, P. Gans, F. Rebeillé, F.A. Wollman, ATP control on state transitions *in vivo* in *Chlamydomonas reinhardtii*, Biochim. Biophys. Acta 1020 (1990) 72–80.
[77] G. Forti, A. Furia, P. Bombelli, G. Finazzi, *In vivo* changes of the oxidation-reduction state of NADP and of the ATP/ADP cellular ratio linked to the photosynthetic activity in *Chlamydomonas reinhardtii*, Plant Physiol. 132 (2003) 1464–1474.
[78] G. Finazzi, F. Rappaport, *In vivo* characterization of the electrochemical proton gradient generated in darkness in green algae and its kinetics effects on the cytochrome b_6f turnover, Biochemistry 37 (1998) 9999–10005.
[79] B. Szal, Z. Dabrowska, G. Malmberg, P. Gardeström, A.M. Rychter, Changes in energy status of leaf cells as a consequence of mitochondrial genome rearrangement, Planta 227 (2008) 697–706.
[80] P. Priault, G. Vidal, R. De Paepe, M. Ribas-Carbo, Leaf age-related changes in respiratory pathways are dependent on Complex I activity in *Nicotiana sylvestris*, Physiol. Plant 139 (2007) 64–78.
[81] G. Vidal, M. Ribas-Carbo, M. Garmier, G. Dubertret, A.G. Rasmusson, C. Mathieu, C.H. Foyer, R. De Paepe, Lack of respiratory chain Complex I impairs AOX engagement and modulates redox signaling during elicitor-induced cell death in tobacco, Plant Cell 19 (2007) 640–655.
[82] M. Stitt, R.M. Lilley, H.W. Heldt, Adenine nucleotide levels in the cytosol, chloroplasts, and mitochondria of wheat leaf protoplasts, Plant Physiol. 70 (1982) 971–977.
[83] S. Krömer, H.W. Heldt, On the role of mitochondrial oxidative phosphorylation in photosynthesis metabolism as studied by the effect of oligomycin on photosynthesis in protoplasts and leaves of barley (*Hordeum vulgare*), Plant. Physiol. 95 (1991) 1270–1276.
[84] P. Gardeström, B. Wigge, Influence of photorespiration on ATP/ADP ratios in the chloroplasts, mitochondria, and cytosol, studied by rapid fractionation of Barley (*Hordeum vulgare*) protoplasts, Plant Physiol. 88 (1988) 69–76.
[85] W. Junge, B. Rumberg, H. Schröder, the necessity of an electric potential difference and its use for photophosphorylation in short flash groups, Eur. J. Biochem. 14 (1970) 575–581.
[86] E. Weis, J. Berry, Quantum efficiency of photosystem II in relation to energy-dependent quenching of chlorophyll fluorescence, Biochim. Biophys. Acta 894 (1987) 198–208.
[87] J.F. Palatnik, V.B. Tognetti, H.O. Poli, R.E. Rodríguez, N. Blanco, M. Gattuso, M.R. Hajirezaei, U. Sonnewald, E.M. Valle, N. Carrillo, Transgenic tobacco plants expressing antisense ferredoxin-NADP(H) reductase transcripts display increased susceptibility to photo-oxidative damage, Plant J. 35 (2003) 332–341.
[88] P. Horton, A.V. Ruban, M. Wentworth, Allosteric regulation of the light-harvesting system of photosystem II, Philos. Trans. R. Soc. Lond. B Biol. Sci. 355 (2000) 1361–1370.
[89] L. Kalituho, J. Rech, P. Jahns, The roles of specific xanthophylls in light utilization, Planta 225 (2007) 423–439.
[90] A. Kanazawa, D.M. Kramer, *In vivo* modulation of non photochemical exciton quenching (NPQ) by regulation of the chloroplast ATP synthase, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 12789–12794.
[91] J.A. Cruz, T.J. Avenson, A. Kanazawa, K. Takizawa, G.E. Edwards, D.M. Kramer, Plasticity in light reactions of photosynthesis for energy production and photoprotection, J. Exp. Bot. 56 (2005) 395–406.
[92] C.H. Foyer, M. Lelandais, J. Harbinson, Control of the quantum efficiencies of photosystems I and II, electron flow, and enzyme activation following dark-to-light transitions in pea leaves, Plant Physiol. 99 (1992) 979–986.
[93] W.L. Butler, Energy distribution in the photochemical apparatus of photosynthesis, Annu. Rev. Plant Physiol. 29 (1978) 345–378.
[94] W. Bilger, O. Björkman, Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*, Photosynth. Res. 25 (1990) 173–186.

Please cite this article as: P. Cardol, et al., The onset of NPQ and $\Delta\mu_H^+$ upon illumination of tobacco plants studied through the influence of mitochondrial electron transport, Biochim. Biophys. Acta (2009), doi:10.1016/j.bbabio.2009.10.002