

LEGENDS TO SUPPLEMENTARY FIGURES

Figure S1. Isolation of *dum22stt7-9* double mutants. A. Analysis of mitochondrial deficiencies by TTC staining in meiotic products of the *dum22xstt7-9* cross. Mitochondrial activity of 19 meiotic products was probed by assessing TTC staining (1) in cells that were cultivated on TAP agar-plate. When placed in anaerobic and dark conditions for few hours, wild-type colonies reduce 2,3,5-triphenyltetrazolium chloride (TTC) to red formazan and stain purple, whereas respiratory mutant colonies remain green. **B. Analysis of state transitions by 77 K fluorescence spectroscopy in TTC- meiotic products.** State transitions were probed by 77K fluorescence emission spectra of cells exposed to different treatments before freezing in liquid nitrogen. Aerobiosis: dark incubation at ambient oxygen concentration; anaerobiosis: dark incubation in nitrogen; Light + DCMU, illumination ($50 \mu\text{moles m}^{-2} \text{s}^{-1}$) in the presence of $10 \mu\text{M}$ DCMU for 10 min. PSII and PSI fluorescence emissions were measured at 685 and 715 nm, respectively. Chl concentration was lower than $2 \mu\text{g mL}^{-1}$. Based on their constant fluorescence ratio, seven *dum22 stt7-9* clones (A1, A7, E6, F4, F5, H8, J1) were isolated among those carrying the dark⁻ TTC⁻ phenotype. **C. Growth phenotype of *dum22stt7-9* mutant isolates.** Drops of cell suspensions ($A_{750\text{nm}} \sim 0.01$) were plated on solid media (TAP or Min media) and incubated for 4 days in the light ($50 \mu\text{moles m}^{-2} \text{s}^{-1}$).

Figure S2. Time course of PSII activity during photoinhibition (A) measured from variable fluorescence (F_v/F_{max}) changes, where $F_v = F_m - F_o$. Cultures of the different strains were exposed to two different intensities ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$) of white light for the indicated times. Fluorescence parameters were measured after 10 minutes of dark recovery. We note that the F_v/F_m of the respiratory mutants was lower in untreated samples, in line with their higher non-photochemical reduction of the plastoquinone pool. Therefore, a better comparison of the light induced decrease in the PSII photochemical yield required normalization of this parameter in untreated samples (B).

Figure S3. Light saturation of P₇₀₀ oxidation, evaluated from P700 redox changes profiles in continuous illumination. Traces were measured in the presence of MV 2 mM and DCMU 20 μM to prevent both PSII driven linear electron flow and PSI driven cyclic flow.

SUPPLEMENTARY METHODS

Isolation of the *dum22stt7-9* strain. The meiotic progeny of the cross was obtained after dissecting tetrads or octads. This was first analyzed for the presence of the mitochondrial *dum22* mutation that prevents growth in the dark, due to the inability to use acetate as an exogenous source of reduced carbon through respiration (dark⁻ phenotype). About 80% (15 out of the 19 clones obtained from 9 germinated zygotes) of the progeny showed a dark⁻ phenotype (Fig. S1), in good agreement with the preferential transmission of the mitochondrial genome by the *mt⁻* parental strain (reviewed in (1)). Respiratory deficiency was further confirmed in the 15 dark⁻ strains by performing an *in vivo* 2,3,5-triphenyltetrazolium chloride (TTC) staining test (1). When placed in anaerobic and dark conditions for few hours, wild-type colonies reduce 2,3,5-triphenyltetrazolium chloride (TTC) to red formazan and stain purple, whereas respiratory mutant colonies remain green (Figure S1 and STable 1). The presence of the nuclear *stt7-9* mutation was then assessed based on the loss of ST among the dark⁻ progeny. To this end, we measured the ratio of PSI over PSII fluorescence emission at 77 K, where light emission by each photosystem can be distinguished. Under conditions where the PQ pool is reduced thus promoting state 2 (*i.e.* in the dark for *dum22* mutant or in the dark under anaerobiosis for the wild type) most of the antenna proteins are connected to PSI. This results in a stimulation of PSI fluorescence emission at the expense of that of PSII. The opposite prevails in state 1 conditions, here achieved by an illumination in the presence of DCMU, to oxidize the plastoquinone pool (reviewed in (2)). In agreement with previous reports, a switch from State 2 to State 1 conditions was accompanied by a decrease in the PSI/PSII fluorescence ratio in the wild-type or *dum22* mutant cells, whereas no changes were seen in *stt7-9* cells (Stable I and Figure S1), which are constitutively locked in state 1 because of the absence of the Stt7 kinase. Based on their constant fluorescence ratio, seven *dum22stt7-9* clones were isolated among the 15 isolates carrying the dark⁻ phenotype, thus confirming the mendelian inheritance of the *stt7-9* nuclear mutation. Two of them (A1, J1) were further analyzed for their photosynthetic features.

1. Cardol P, Remacle C (2008) in *The Chlamydomonas Source Book 3*, eds. Stern D, Harris EE (Elsevier, Vol. 2, Organellar and Metabolic Processes).

2. Wollman FA (2001) State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. *EMBO J* **20**:3623-30.