Disentangling chloroplast ATP synthase regulation by proton motive force and thiol modulation in Arabidopsis leaves

Felix Buchert1, 2, Benjamin Bailleul1 and Pierre Joliot1

1Laboratory of Chloroplast Biology and Light-Sensing in Microalgae - UMR7141, IBPC, CNRS-Sorbonne Université, Paris, France.

2 Institute of Plant Biology and Biotechnology, University of Münster, Schlossplatz 8, 48143 Münster, Germany.

Supporting Information – Supplementary Figures S1-S8

Supplementary Table 1

Supplementary discussion

Supplemental reference list

Supplementary Figure 1



Figure S1: Decay of electrochromic shift signals measured at 520 nm (dark squares) and 546 nm (red circles), respectively, after a 12-ms saturating light pulse on a logarithmic time scale. The differential signals is shown in blue triangles and, given that the ΔI/I level at 10 ms darkness served as a reference for our study, is normalized to the 520 nm at 10-ms (magenta downward triangle). The very minor deviations in the kinetics (magenta vs dark) supported the validity of single-wavelength measurements when using short pulses in studies of the dark dynamics.

Supplementary Figure 2

Ein Bild, das Sport, Sportwettkampf enthält.

Mit sehr hoher Zuverlässigkeit generierte Beschreibung

Figure S2: Conservation of physiological properties upon infiltration of leaf samples. ΔI/I signals dropped by ~30% upon infiltration due to longer light trajectory in non-infiltrated samples. However, once the signals were normalized to the ECS increase following a saturating laser flash (see Methods), the behavior with (open red) and without (close dark) water infiltration were very similar, following a single turnover flash (triangles) or a saturating pulse in fully dark-adapted states (squares). This was also true after applying a saturating pulse to samples that were pre-illuminated for 30-s and kept in the dark for 3-min (circles).

Supplementary Figure 3



Figure S3: Analysis of the initial decay kinetics upon generating a maximum membrane potential by light pulses of various intensity. The ECS decay rates in the first 10 ms of darkness (gray area, termed leak phase) depended on the intensity of the light pulse. We assume that the plateau value was determined by H+ leaks that were triggered above a critical potential (leak). Above this potential, the rate of ATP synthesis at the level of the F1 complex might become a rate limiting process, inducing temporary decoupling of H+ translocation from ATP synthesis. As shown previously for spinach [cf. Fig. 1 in supplemental reference 1], the decay kinetics after 10 ms were identical and reflected H+ translocation via CF1Fo.

Supplementary Figure 4



Figure S4: The effect of mitochondrial inhibitors and uncouplers on the dark. (A) Comparison of the pulse-induced ECS kinetics in three treatments, nigericin (black squares), nigericin + nonactin (red circles) and FCCP (blue triangles) demonstrate similar dark. The three curves were obtained on leaves from the same plant and digits in parenthesis indicate the duration of the pulse in ms. When the ΔΨ10ms is set to 0, the dark is visualized as dashed lines, which is the ECS value obtained after ~1 min darkness. (B) Representative example of comparison between antimycin-A and nigericin treatment on leaves from the same plant. The similar values of dark indicate a low AOX activity. (C) Representative example of comparison between antimycin-A and nigericin treatment on leaves from another plant. The very high dark in AA-infiltrated leaf indicates a high AOX activity.

Supplementary Figure 5

Figure S5: Typical kinetics of (A) NPQ and (B) ΦPSII following a dark-to-light transition in leaves of Arabidopsis wild type (red) and *gamera* mutant (black). During illumination of the mutant, a prolonged build-up of NPQ and a delayed activation of photosynthesis was observed.

Supplementary Figure 6



Figure S6: Pulse-induced ECS decay kinetics are shown to indicate that the effect by TCEP infiltration (50 mM) was already fully established before the light treatment (Figure 3A) and remained stable afterwards. Dark-adapted (D) leaves were illuminated (L) for 30 s (green-orange LED, 1000 µmol photons m-2 s-1), followed by a dark re-adaptation for tens of minutes until reaching a dark (lines on the right) close to the initial level. The small differences in the ECS decay kinetics were comparable to individual differences in water-infiltrated WT and *gamera*, supported by rate constants (in parenthesis) calculated as described in Figure 5B of the main text.

Supplementary Figure 7



Figure S7: The ECS relaxation kinetics in the *gamera* mutant and TCEP-reduced WT are shown where the ΔΨ10ms values of all curves were set to 0. The value reached after 1 min relaxation is indicated with three dots on the right. The non-infiltrated *gamera* curves show the unusual dark developments after an illumination (filled squares, see also Fig 3A in the main text). Compared to WT TCEP samples (black), the mutant produced a slower decay phase in the presence of antimycin-A (AA), indicating a smooth inactivation transition in the fully reduced state when the dark is low. In the latter conditions, WT TCEP/AA samples displayed an abrupt cessation of ECS decay (see Figure 5A and possible explanations that are discussed in the main text).

Supplementary Figure 8



Figure S8: A results comparison of *in vitro* and *in vivo* thiol modulation is shown, using spinach chloroplasts and *Arabidopsis thaliana* (WT/*gamera*) plants. (A) The digitized plot from spinach [2] shows the reduced ATP synthase in red color, and the oxidized form is in black. The ΔpH values where half-maximal rates were obtained are highlighted by the dashed lines. (B) The conversion of the data from panel A in membrane potential (ΔΨ) equivalents is shown (1 ΔpH = 59 mV ΔΨ), as well as the ECS results converted from Fig 5A in the main text. The estimated ECS-based values relied on assumptions that 1 charge separation/PSI produced a ΔΨ of 20 mV [3-5]. An additional source of uncertainty was that the lowest dark-equilibrated dark obtained was significantly higher than the absolute minimum (dark = 0) used as the *in vitro* reference.

Supplemental discussion

To determine the best protocol to collapse the dark in chloroplasts, we compared different pharmacological treatments (Fig S4). A very potent dark inhibition was achieved by membrane-embedded chemicals which modify the electric permeability but hinder CF1Fo characterization by altering fast and slow ECS decay kinetics.

Fig S4 displays various inhibitors that affect the dark. The shown ECS decay kinetics after a pulse in dark-adapted leaves were obtained using two treatments which should fully collapse the dark: Nonactin is a specific K+ transporter which should remove both the ΔΨ and ΔpH across the thylakoid in the dark in combination with nigericin (Nig), as should FCCP which is an uncoupler. The dark-adapted values of the were very similar between the two treatments as well as with the treatment with Nig alone, indicating that Nig was sufficient in this experiment to suppress the mitochondrial ATP production and, in turn, the dark. However, it is important to note that the pulse-induced had a very long lifetime in Nig-infiltrated leaves (~45 - 60 s). This reflected, in the absence of mitochondrial ATP supply, the interplay of Nig-dependent ΔpH/ΔΨ exchange and the consumption of the ATP generated during the pulse. We then compared antimycin-A (AA) treatment to the Nig treatment and obtained variable results in different plants. In some cases (Fig S4B), the dark-adapted value of the in AA was very similar to the one in Nig, indicating that AA inhibited most of the mitochondrial ATP production whereas in other cases (Fig S4C) Nig and AA treatments gave very different results, suggesting that the activity of the alternative oxidase was still significant [6]. We are aware of this AA unpredictability and besides the slight variance between WT samples, concentration-dependent AA effects may also tie in with chloroplast ATP balancing genotypes as seen in the *gamera* mutant (Figs 3A and 5A in the main text).

To conclude, when one wants to express dark in absolute values (i.e. to measure the “total volume” of the opaque bottle), the reference for dark = 0 should be obtained with Nig, which suppresses mitochondrial ATP production, or with thylakoid uncouplers collapsing the dark across the thylakoid (e.g. FCCP or the combination of Nig and nonactin). At the contrary, a proper kinetic characterization of CF1Fo at low dark should preferably be performed in the presence of AA.

Supplemental reference list

[1] P. Joliot, A. Joliot, Quantification of the electrochemical proton gradient and activation of ATP synthase in leaves, Biochim. Biophys. Acta, 1777 (2008) 676-683.

[2] U. Junesch, P. Gräber, Influence of the redox state and the activation of the chloroplast ATP synthase on proton-transport-coupled ATP synthesis hydrolysis, Biochim. Biophys. Acta, 893 (1987) 275-288.

[3] W. Schliephake, W. Junge, H.T. Witt, Correlation between field formation, proton translocation, and the light reactions in photosynthesis, Z Naturforsch B, 23 (1968) 1571-1578.

[4] A.A. Bulychev, W.J. Vredenberg, Effect of ionophores A23187 and nigericin on the light-induced redistribution of Mg2+, K+ and H+ across the thylakoid membrane, Biochim. Biophys. Acta, 449 (1976) 48-58.

[5] 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.

[6] B. Bailleul, N. Berne, O. Murik, D. Petroutsos, J. Prihoda, A. Tanaka, V. Villanova, R. Bligny, S. Flori, D. Falconet, A. Krieger-Liszkay, S. Santabarbara, F. Rappaport, P. Joliot, L. Tirichine, P.G. Falkowski, P. Cardol, C. Bowler, G. Finazzi, Energetic coupling between plastids and mitochondria drives CO2 assimilation in diatoms, Nature, 524 (2015) 366-369.