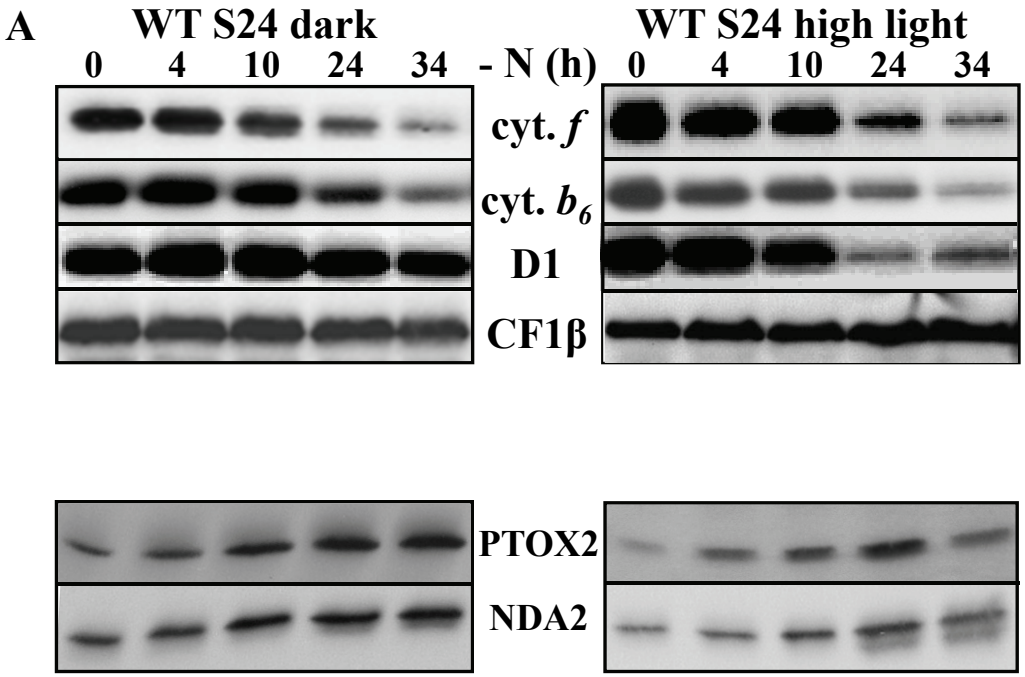
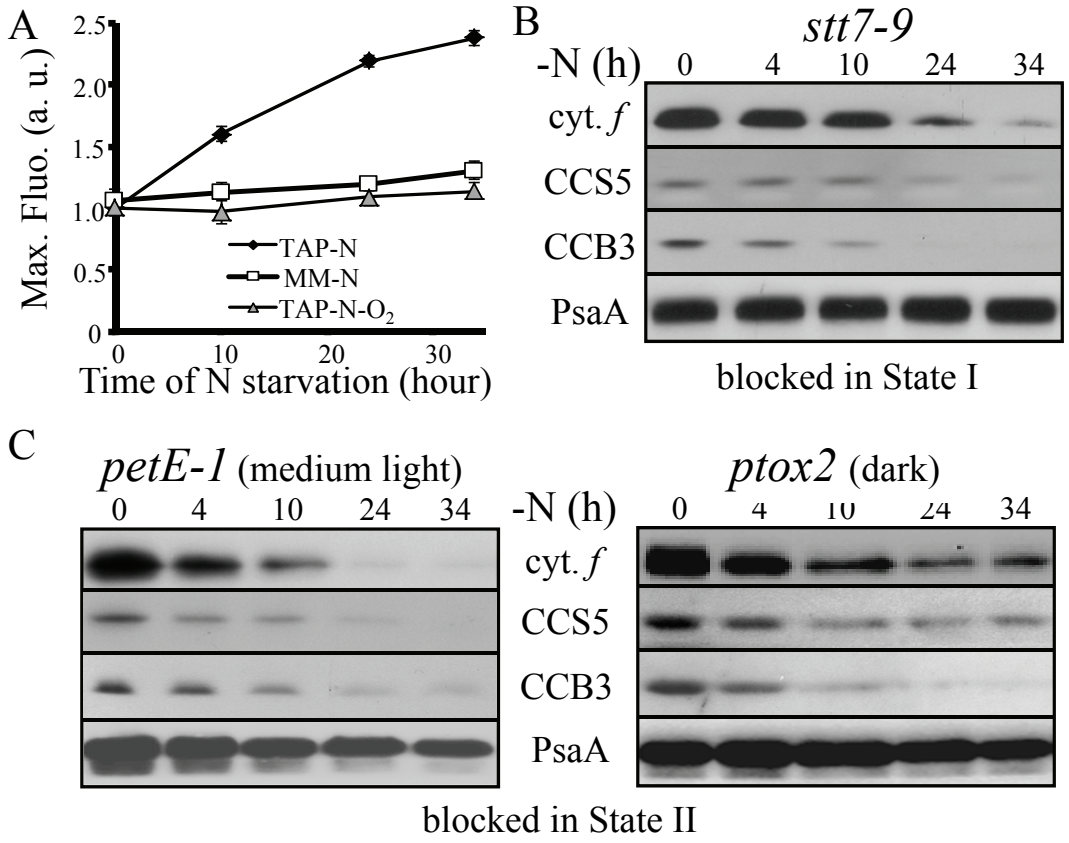


Supplemental Figure 1: the induction of PTOX2 and NDA2 does not depend on the incident light.

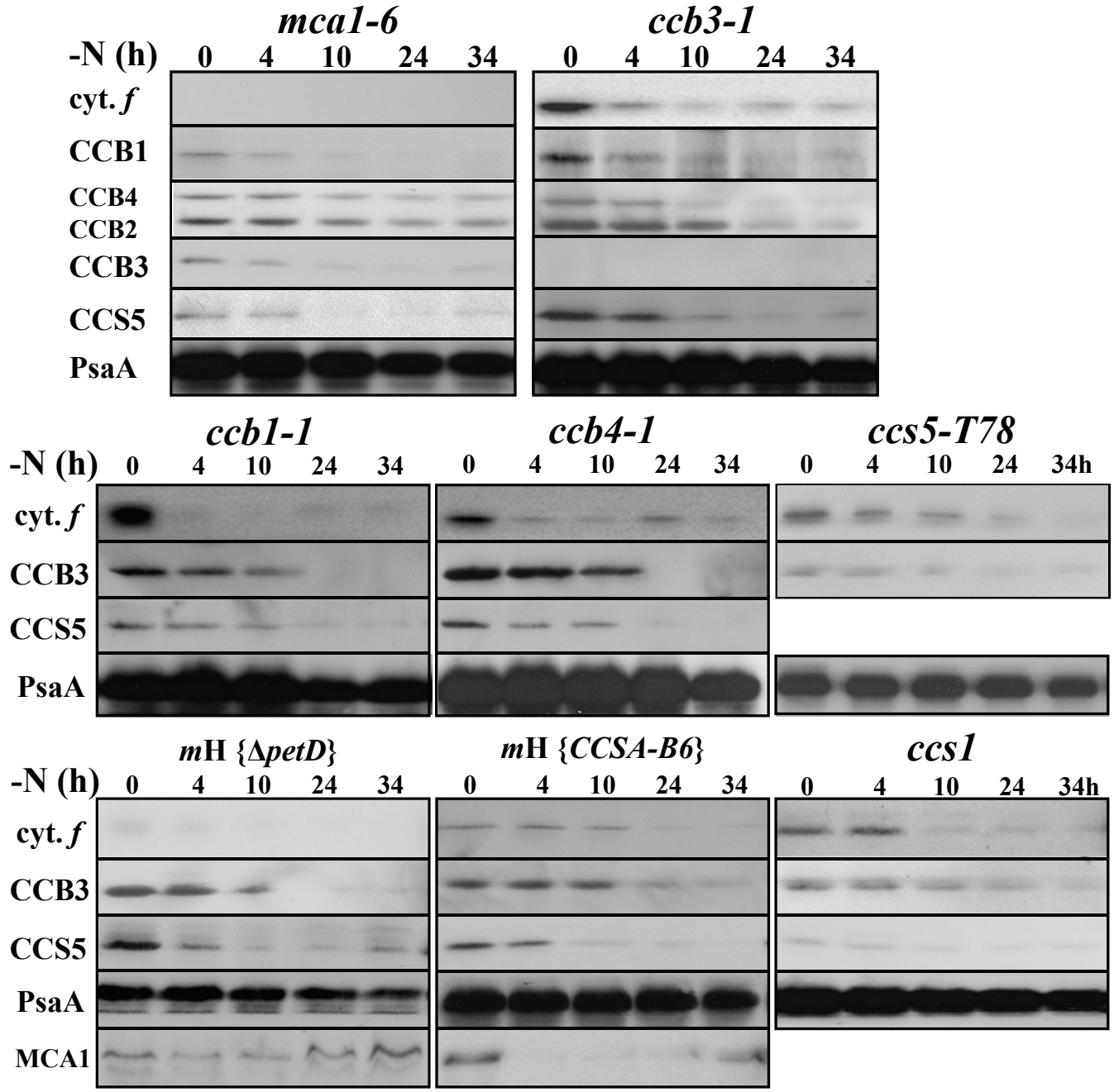
Whole cells protein extracts from WT S24 deprived of nitrogen under total darkness (left panel) or high light (120 µE.m^{-2}.s^{-1}, right panel), analysed in Fig. 2A, were immunodetected using antibodies raised against the PTOX2 and NDA2 proteins. We note that in the cells starved for nitrogen under high light, the amount of PTOX2 and to a lesser extent of NDA2 slightly decrease at the end of the experiment.



Supplemental Figure 2: The redistribution of the cytochrome b_6f complex during State Transitions does not contribute to its loss during nitrogen deprivation.

A) State transition during nitrogen starvation. Evolution of the maximum fluorescence level recorded in the presence of DCMU of WT S24 starved for nitrogen in aerobic, anaerobic or photoautotrophic conditions, normalised to the initial value measured at the onset of nitrogen deprivation. As commonly observed with cells grown in nitrogen-replete conditions, re-suspension into a fresh medium containing acetate - highly reducing-, whether it contains or not a nitrogen source, triggers a massive transition to State II. As shown by the increase in the maximum fluorescence level, cells starved for nitrogen sources in the presence of acetate and in aerobic conditions progressively consumed acetate and reverted to State I. By contrast, the maximum fluorescence level remained constant in cells starved for nitrogen in phototrophic conditions because of the absence of fresh acetate, or in cells deprived of nitrogen under anaerobiosis in darkness or low light that remained blocked in State II.

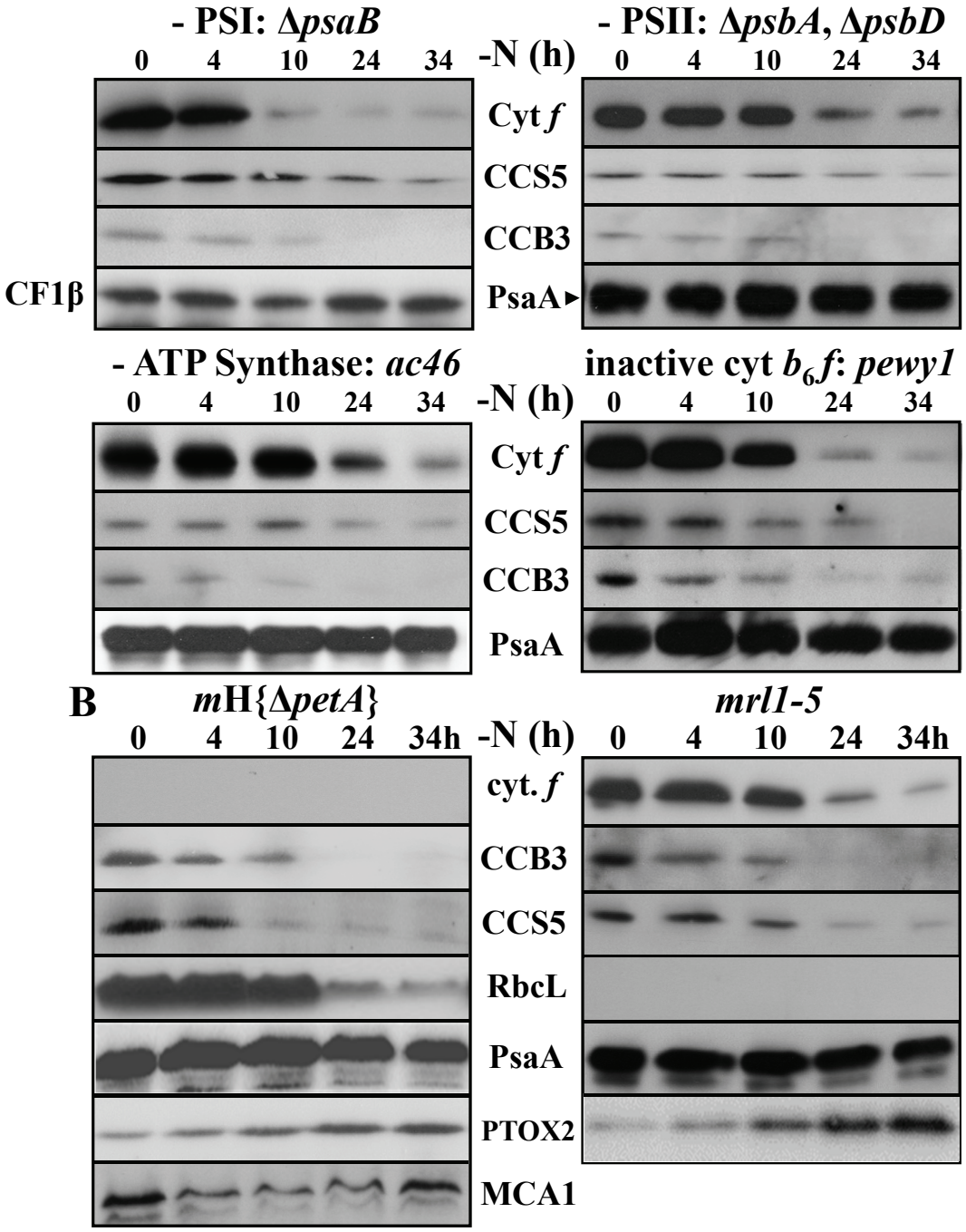
B-C) A strain locked in State I (B) - (the *stt7-9* mutant that lacks the STT7 kinase responsible for transition to State II Bellafiore et al., 2005)- and two strains locked in State II (C) because of the full reduction of their PQ pool (the *petE-1* mutant lacks plastocyanin required for the reoxidation of the PQ pool by PSI under dim light ($80\mu E.m^{-2}.s^{-1}$) and the *ptox2* mutant, kept in darkness) were deprived in nitrogen sources. Variations of the accumulation of cytochrome *f*, CCB3 and CCS5 during nitrogen starvation were analysed as in Fig. 1A and proved that the loss of cytochrome b_6f subunits and related proteins upon nitrogen starvation was independent of State I or State II since it developed similarly in these three strains. Accumulation of PsaA provides a loading control.



Supplemental Figure 3: The degradation of cytochrome b_6f subunits and related proteins does not result from a "cascade" of proteolytic events.

The hypothesis of a degradation "cascade", where the degradation of a subunit would trigger the degradation of the other proteins, was tested using all available mutant strains specifically lacking either a cytochrome b_6f subunit or one of the cytochrome b_6f related proteins, whether it being a CCB or a CCS factor. Nitrogen deprivation assays were performed in the mutants *mca1-6*, *ccb3-1,* m{Δ*petD*}, *ccb1*, *ccb3*, *ccb4*, m{*CCSA-B6*} and *ccs1* (see supplemental table SI). Total protein extracts from nitrogen-depleted cells were collected at indicated time points and analysed with the specific antibodies marked in the figure to follow the loss of cytochrome b_6f subunits and related proteins. PsaA provides a loading control.

In all instances, the remaining cytochrome b_6f subunits and related proteins were still lost upon nitrogen starvation, excluding this "cascade hypothesis" and showing that all cytochrome b_6f complex subunits and related proteins are lost independently from each other.

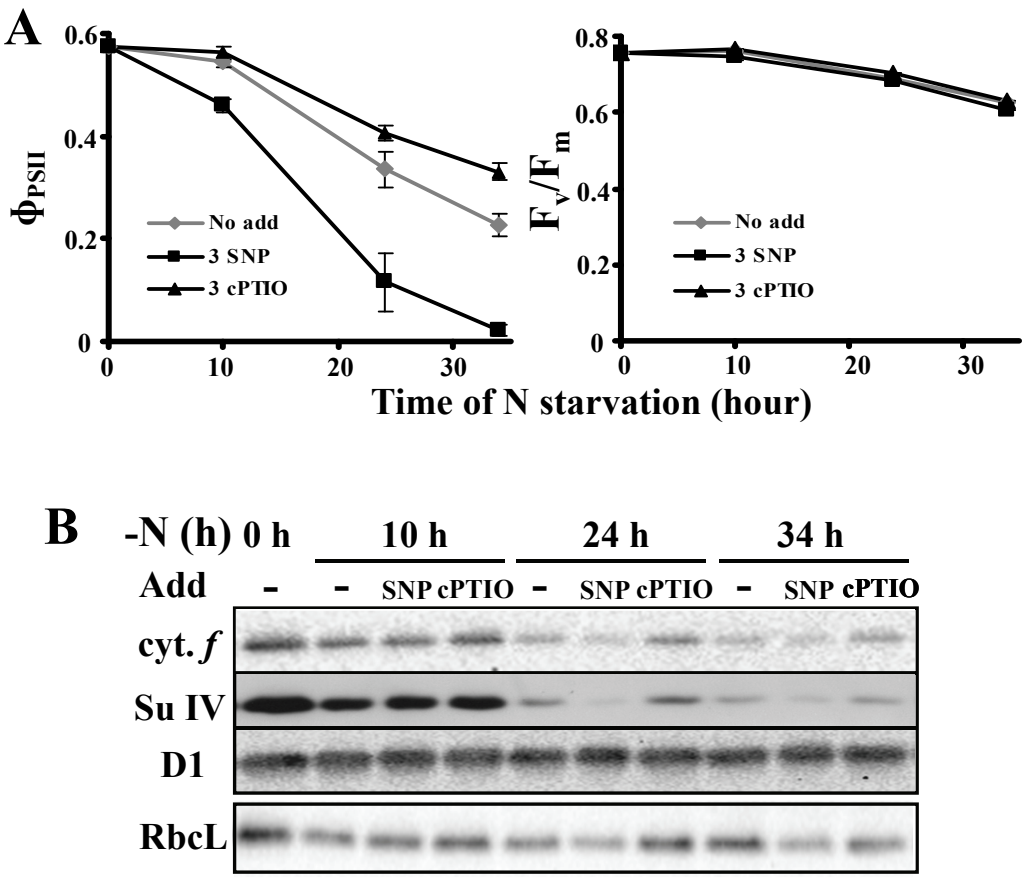


Supplemental Figure 4: the degradation of cytochrome *b*$_6$*f* complex subunits and related protein is independent of the photosynthetic activity of the cells.

The presence of any major photosynthesis proteins or the activity of the cytochrome *b*$_6$*f* complex at the beginning of nitrogen starvation is not a prerequisite for the loss of the cytochrome *b*$_6$*f* complex subunits and related proteins. A) Cytochrome *b*$_6$*f* subunits and related proteins were assessed as in Fig. 1A in strains deficient for PSI (Δ*psaB*), PSII (Δ*psbA*, Δ*psbD*), ATP synthase (*ac46*) assembly and accumulation, as well as in a strain accumulating wild-type level of an inactive cytochrome *b*$_6$*f* complex (*pwye*), because of a mutation in its Qo quinol binding site (Zito et al., 1999). The accumulation of the PSI subunit PsaA or of the CF1 subunit b provides a loading control.

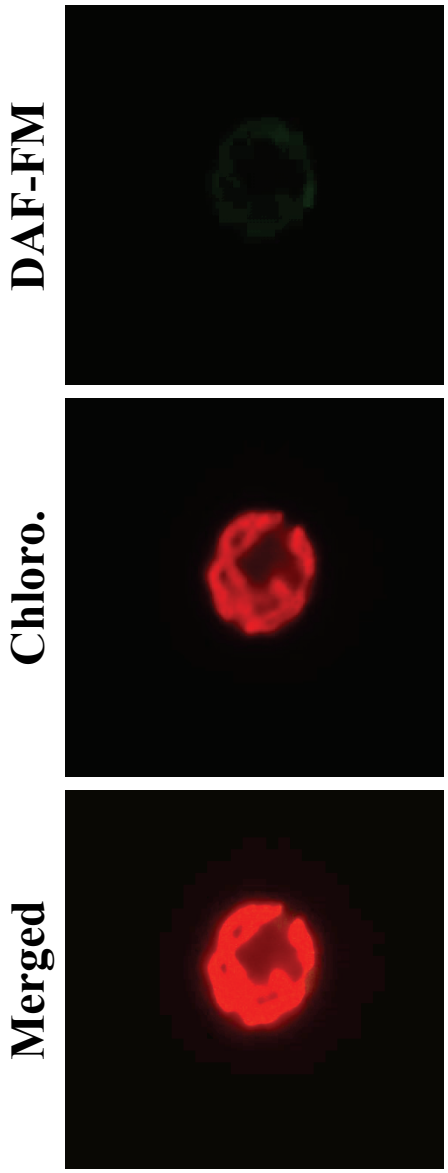
B) The cytochrome *b*$_6$*f* complex and the RuBisCO are lost independently from each other.

Left panel: accumulation of RuBisCO LS and of cytochrome *b*$_6$*f* related proteins assessed in strain *m*H {D*petA*} expressing the tagged version of MCA1 but lacking accumulation of the cytochrome *b*$_6$*f* complex, because of a deletion of the *petA* gene. Right panel: accumulation of the cytochrome *b*$_6$*f* subunits and related proteins in the RuBisCO-defective *mrl1* mutant strain. The PSI subunit PsaA provides a loading control.

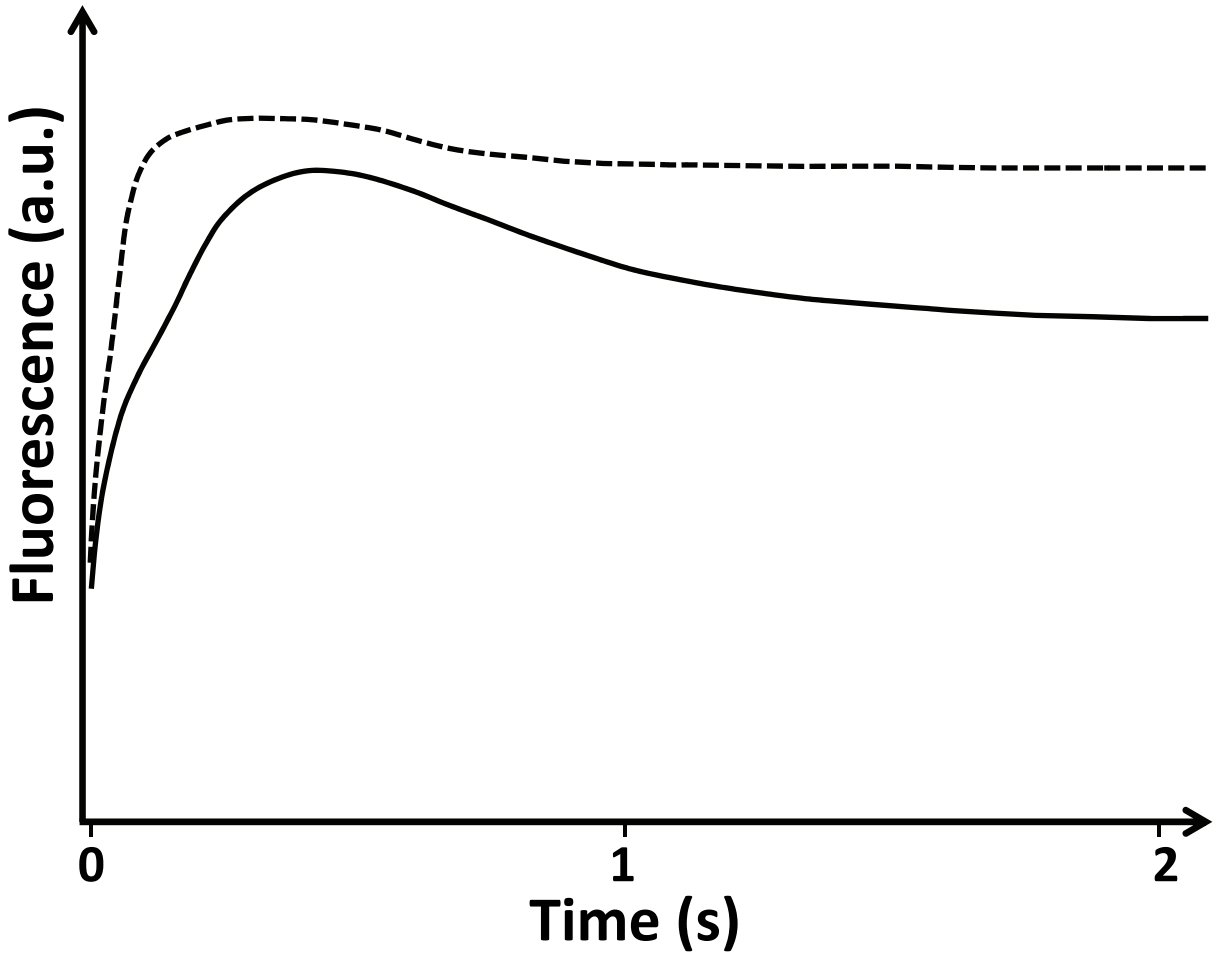


Supplemental Figure 5: the loss of the cytochrome b_6f complex is accelerated upon multiple addition of SNP.

A) Same experiment than that presented in fig. 8, panels A and B, except that SNP and cPTIO were added three times during the starvation, 1, 3 and 6 hours after the transfer to N-free medium. Note the faster decline of Φ_{PSII} in the SNP treated cultures, compared to cultures where SNP was added only once (compare panels A, right part in this figure and in Fig. 10), which cannot be attributed to PSII inactivation (panel A, right part).



<u>Supplemental Figure 6:</u> Confocal imaging of a *Chlamydomonas* wild-type cell grown in the presence of acetate but not pre-incubated with the DAF-FM fluorochrome.



<u>Supplemental Figure 7:</u> complementation of the *nit1-305* mutation restores the degradation of the cytochrome b_6f complex.
Fluorescence induction kinetics of the mutant strain *nit1-305* (continuous line —) and of the transformant Tft5 (*nit1-305::NIT1*; dashed line ---) after 48 h of N-starvation. The *nit1* mutant shows evidence for cytochrome b_6f activity (delay in fluorescence rise, decay phase after 0.5 s) which are almost completely abolished in the nit+ transformant.

strain	genotype	phenotype	reference
{Δ*psaB*}	*nit1-137 nit2-124 mt*$^{+}$ {Δ*psaB*}	Lacks PSI complex	(Redding et al., 1998)
{Δ*psbA*, Δ*psbD*}	*nit1-137 nit2-124 mt*$^{+}$ {Δ*psbA* Δ*psbD*}	Lacks PSII complex	(Minai et al., 2006)
pwye	*nit1-137 nit2-124 mt*$^{+}$ {*petD-*$_{77}$*PWYE*$_{80}$}	Accumulates wild-type amounts of an inactive cyt. b_6f complex	(Zito et al., 1999)
mca1-6	*mca1-6 nit1-137 nit2-124 mt*$^{-}$	cyt. b_6f mutant lacking pet*A* mRNA accumulation.	(Boulouis et al., 2011)
petE-1	*petE::aphVIII nit1-137 nit2-124*	Knock-out the *PETE* gene. Lacks plastocyanin.	X. Johnson, unpublished
stt7-9	*stt7-9 nit1-137 nit2-124*	Defective for the STT7 kinase	(Fleischmann et al., 1999)
mdh1-ac46	*mdh1-ac46 nit1-137 nit2-124 mt*$^{+}$	Lacks ATP synthase complex.	(Lemaire and Wollman, 1989)
mrl1-5	*mrl1-5 nit1-137 nit2-124 mt*$^{-}$	Lacks *rbcL* mRNA and RuBisCO.	(Johnson et al., 2010)
ccb1-1	*ccb1-1 nit1-137 nit2-124 mt*$^{+}$	Defective binding of *c'*$_i$ heme to apocytochrome b_6	(Kuras et al., 2007)
ccb3-1	*ccb3-14 nit1-137 nit2-124*		
ccb4-1^{+}	*ccb4-1 nit1-137 nit2-124 mt*$^{+}$		
CAL28.01.07	*ccs1-CAL28.01.07 nit1-137 nit2 mt*$^{+}$	Defective binding of *c* heme to apocytochrome *f*	(Dent et al., 2005; Inoue et al., 1997)
ccs5-T78^{+}	*ccs5-T78 nit1-137 nit2-124 mt*$^{+}$		(Xie et al., 1998; Gabilly et al., 2010)
nit1-305	*nit1-305 NIT2 mt*$^{-}$ (/*mt*$^{+}$)	Lacks NR diaphorase activity; cannot grow on nitrate nor nitrite; does not lose the cytochrome b_6f complex upon Nitrogen starvation	CC-2453
nitA	*nit1-A NIT2 mt*$^{-}$	Lacks NR diaphorase activity; cannot grow on nitrate nor nitrite does not lose the cytochrome b_6f complex upon Nitrogen starvation	CC-798
nit1-e18	*nit1-e18 NIT2 sr1 ac17 mt*$^{+}$	Lacks NR; cannot grow on nitrate or nitrite does not lose the cytochrome b_6f complex upon Nitrogen starvation	CC-2929
nit7	*nit7 NIT2 sr1 ac17 mt*$^{+}$	Lacks the MoCo cofactor of NR, SO, AO and XOR; cannot grow on hypoxanthine, nitrate nor nitrite does not lose the cytochrome b_6f complex upon Nitrogen starvation	CC-2903
nit1^{+}	*nit1-137 NIT2 mt*$^{+}$	Lacks NR, no growth on nitrate does not lose the cytochrome b_6f complex upon Nitrogen starvation	CC-1085
nit2^{+}	*NIT1 nit2-137 mt*$^{+}$	Lacks expression of NR, NiR and HANiT; cannot grow on nitrate nor nitrite loses the cytochrome b_6f complex upon Nitrogen starvation	CC-1086
nit2^{-}	*NIT1 nit2-137 sr-u-sm2 mt-*	Lacks expression of NR, NiR and HANiT; cannot grow on nitrate nor nitrite	CC-1065

		loses the cytochrome b_6f complex upon Nitrogen starvation	
A3	*nit1-137 mt-*	Lacks NR, no growth on nitrate does not lose the cytochrome b_6f complex upon Nitrogen starvation	From cross 21gr X 137c mt-
H1	*nit1-137 nit2-124 mt-*	Lacks expression of NR, NiR and HANiT; cannot grow on nitrate nor nitrite loses the cytochrome b_6f complex upon Nitrogen starvation	From cross 21gr X 137c mt-
WT 7.4	*NIT1 NIT2 mt*$^-$	Wild-type for photosynthesis and nitrogen assimilation. moderate loss of the cytochrome b_6f complex upon nitrogen starvation	From cross 21gr x H1

Supplemental Table SI : Strains used for the crosses and experiments presented as Supplemental Data.
By convention, chloroplast genotypes, when relevant, follow the nuclear genotype and are written between brackets. CC- numbers refer to strains obtained from the Chlamydomonas Resource Center (http://chlamycollection.org).

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cross # [b]	mt+	mt-	tetrads analysed	Result[a]:
1	*nit1* (*nit1-137*)	H1 (*nit1-137 nit2-124*)	11 2:2 segregation	2 Nii+ Nit- Lbf- 2 Nii- Nit- Lbf+
2	*nit1*$^{+}$ (*nit1-137*)	*nit2*$^{-}$ (*nit2-137*)	28 (8 PD, 9 NPD, 11 T)	Nii+ Nit+ were Lbf+ Nii+ Nit- were Lbf- Nii- were Nit- Lbf+
3	21gr (*NIT1 NIT2*)	H1 (*nit1-137 nit2-124*)	18 (7 PD, 1 NPD, 10 T)	Nii+ Nit+ were Lbf+ Nii+ Nit- were Lbf- Nii- were Nit- Lbf+
4	*nit2*$^{+}$ (*nit2-137*)	A3 (*nit1-137*)	3 (2 DR, 1 T)	Nii+ Nit+ were Lbf+ all Nii+ Nit- were Lbf- all Nii- were Nit- Lbf+
5	21gr (*NIT2*)	*nit2*$^{-}$ (*nit2-137*)	16 2:2 segregation	2 Nii+ Nit+ Lbf+ 2 Nii- Nit- Lbf+
6	21gr (*NIT1*)	A3 (*nit1-137*)	6 2:2 segregation	Nit+ progeny, except 1, were Lbf+ Nit- progeny, except 1, were Lbf-
7	*nit4-104*	WT 7.4 (*NIT4*)	5 2:2 segregation	Nit+ progeny, except 1, were Lbf+ Nit- progeny were Lbf-
8	21gr (*NIT1 NIT2*)	*nit1-305*	20 2:2 segregation	Nit+ progeny, except 1, were Lbf+ Nit- progeny were Lbf-
9	*nit1-305*	A3 (*nit1-137*)	none	20 random spores recovered on NH_4 were Nit- Lbf- 19 random spores recovered on NO_3 were Nit+ Lbf+

Supplemental Table 2: Genetic analysis of the determinism of the cytochrome *b_6f* loss in nitrate assimilation mutants.

[a] Reported phenotypes are Nit (growth on 5 mM KNO_3, indicative of a wild-type phenotype for nitrogen assimilation: *NIT1, NIT2*), Nii (growth on 5mM $NaNO_2$, indicative of a wild-type *NIT2* gene), Lbf (Loss of the cytochrome *b_6f* during N-starvation, assessed by Φ_{PSII} recorded with a home-built fluorimeter on cells spotted on N-free agar plates for 48h). Growth tests were performed in microtiter plates under 50 $\mu E.m^{-2}.s^{-1}$ fluorescent light, with low cell density inoculum.

[b] The upper part of the table (crosses #1 - #5) describes crosses aimed addressing the interplay between Nitrate Reductase and Nitrite Reductase in the loss of the cytochrome *b_6f* complex, while the lower part (crosses #6 - # 9) shows crosses done to study the role of Nitrate Reductase in this loss.

Name	Sequence (5'->3')
LAO1QC1*	GAGACTGTGATGCCCAAAAAGTG
LAO1QR1	GCTTGCCCAGGCCGCGAATGGAA
Mca1QC2*	CGTCCGCAGGAGCAGGGTGAGC
Mca1QR2	CGGCGTGCTGCATGGACAG
Ccb3QC1	ACAATTCGCCCTGCGTCCTCGT
Ccb3QR1	ATCGAGCGAGTGGGTGTGCCTG
PetCQC2	ACCGCCGACAGCACCATCGAGA
PetCQR2*	TGGGCCAGGGCCAGCGACAGGG
Nac2QC2*	CTGGAGGCCAGCAACCTTGAGC
Nac2QR2	TGTCGTACAGCTCTGCCGCTTC

Supplemental Table 3: Oligonucleotides Used in This Work.

The star indicates oligonucleotides that encompass the junction between two exons, in order to prevent amplification from genomic DNA, would this later still be present in the reverse transcription reaction.