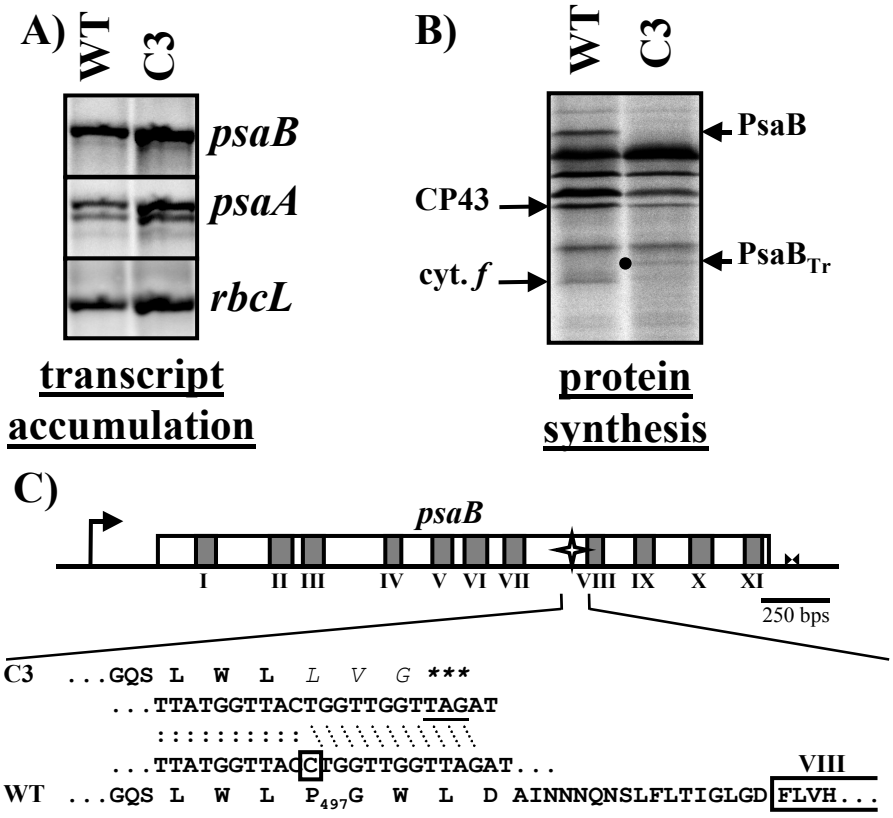
Supplementary data

Molecular characterisation of the C3 recipient strain.

The chloroplast mutant strain C3 lacks translation of PsaB and PsaA (Girard-Bascou et al., 1987). Before using it as a recipient strain for transformation, we characterised its molecular defect. That strain shows a low frequency of spontaneous reversion and accumulates mature *psaB* and *psaA* messengers, even if *psaB* transcript accumulates 1.8 fold more than in wild type (figure IA). Recombination studies suggested a mutation in the *psaB* coding sequence since the C3 mutation mapped close to Fud26 and g2.3 mutations (Girard-Bascou, 1987). These two mutations correspond to frameshifts at the end of the *psaB* coding sequence that lead to premature translational arrests, respectively 94 and 157 residues before the stop codon of the wild type protein (Bingham et al., 1991; Girard-Bascou et al., 1987). PCR amplified *psaB* gene from the C3 strain was sequenced in the region mutated in Fud26 and g2.3 strains. A deletion of a cytosine in the CCT codon encoding residue P_{497} induces a frameshift and yields a premature termination codon at position 500, before helix VIII (see figure IC). Indeed, in 5 min pulse-labelling experiments, we observed in the C3 strain a truncated PsaB polypeptide that migrates close to cytochrome *f* (figure IB). This polypeptide was not detected in 45 min pulse-labelling experiments in the previous study (Girard-Bascou et al., 1987), probably because of a very rapid turn-over. Its apparent molecular weight is lower than predicted from its amino acid composition (~30 kDa instead of 55 kDa), a property shared by wild type PsaA and PsaB that migrate with an apparent molecular weight of 60kDa (instead of 85 kDa). This truncated PsaB is inserted in the membrane as it is detected in membrane preparation (data not shown). However, it lacks the last 237 residues of the protein that provide, through the five last transmembrane helices, the major contacts with the PsaA subunit, and is therefore unstable.

Figure I



Legend of figure I: ***Molecular characterisation of the C3 mutant strain***

A) Transcript accumulation in the C3 strain.

Total RNA, extracted from wild type and C3 strains was probed with specific probes for accumulation of *psaB*, *psaA* exon 3 and *rbcL* (as a loading control) messengers.

B) A truncated PsaB polypeptide is synthesized in the C3 mutant strain.

Membranes were purified from pulse labelled WT and C3 cells, solubilised and separated by electrophoresis on a denaturing 12-18% acrylamide-urea gel. Positions of cytochrome *f*, CP43 and of truncated PsaB polypeptide ($PsaB_{Tr}$) detected in the C3 strain, are indicated.

C) In the C3 strain, a single base pair deletion in the *psaB* coding sequence results in a frame-shift leading to a premature termination codon at position 500.

Assessment of a regulation of translation initiation in the CES behaviour of PsaA, using the aadA reporter gene

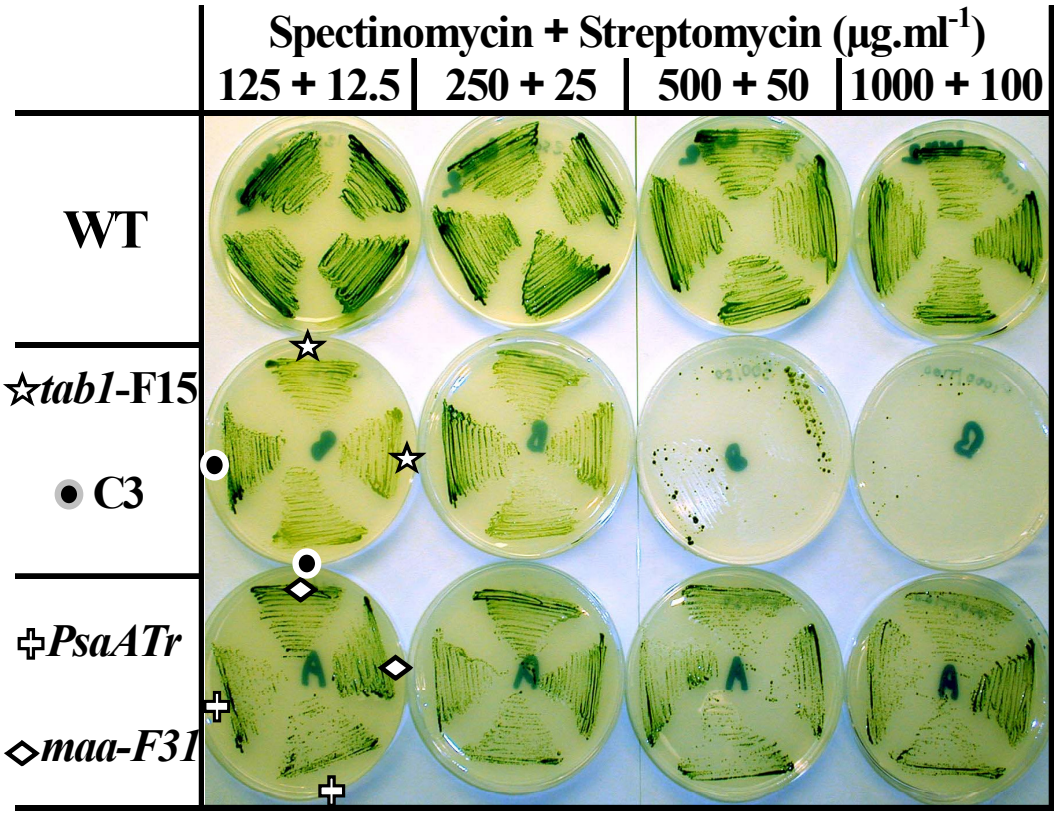


Figure II: *The psaA 5'UTR confers a PsaB-dependant expression to the reporter gene 5'psaA-aadA*

It can be seen on this coloured version of fig. 1B that tab1 and C3 transformants present a robust growth in the presence of low antibiotic concentrations, but have a small pigmentation defect and therefore appear more yellow than the other transformed strains.

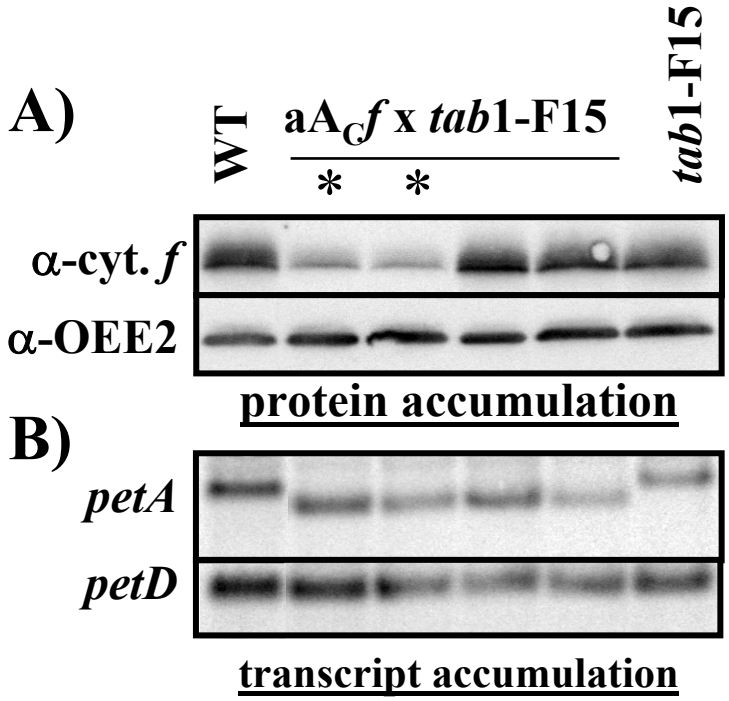
Table I

Genotype of the strain	Antibiotic concentrations Spectinomycin + Streptomycin (µg.ml^{-1})					
	125 + 12.5	250 + 25	500 + 50	1000 +100	2000 + 200	2000 + 400
WT::5'*psaA-aadA*	8 +++	8 +++	8 +++	8 +++	5 +++ 3 +	5 +/- 3 -
tab1-F15::5'*psaA-aadA*	8 +++	6 +++ 2 +	5 +/- 3 -	2+/- 6 -	8 -	8 -
C3::5'*psaA-aadA*	8 +++	7 +++ 1 +	1 + 5 +/- 2 -	1+/- 7 -	8 -	8 -
PsaA$_{Tr}$::5'*psaA-aadA*	8 +++	8 +++	8 +++	8 +++	7 +++ 1 +	1 +++ 2 + 5 +/-
maa-F31::5'*psaA-aadA*	8 +++	8 +++	8 +++	8 +++	8 +++	4 +++ 3 + 1 +/-

Table I: ***growth of the transformed strains listed in the left column on TAP medium supplemented with increasing concentrations of antibiotics, as indicated in the top line***

Eight independent transformants were tested for each genotype. +++: growth is as on TAP plates without antibiotics ; +: most of the cells turn brownish with patches of actively dividing green clones ; +/-: only a few green clones; -: no growth.

Figure III: *accumulation of the chimeric transcript is not correlated with the expression of the 5'psaA$_C$-petA gene.*

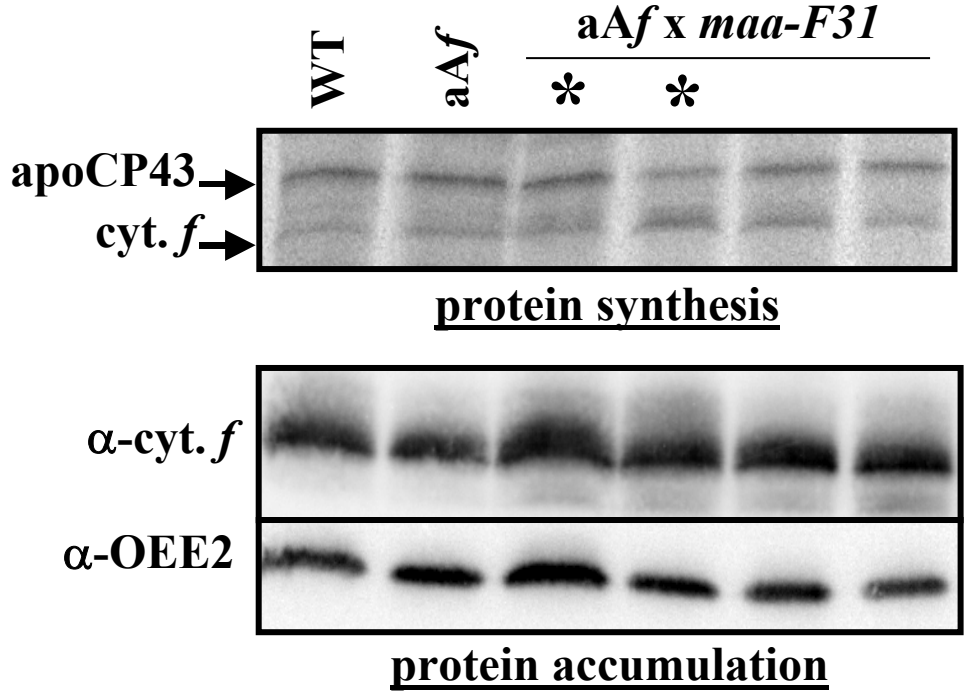


A) Accumulation of cytochrome *f* and OEE2 (as a loading control), detected with specific antibodies in a representative tetrad progeny (out of five) of the cross *aA$_C$f* x *tab1*-F15. Wild type and *tab1*-F15 strains are also presented. * designates the *tab1* tetrad progeny, identified through their typical kinetics of fluorescence induction.

B) Accumulation of 5'*psaA$_{Cod}$-petA* and *petD* (as loading control) transcripts detected with specific probes in the strains from panel E.

Figure III: *the maa mutation does not alter the expression of the 5'psaA-petA reporter gene*

Cytochrome *f* synthesis and accumulation in the progeny of a representative tetrad from the cross aA*f* x *maa*-F31. * designates the *maa* progeny, identified by fluorescence transients. Accumulation of OEE2 provides a loading control



DNA constructs

A 245 bps DNA fragment containing the *psaA* promoter and 5'untranslated regions was amplified by PCR using primers $PsaA_{Prom}$ and PsaA (see Table II) and template plasmid ps1A1 (Kuck et al., 1987). Primers $PsaA_{Prom}$ and $PsaA_C$ were used with the same template to amplify a similar DNA fragment of 310 bps containing in addition the first 60nts of *psaA* coding sequence. A 750 bp DNA fragment containing the *psaC* promoter region and 5'UTR as well as the 30 first nucleotides of *psaC* coding sequence was amplified by PCR using primers $PsaC_{Prom}$ and PsaC and restriction fragment R23 (Rochaix, 1978) as a template. All PCR products were digested with *Cla*I and *Nco*I, two restriction sites introduced in the sequence of the oligonucleotides, respectively upstream of the 5'UTR and downstream of the *psaA* or *psaC* initiation codon. Digested fragments were then ligated into plasmid pAFFF (Choquet et al., 1998) digested with the same enzymes to yield plasmid paA*f*, p*aA*$_C$*f* and paC*f*, respectively, where cytochrome *f* was translated under the control of *psaA* or *psaC* 5'UTRs, in frame with the initiation codon of these genes. The spectinomycin resistance cassette (Goldschmidt-Clermont, 1991) was then introduced in reverse orientation with respect to the *petA* gene in these plasmids, linearized by the unic cutter *Hinc*II, to create plasmids pKaA*f*, pK*aA*$_C$*f* and pKaC*f*.

The DNA fragment amplified with primers $PsaA_{Prom}$ and PsaA was also cloned, after digestion with *Cla*I and *Nco*I, into the pUC-atpX-AAD (Goldschmidt-Clermont, 1991) vector digested by the same enzymes to yield plasmid paAK. The 5'*psaA-aadA* chimeric gene was excised by digestion with *Sma*I And *Eco*RV and cloned in the unic EcoRV site of plasmid pWF (Kuras and Wollman, 1994) to create plasmid p*f*aAK.

A 5.3 kbps *Bam*HI-*Eco*RI sub-fragment of the chloroplast restriction fragment R17 (Rochaix, 1978) was subcloned into vector pCR1 to yield plasmid pBR17-1. This plasmid was then digested with the unic cutter *Spe*I, filled up with Klenow enzyme and religated on itself, to create plasmid $pPsaA_{Tr}$. This introduced of a 4 pbs frameshift, 191 pbs after the beginning of *psaA* exon 3. The *aadA* recycling cassette (Fischer et al., 1996) was then introduced, in the reverse orientation with respect to the third exon of *psaA*, in plasmid $pPsaA_{Tr}$ linearised with the unique cutter *Sma*I, to yield plasmid pK_rPsaA_{Tr}.

Mutation in the *psaB* gene from the C3 strain was characterised by sequencing a PCR fragment amplified using oligonucleotides primers $PsaB_{Cod}$ and $PsaB_{Rev}$ and total DNA from the C3 strain, isolated as described in (Schroda et al., 2001), as template.

name	**Sequence**	***Restriction sites*** [a]
psaA$_{Prom}$	CGC***GAATTC***ATCGATCTTTTACGAATACACATATGGT	***EcoRI***, ClaI
psaA	CGC***GGATCC***ATGGTCATGGATTTCTCCTTAT	***BamHI***, NcoI
psaA$_{C}$	CGC***GGATCC***ATGGGATTACGATCAACCGCA	***BamHI***, NcoI
psaC$_{Prom}$	GCG***ATCGAT***GAATTCTTCTTGCCTGTAGTGGAAACTGTTCAGT	***ClaI***, EcoRI
psaC	CGC***GGATCC***ATGGTATCGTAAATTTTAACGATATGAGCCATATTTT	***BamHI***, NcoI
psaB$_{Cod}$	TATTGAGCCTGTATTTGCTCAATGG	
psaB$_{Rev}$	CAAATAAGAAAGTCCAAGCCCAAAC	

Table II: ***primers***

[a] restriction sites introduced in the sequence of the oligonucleotide, used for cloning strategies or RFLP analysis.

References

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