## Interaction between starch breakdown, acetate assimilation and photosynthetic cyclic electron flow in *Chlamydomonas reinhardtii*\*

## Xenie Johnson, Jean Alric

From the UMR 7141, CNRS et Université Pierre et Marie Curie (Paris VI), Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie 75005 Paris, France

**Table S1**. Quantification of the components involved in photosynthetic electron transport, in the wild type strain and the various mutant strains used in this study. The concentrations are given relative to the concentration in Photosystem I [PSI] (50 nM, see text), data are averages of three independent experiments. It should be noted that, in our study, the concentration of active PSII is null due to the systematic treatment of our samples with 10  $\mu$ M DCMU.

Strain	[PSI]	[PSII] <sup>*</sup>	$[b_6f]^{\ddagger}$	[PQ] <sup>§</sup>	$[PC]^{\dagger}$	[Chl] <sup>¶</sup>
WT	1	$1.14 \pm 0.17$	0.47 ± 0.16	$4.5 \pm 0.3$	$1.5 \pm 0.3$	$1035 \pm 123$
sta6	1	$0.94 \pm 0.15$	$0.45 \pm 0.17$	$4.5 \pm 0.4$	$1.8 \pm 0.4$	$1031 \pm 260$
$\Delta Rbcl$	1	$0.98 \pm 0.26$	$0.44 \pm 0.15$	$4.1 \pm 0.5$	$1.2 \pm 0.1$	$1113 \pm 235$
stt7-9 dum11	1	$1.18 \pm 0.23$	$0.50 \pm 0.19$	$2.7 \pm 0.3$	$1.3 \pm 0.3$	$1069 \pm 265$

<sup>\*</sup> The Photosystem II to Photosystem I ratio was measured directly *in vivo* by comparing the amplitude of the electrochromic bandshift of carotenoids at 520 nm at 100  $\mu$ s after a saturating flash giving a single turnover of PSI + PSII (untreated sample) or PSI alone in a sample treated with 1mM hydroxylamine and 10  $\mu$ M DCMU (3(3,4-dichlorophenyl)-1,1-dimethylurea), and preilluminated (1).

<sup>\*</sup> The cytochrome  $b_6 f$  complex to PSI ratio was measured directly *in vivo* by comparing the light-induced absorbance change at 700 nm (P<sub>700</sub>,  $\Delta \varepsilon_{700 \text{ nm}} = -60 \text{ mM}^{-1} \text{ cm}^{-1}$ ) (2) and 554 nm (cyt *f*,  $\Delta \varepsilon_{554 \text{ nm}} = -25 \text{ mM}^{-1} \text{ cm}^{-1}$ ) (3,4) for samples treated with hydroxylamine, DCMU and 20 µM DBMIB (2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone), *i.e.* when PSI is the only active complex.

<sup>§</sup> The pool-size of PSII electron acceptors (oxidized plastoquinones) was determined directly *in vivo* according to the following procedure, previously described in (5). Cells were placed under osmotic shock (1M sucrose) to block electron transfer downstream of PSII, plastocyanin diffusion being blocked (6). The area over the fluorescence rise observed under continuous illumination, being proportional to the product of photochemical rate and time, is related to the number of PSII electron acceptors (7,8). This area was divided by the area over the fluorescence rise measured in the presence of DCMU (corresponding to one electron borne by  $Q_A$ ), therefore giving the number of electron acceptors per PSII (including  $Q_A$ ). After discounting  $Q_A$ , this number was divided by a factor of two (plastoquinones are two-electron carriers) and further related to the amount of PSI, provided the PSI:PSII stoichiometry. The amount of oxidized plastoquinones was found similar in all the first 3 strains, with the exception of *stt7-9 dum11*, a respiratory-deficient mutant having a more reduced pool of plastoquinones, similarly to that reported in (9).

<sup>†</sup> The relative concentration of PSI secondary electron donors was determined similarly to the PQ pool (see <sup>§</sup>), but this time on the basis of  $P_{700}$  oxidation curves in the presence of hydroxylamine, DCMU and DBMIB. The photochemical rate of PSI was determined from the rise-time of  $P_{700}^+$  under osmotic shock (1M sucrose) (6). The concentration of plastocyanin was estimated from the pool-size of PSI electron donors after discounting one electron per PSI ( $P_{700}$ ) and two electrons per cyt  $b_6 f$  complex (cyt *f* and Rieske protein).

<sup>¶</sup> In order to be consistent with the data of Gibbs (10) we refer to in the discussion, the total chlorophyll concentration was determined similarly by absorbance spectrophotometry using the method of Arnon (11) rather than the more recent method of Porra (12).

- 1. Joliot, P., and Delosme, R. (1974) Biochimica et Biophysica Acta (BBA) -Bioenergetics 357, 267-284
- 2. Hiyama, T., and Ke, B. (1972) *Biochimica et Biophysica Acta (BBA) Bioenergetics* 267, 160-171
- 3. Alric, J., Pierre, Y., Picot, D., Lavergne, J., and Rappaport, F. (2005) *Proceedings of the National Academy of Sciences of the United States of America* **102**, 15860-15865
- 4. Metzger, S. U., Cramer, W. A., and Whitmarsh, J. (1997) *Biochimica et biophysica* acta 1319, 233-241
- 5. Houille-Vernes, L., Rappaport, F., Wollman, F. A., Alric, J., and Johnson, X. (2011) Proceedings of the National Academy of Sciences of the United States of America **108**, 20820-20825
- 6. Cruz, J. A., Salbilla, B. A., Kanazawa, A., and Kramer, D. M. (2001) *Plant physiology* **127**, 1167-1179
- 7. Bennoun, P. (1982) Proceedings of the National Academy of Sciences of the United States of America **79**, 4352-4356
- 8. Forbush, B., and Kok, B. (1968) *Biochimica et Biophysica Acta (BBA) Bioenergetics* 162, 243-253
- 9. Cardol, P., Alric, J., Girard-Bascou, J., Franck, F., Wollman, F. A., and Finazzi, G. (2009) Proceedings of the National Academy of Sciences of the United States of America 106, 15979-15984
- 10. Gibbs, M., Gfeller, R. P., and Chen, C. (1986) Plant physiology 82, 160-166
- 11. Arnon, D. I. (1949) *Plant physiology* 24, 1-15
- 12. Porra, R. J., Thompson, W. A., and Kriedemann, P. E. (1989) *Biochimica et Biophysica Acta (BBA) Bioenergetics* 975, 384-394