the plant journal

SEB Society for Experimental Biology

The Plant Journal (2012) **72**, 652–661 doi: 10.1111/j.1365-313X.2012.05111.x

Identification of an OPR protein involved in the translation initiation of the PsaB subunit of photosystem I

Michèle Rahire[1], Fabrice Laroche[1], Lorenzo Cerutti[2] and Jean-David Rochaix[1,*]

[1]*Departments of Molecular Biology and Plant Biology, University of Geneva, 30, Quai Ernest Ansermet, Geneva 1211, Switzerland, and*

[2]*Swiss Swiss Institute of Bioinformatics, CMU-1, rue Michel Servet, CH-1211 Geneva 4, Switzerland*

Received 6 June 2012; revised 29 June 2012; accepted 16 July 2012; published online 13 September 2012.

*For correspondence (e-mail jean-david.rochaix@unige.ch).

SUMMARY

Genetic analysis of mutants deficient in the biosynthesis of the photosystem I complex has revealed several nucleus-encoded factors that act at different post-transcriptional steps of chloroplast gene expression. Here we have identified and characterized the gene affected in the *tab 1-F15* mutant, which is specifically deficient in the translation of the photosystem I reaction center protein PsaB as the result of a single nucleotide deletion. This gene encodes Tab 1, a 1287 amino acid protein that contains 10 tandem 38–40 amino acid degenerate repeats of the PPPEW/OPR (octatricopeptide repeat) family, first described for the chloroplast translation factor Tbc2. These repeats are involved in the binding of Tab 1 to the 5′-untranslated region of the *psaB* mRNA based on gel mobility shift assays. Tab 1 is part of a large family of proteins in *Chlamydomonas* that are also found in several bacteria and protozoans, but are rare in land plants.

Keywords: chloroplast biogenesis, translation, photosystem I, *Chlamydomonas reinhardtii*, OPR protein.

INTRODUCTION

The primary reactions of photosynthesis in algae and plants occur in the thylakoid membrane and are mediated by the two photosystems PSI and PSII, and their light-harvesting systems. They are connected in series through the electron transport chain, which includes the plastoquinone pool, the cytochrome b_6f complex and plastocyanin. Absorbed light excitation energy is used by the two photosystems to create charge separations across the membrane, with PSII oxidizing water at one end of the chain and PSI reducing ferredoxin at the other end, followed by the reduction of $NADP^+$ to NADPH. Electron transport is coupled with proton translocation across the membrane, giving rise to a proton gradient that is used by the ATP synthase to generate ATP. Both ATP and NADPH drive the Calvin–Benson cycle that promotes the assimilation of CO_2 and the formation of sugars.

Each of the photosynthetic membrane complexes contains numerous subunits of nuclear and chloroplast genetic origin (Eberhard *et al.*, 2008; Stern *et al.*, 2010). The mRNAs of nucleus-encoded subunits are translated on cytoplasmic ribosomes, and their products are imported into chloroplasts, whereas the mRNAs of chloroplast-encoded subunits are translated on chloroplast ribosomes, and finally assembled together with their nucleus-encoded partners into functional photosynthetic complexes. Whereas the composition, structure and function of these complexes are well understood, the mechanisms underlying their coordinate assembly are still largely unknown.

A genetic analysis of numerous mutants of *Chlamydomonas*, Arabidopsis and *Zea mays* (maize) that are deficient in photosynthesis or in pigmentation has revealed a large set of nuclear loci that are involved in different post-transcriptional steps of chloroplast gene expression, such as RNA processing and stability, RNA editing, splicing, translation and assembly of the complexes in the thylakoid membrane (Eberhard *et al.*, 2008; Stern *et al.*, 2010). These loci encode factors that act mostly in a gene-specific manner in *Chlamydomonas*, whereas in land plants some of them are involved in the expression of several chloroplast genes, and a loss of these factors generally introduces more pleiotropic effects (Eberhard *et al.*, 2008; Stern *et al.*, 2010).

Photosystem I acts as a light-driven plastocyanin ferredoxin oxidoreductase (Nelson and Yocum, 2006). The PSI core complex consists of the two large reaction center polypeptides PsaA and PsaB and the small PsaC protein, which are encoded by the chloroplast genome. These three proteins bind most of the redox co-factors of PSI involved in electron transport, as well as many chlorophyll, carotenoid and lipid molecules. In addition, the complex contains nine

or 10 subunits, PsaD–PsaN, which are generally nucleus-encoded except for PsaJ, which is chloroplast-encoded. In this study we have characterized *tab 1-F15*, a PSI-deficient nuclear mutant of *Chlamydomonas*. It is specifically affected in the translation of the two PSI reaction center polypeptides PsaA and PsaB, based on protein pulse-labeling experiments (Girard-Bascou *et al.*, 1987). Further analysis with a chimeric chloroplast transgene, in which the *psaB* 5′ untranslated region (5′-UTR) was fused to a reporter gene, revealed that the reporter gene was expressed in the wild-type but not in the mutant nuclear background, indicating that this mutant is specifically affected at the level of initiation of translation of the chloroplast *psaB* mRNA (Stampacchia *et al.*, 1997). Because PsaB appears to be the major anchor protein for the assembly of PSI, it is particularly important to identify the factors required for its expression and integration into the thylakoid membrane. Besides *tab 1-F15*, another mutant *tab 2-F14* is also specifically unable to translate the *psaB* mRNA. The Tab 2 locus has been identified, and codes for a protein that is highly conserved in plants and cyanobacteria (Barneche *et al.*, 2006; Dauvillée *et al.*, 2003). Here, we show that Tab 1 encodes a large protein with 10 tandem 38–40 amino acid degenerate repeats with RNA binding properties. These octatrico peptide repeats (OPRs) are also found in other proteins involved in post-transcriptional steps of chloroplast gene expression. Together these proteins form a new family of proteins, and they are also present in other unicellular organisms and some bacteria, but are rare in land plants.

RESULTS

Isolation of the nuclear gene deficient in the PSI mutant *tab 1-F15*

Previous work has shown that the *tab 1-F15* mutant is specifically deficient in the translation of the PsaB subunit of PSI (Girard-Bascou *et al.*, 1987; Stampacchia *et al.*, 1997). To understand how the corresponding factor acts, a first step was to isolate its gene. The *tab 1-F15* mutant was transformed with an indexed cosmid library of *Chlamydomonas* (Zhang *et al.*, 1994) by selecting for photoautotrophic growth or for growth on acetate-containing medium under 60 μmol photons m^{-2} s^{-1}. PSI mutants are light sensitive and unable to grow under these conditions. Only a single cosmid, called c35D7, could be identified that complemented the mutant (see Experimental procedures for details). Analysis of this cosmid revealed that it contained a 6-kb genomic *Not*I fragment that was considerably rearranged based on sequence comparison with the corresponding genomic locus. We therefore used an 889-bp *Not*I-*Nde*I fragment from c35D7 to rescreen the cosmid library. A new cosmid of 40 kb, called c102A3, was identified that rescued the *F15* mutant with a high yield by transformation. To identify the gene affected in *F15*, dubbed Tab 1, the cosmid was subcloned in smaller fragments that were then tested for their ability to rescue the mutant by transformation on minimal medium. A 5.4-kb *Not*I fragment complemented the mutant and was used as a probe for isolating 3.6 kb of cDNA from a *Chlamydomonas* cDNA library, which was also able to rescue the mutant. A BLAST search of the *Chlamydomonas* genome with this cDNA as a probe revealed that the *Tab 1* gene is located on chromosome 7 of the algal genome (Merchant, 2007) (http://genome.jgi-psf.org/chlamy/chlamy.info.html; GenBank, HQ683741).

Tab 1 encodes a PPPEW/OPR protein

The sequence of the full-length 4.8-kb *Tab 1* open reading frame was assembled from the partial 3.6-kb cDNA and the 1.2-kb genomic region corresponding to the 3′ end of the gene, which was covered by an expressed sequence tag (EST) sequence (estExt_fgenesh2_pg.C_160172). Comparison of the genomic and cDNA sequence revealed five introns within the 6-kb *Tab 1* gene with a 3′-UTR of 658 nucleotides (Figure 1). Data on the mapping of *Tab 1* transcripts were also obtained from the ∼5 million-plus EST reads from Joint Genome Institute, University of California (JGI-UCLA) and Genoscope (http://genomes.mcdb.ucla.edu). In this way the 3′ end was confirmed and the 5′-UTR was estimated to be close to 280 nucleotides. High-throughput RNA sequencing (RNA-seq) revealed that *Tab 1* is expressed at very low levels: ∼10-fold less than the average *Chlamydomonas* gene (http://genomes.mcdb.ucla.edu).

The *Tab 1* gene encodes a protein of 1287 amino acids, with a predicted molecular weight of 129.093 (Figure 1) (GenBank ADY68544.1). It differs from the predicted protein Cre07.g348800.t1.2 in Augustus u10.2, a gene prediction set based on the *Chlamydomonas reinhardtii* genomic sequence, EST and RNA-seq data (http://genomes.mcdb.ucla.edu), by the presence of an additional exon (exon 2, encoding 53 amino acids; Figure 1). The protein is predicted by TARGETP to be localized in the chloroplast, with a putative pre-sequence of 27 amino acids. The protein has a high content of A (21%), S (10.7%), L (9.7%), P (9.0%) and G (8.6%). A BLAST search revealed that Tab 1 shares common features with Tbc2, a nucleus-encoded protein that is required for the translation of the *psbC* mRNA (Auchincloss *et al.*, 2002), and with the chloroplast splicing factor Raa1 (Merendino *et al.*, 2006). This common feature of Tab 1 is the presence of 10 tandem 38–40 amino acid PPPEW repeats located between residues 400 and 782 (Figures 1 and 2a). Repeats of this sort were first identified in Tbc2 (Auchincloss *et al.*, 2002), and they are also present in Raa1 (Merendino *et al.*, 2006) and Tda1 (Eberhard *et al.*, 2011). A BLAST search of the *C. reinhardtii* nuclear genome with Tab 1, Tbc2 and Raa1 as queries revealed a total of 15 proteins that were significantly related to one of these proteins. These proteins were then analyzed for the presence of repeated sequence motifs. A multiple sequence alignment of these repeats was

MMPSLKNLGGGFGRVSVGTTPFRKCARAAGRRAAPGLCSAHSAGESPLLS
NTELERRQTGAQRQVVPSRRQSGSGGPGAPPLAPSQLTAAIHACTSTAQL
LALYRQHGKRFTHVQASAALRQLVNVAPLPAPESEEDAQGGASSSSNSAR
SPEALAARQVTGRLTVRQGEGEVVAEQRRNSNYEASTSAAAPAPAQPPSK
GKGKRPLLQDPEPTTTADLALTEQPSDASTIGTLAPQPDVAAALPAAVPQ
PQPSRRQITSMAAGLAAVLSHSCGVMDGRGVATAANAMARLRYDDLALLE
QLEQRSLVLMGVPAEALPSASASSARHAAVAVGRQQQQEQQQQTRWAVKP
DRRRRDRASPAAASASAGAASSSAAVSAAVEASPAGAAAEAAAPSAAGPM
TASELLALVSAFGSLGYRPSQTWLLSFTRCTAPHLTTYASTPESLPTLLS
SLGNTGHRPPPAWLQSACAAAAPHLPSYSSAQLKALAAGLCAMRHLPDDP
WVAAYLGASAQLLPGYSAAELTVTISSLAGLGCRPGDEWMEGFYARATAV
VGTTGGLTGPQAASILASLSKLNCRPTSDWLNTVLLGTRRSLSDASAQQL
TELAASLARLRFRPPEPWLQQYFNASFQRLPFYTPAQACTAAQALARLGR
RPTKLWMEEFGRLLGAKLPLMPGGQQCEAVAALVDLGYVPSPAWLAGYEQ
HSNAQLASCTSDQLCLVLPALAKVNFRPQVSWLYSFIMSAYSQLDAESSP
QLALVFECLPALTPHGSWLDEIIQICAAEAAMRGAGPVGSSSAAAVSAAE
VAEPDAVVVPVPGIAAAAAAVAAAPAAAPAQPASAGLFATGPLYGDGPGA
SAAAGNGVASTAAVGPIDANVPINGAAAPTDAALSAAATLPDTSTIQISS
ELVPSSDAVTLSAPYRFSASAGYGSAASEAAANAAAAATVLDLTDMQLAA
VPIATPAAAAAAAAAGGRAAEGMNGRREGRGGRSDSGWGGPGGGGGAGGG
SVGRGDLSSPSADLLRRPARVPRPAVAVLASSIEAADAVPTSSASASAPN
PAPSVTPSSVTAGSAISSPYAPASPTASWSDADLLLPGSPDQQPQLLAQP
RPPAQLLQRPRLRSAFSALPASSPAMEPIVAAGSSAAAASSARLDAVAAA
TAARVAAAAASRRPLTSAAPLGLQAMDATAAAAMAAGSAVAAALAAGAAD
DEVRVQYLDSASLNRLIGPPDGPPGPDFSGCQKRAMEAAARKARRGTNNL
FSLGRVWTNELGGSDAGPDIVGGTGGGMPSPVPSPVG*

Figure 1. Structure of the *Tab 1* gene and its product. The *Tab 1* gene is shown with exons (black boxes) and introns (lines). The 5′ and 3′ UTRs are indicated as open boxes at both ends of the gene. The region encoding the PPPEW/OPR repeats is indicated by open yellow boxes at the top. Relevant restriction sites are indicated: St, *Stu*I; A, *Afl*II; X, *Xmn*I; C, *Cla*I; S, *Sma*I; N, *Not*I. The Tab 1 protein amino acid sequence is shown below. Sites corresponding to the five intron insertions are indicated by wedges. The 10 PPPEW/OPR repeats arranged in tandem are indicated alternatively in red and blue and underlined. The *tab 1-F15* mutation creates a stop codon at L_{670}, highlighted in yellow. Residues highlighted in green (L299 and P952) indicate the ends of the fragment fused to the PsaD transit peptide for testing *in vivo* complementation. Residues highlighted in blue (E390 and F747) indicate the ends of the fragment used for the *in vitro* RNA binding experiments. Residues E771 and P866, highlighted in gray, encompass the end of the region that is essential for Tab 1 activity (see Table S2).

performed to derive a consensus sequence named PPPEW (Figure 2b) (for details, see Experimental procedures). Searching the entire *Chlamydomonas* proteome with the PPPEW profile identified 39 additional proteins with several repeats besides Tab 1, Tbc2, Raa1 and Tda1 (Figure 2c). These repeats were found in several copies, often arranged in tandem, in the 43 proteins (Figure 2c). Searching the UniprotKB/Swissprot database for proteins containing similar motifs with the MAST search tool (http://meme.sdsc.edu/meme/intro.html) indicated that this motif is mainly found in protozoans, such as apicomplexans, and in a small family of proteins in the parasitic alpha-proteobacterium *Coxiella burnetii*. Another feature of Tab 1 that is shared with numerous *Chlamydomonas* proteins is the presence of several stretches of four or more Ser, Arg, Gln, Ala and Gly residues, which are interspersed throughout the protein.

Tab 1 lacks a single nucleotide in the *tab 1-F15* mutant

Sequencing of the entire cDNA of Tab 1 in *tab 1-F15* by reverse transcription polymerase chain reaction (RT-PCR) revealed a single base deletion within one of the PPPEW/OPR repeats that changes Leu_{670} to a stop codon (Figures 1 and S2). The 5.4-kb *Not*I genomic fragment that complements *tab 1-F15* lacks the 187 amino acid C-terminal region of Tab 1, which is apparently not essential for the function of the protein. To further test which part of Tab 1 is essential for photoautotrophic growth, the 4.5-kb *Not*I fragment was digested with several restriction enzymes that cut at different sites within the *Tab 1* gene. The digested DNA was then used to assess the transformation yield, which remained high for enzymes cutting downstream of the PPPEW/OPR repeats (Table S2). Digestion with *Cla*I, which cuts at a single site corresponding to P_{866}, located 84 residues downstream of the last OPR repeat, did not significantly affect the transformation yield, suggesting that the remaining C-terminal 421 residues of Tab 1 are not essential (Figure 1). However, digestion with *Xmn*I, which cleaves at a unique site corresponding to E_{771} within the last OPR repeat, significantly diminished the transformation yield, indicating that the region of Tab 1 essential for its activity ends between E_{771} and P_{866}. These results suggest that the entire set of OPR repeats is required for the full activity of Tab 1. The protein contains several hydrophobic regions, some of which are predicted to be transmembrane domains by some, but not all, prediction programs.

Tab 1 accumulates to low levels and is highly unstable

In order to determine the level of Tab 1 an antibody was produced against a recombinant subfragment of Tab 1 (see Experimental procedures). Immunoblot analysis of total wild-type cell extracts with Tab 1 antiserum revealed two bands at 180 and 185 kDa, which were undetectable in *tab 1-F15* and which are significantly larger than the predicted mass of 130 kDa (Figure 3). It is not clear whether the smaller form results from proteolytic cleavage or from authentic processing of the protein. The abnormal migration of Tab 1 may result from its long hydrophobic stretches. After separation of the soluble and insoluble fractions by centrifugation of the total cell extract, most of Tab 1 was found in the latter fraction, suggesting that it is associated with membranes or with the nucleoid (Figure 3). Attempts to localize the protein in the chloroplast after subcellular fractionation failed because the protein was highly sensitive to proteolysis, even in the presence of a cocktail of protease inhibitors. The number of Tab 1 molecules per cell was estimated at 12 000, and the molar ratio between Tab 1 and PsaD, a representative PSI protein, was 1/500 (see Appendix S1 for details; Figure S3).

Because Tab 2 is also required for translation initiation of the *psaB* mRNA in *C. reinhardtii*, it is possible that this protein may interact with Tab 1 (Dauvillée *et al.*, 2003).

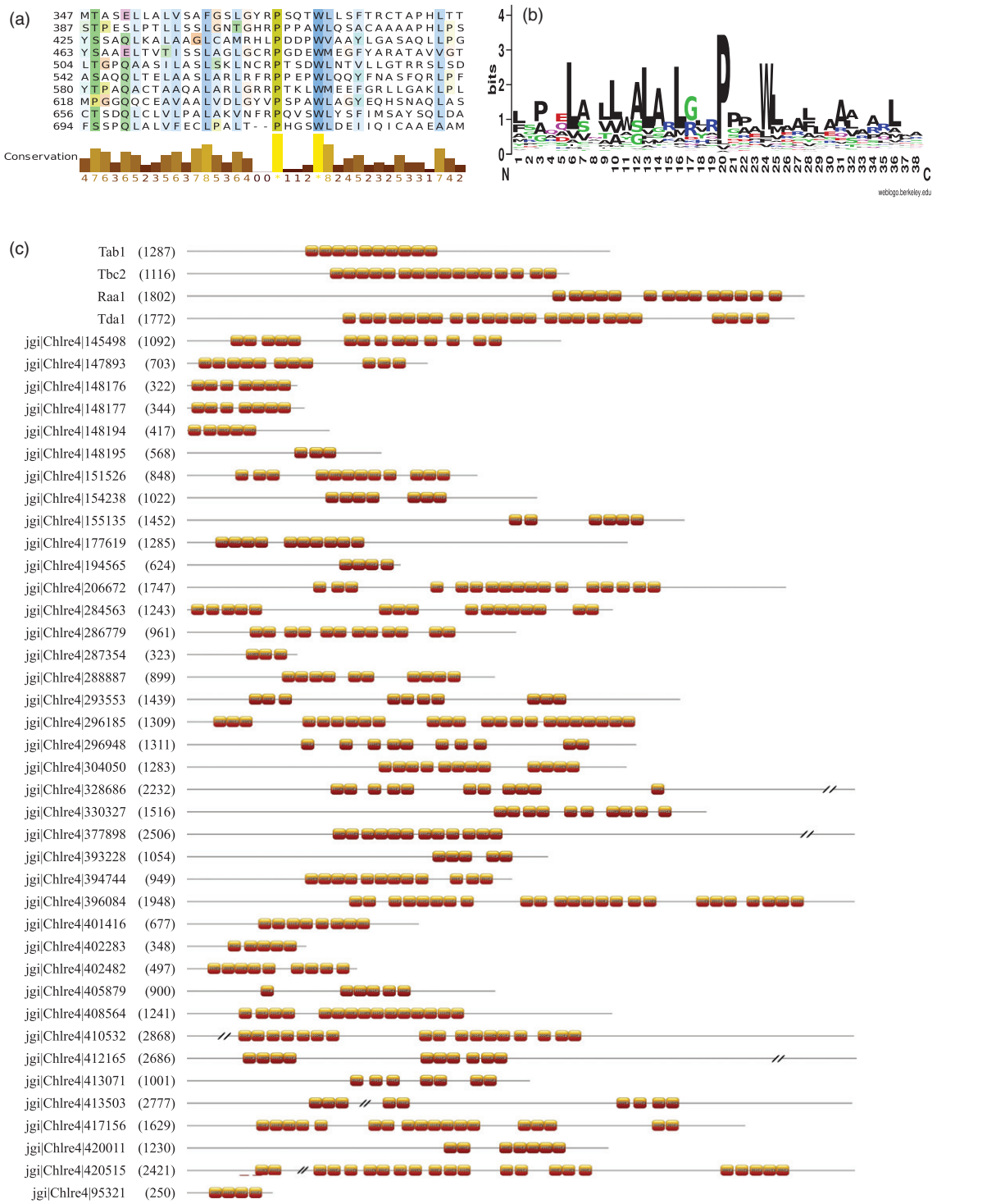

Figure 2. PPPEW/OPR repeats of Tab 1 (a) Alignment of the 10 PPPEW/OPR repeats of Tab 1 (color scheme: CLUSTALX, using 30% identity threshold). Note that in the last repeat two amino acids have been deleted. (b) Consensus PPPEW/OPR sequence derived from the repeats of 43 proteins from the PPPEW family. (c) The PPEW/OPR repeat family in *Chlamydomonas reinhardtii*. Numbers in parenthesis indicate the number of amino acids. The // symbol indicates that the protein sequence has been cut to fit the page.

However, attempts to show an interaction between these two proteins by co-immunoprecipitation failed. If these two proteins were part of the same complex the level of one of these proteins might be decreased in the absence of the other. Contrary to this expectation, the loss of Tab 2 in the *tab 2-F14* mutant did not significantly affect the level of Tab 1.

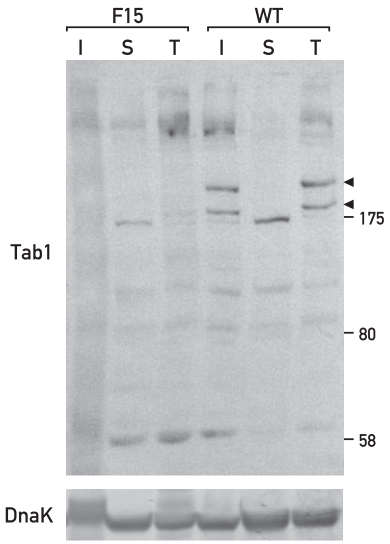

Figure 3. Tab 1 is associated with the insoluble cell fraction. Total cell extracts (T) from wild-type and *tab 1-F15* were prepared and separated into pellet (I) and supernatant (S) fractions. Proteins were fractionated by PAGE and immunoblotted with Tab 1 antibodies. The two TAB 1 bands at 180 and 185 kDa are indicated with arrowheads. I, insoluble fraction; S, soluble fraction; T, total proteins.

Tab 1 is an RNA binding protein

The fact that Tab 1 is involved in the translation of the *psaB* mRNA and that its target is located within the *psaB* 5′-UTR raises the possibility that it is a RNA binding protein. To test this possibility, the Tab 1 cDNA was fused to a T7 promoter, but no recombinant protein could be detected in *Escherichia coli*. To circumvent this problem we fused the first nine PPPEW/OPR repeats of Tab 1 to the maltose binding protein (MBP-Tab 1; for details see Experimental procedures). Under these conditions ~50% of the recombinant protein produced in *E. coli* was soluble. Incubation of increasing quantities of MBP-Tab 1 with ^{32}P-labeled *psaB* 5′-UTR revealed efficient binding of the protein to the RNA, as determined by electrophoretic band shift assays (Figure 4a). We estimate the K_d of this interaction close to 33 nM based on the data shown in Figure 4(a). To test the specificity of binding, competition experiments were performed with an excess quantity of unlabelled *psaB* 5′-UTR or with an unrelated RNA originating from the pKS plasmid. Competition of RNA binding was observed with a 100-fold excess of *psaB* RNA, but not with KS RNA, indicating that Tab 1 shows RNA binding specificity (Figure 4b). To test the specificity amongst different chloroplast mRNAs, competition experiments were performed with the *psbB* and *psbC* 5′-UTRs (Figure 4c). Whereas the *psbB* 5′-UTR did not compete with the *psaB* 5′-UTR, the *psbC* 5′-UTR competed very efficiently, indicating that the Tab 1 RNA binding properties cannot account for the specificity of this protein for *psaB* mRNA translation. Further competition experiments with oligo rA, rG, rU and rC showed that only oligo rU competed with the binding of *psaB* RNA (Figure 4e). The sequence of the *psaB* 5′-UTR is indeed U-rich (43%), and contains U-rich tracts. However, one has to take into account that one of the PPEW/OPR repeats and all of the flanking protein regions were missing from the assayed protein, which might alter the RNA binding properties of Tab 1.

Association of Tab 1 mRNA with large complexes

Because Tab 1 is likely to be involved in the initiation of translation of the *psaB* mRNA we compared the polysome profiles of Tab 1 in the wild type and *tab 1-F15* mutant. After sucrose density centrifugation of the polysomes, RNA was isolated from the different fractions and hybridized with a labeled *psaB* probe (Figure 5). Comparison of the *psaB* mRNA distribution between the wild type and *tab 1-F15* revealed a shift towards smaller sizes in the mutant. However, a significant portion of *psaB* mRNA was still associated with large complexes in *tab 1-F15* (Figure 5, fractions 5–9). A similar observation was made by Eberhard *et al.* (2011) with *atpA* mRNA in the *tda1-F54* mutant, which is deficient in the initiation of translation of this mRNA. These authors noticed that *atpA* mRNA is associated with large non-polysomic complexes that co-sediment with polysomes but are more resistant to EDTA. We therefore examined the sedimentation of *psaB* RNA in wild type and *tab 1-F15* in the presence of EDTA. A drastic shift towards the top of the gradient occurred (Figure 5, fractions 2–4), indicating that the RNA protein complexes detected are all sensitive to EDTA. To further check the specificity of the *tab 1-F15* mutation, the polysome profile of *psbD* mRNA was determined and shown to be very similar in the wild type and *tab 1-F15* (Figure 5).

DISCUSSION

Tab 1 is involved in the translation of the PsaB photosystem I subunit

The origin of chloroplast genomes can be traced to an early symbiotic event in which a cyanobacterium invaded a eukaryotic cell and gradually lost its genetic autonomy by transferring the majority of its genes to the nucleus of the host. Chloroplast genomes therefore represent remnants of cyanobacterial genomes, and code for a limited set of chloroplast proteins involved in photosynthesis, plastid gene expression and in a few other functions. However, expression of these genes requires a surprisingly large number of nucleus-encoded proteins (Barkan and Goldschmidt-Clermont 2000, Eberhard *et al.*, 2008). As an extreme example, trans-splicing of the *psaA* gene of *C. reinhardtii* requires more than 14 nucleus-encoded factors (Goldschmidt-Clermont *et al.*, 1990). Several factors specifically required for the processing/ stability of chloroplast mRNAs have been identified (Stern *et al.*, 2010). Similarly, nucleus-encoded proteins involved in the translation of specific mRNAs have

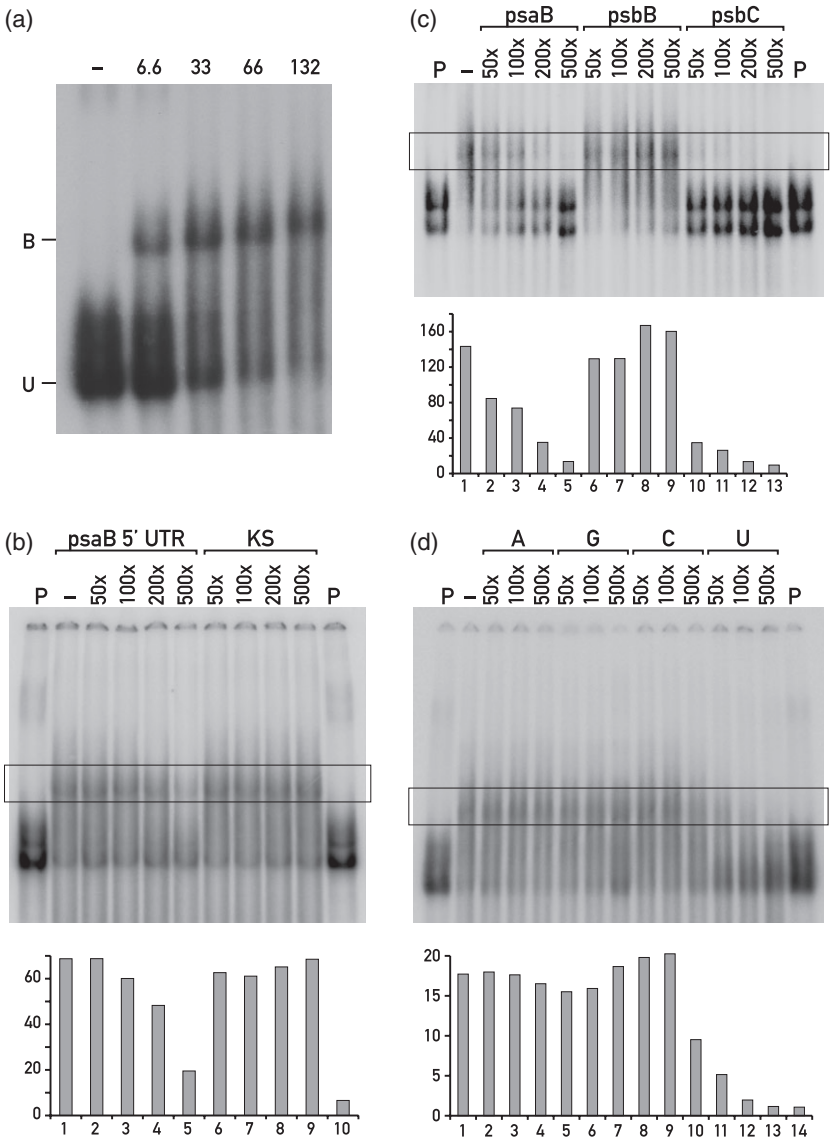

Figure 4. Tab 1 binds to the *psaB* 5′-UTR.
(a) Electrophoretic mobility shift assay (EMSA) of recombinant PPEW-Tab 1 (E390–F747) and ^{32}P-labeled *psaB* 5′-UTR (373 nucleotides). The increasing concentration of recombinant protein used is indicated in nM. U, unbound RNA probe; B, bound RNA probe.
(b) RNA binding competition experiments. EMSA with 100 nM PPEW-Tab 1 and increasing quantities of unlabeled *psaB* 5′-UTR was performed as indicated. KS refers to a transcript from the Bluescript vector (150 nt).
(c) RNA binding competition experiments with 200 nM PPEW-Tab 1 and increasing quantities of unlabeled *psaB* (373 nt), *psbB* (147 nt) and *psbC* (372 nt) 5′-UTR RNA.
(d) RNA binding competition experiments with 100 nM PPEW-Tab 1 and increasing quantities of oligo rA, rG, rC and U, as indicated. The autoradiograms were quantified with a phosphorimager and the results are shown below. Numbers refer to the excess molar quantities of competitor RNA as compared with the labeled *psaB* 5′-UTR. P, ^{32}P-labeled *psaB* 5′-UTR. The RNA binding experiments were repeated three times and gave rise to similar patterns.

been characterized (Eberhard *et al.*, 2008). Coordination of the accumulation of the subunits of a photosynthetic complex occurs mainly through two distinct mechanisms. First, subunits made in excess that cannot assemble are rapidly degraded. Second, coordination of synthesis is achieved through the control of epistasy of synthesis (CES) process, mainly studied in *C. reinhardtii*, in which unassembled subunits directly or indirectly inhibit their own synthesis (Choquet *et al.*, 1998; Choquet and Vallon, 2000; Wostrikoff and Stern, 2007). In the case of PSI the first subunit to be inserted into the thylakoid membrane is PsaB (Nelson and Yocum, 2006). In the absence of PsaB, PsaA cannot assemble and inhibits its own translation (Wostrikoff *et al.*, 2004). Similarly, in the absence of PsaA, the PsaC subunit cannot assemble and inhibits its own translation. In this way synthesis of the subunits is dependent on the ordered assembly of the complex. Because the first subunit to be integrated within the thylakoid membrane is PsaB, it is particularly important to identify and characterize the factors required for the assembly of this anchor protein of PSI that acts as a scaffold for the other subunits. In this study we have identified Tab 1 as a factor required for translation of the *psaB* mRNA. Based on the previous finding that expression of a chimeric gene containing the *psaB* 5′-UTR fused to a reporter coding sequence is dependent on Tab 1 (Stampacchia *et al.*, 1997), this factor probably acts mainly at the level of initiation of translation. We previously characterized the Tab 2 protein that is also specifically required for the initiation of translation of PsaB (Dauvillée *et al.*, 2003). One possibility is that Tab 2 and Tab 1 act in a concerted way, although we were unable to obtain any evidence for an interaction between these two proteins.

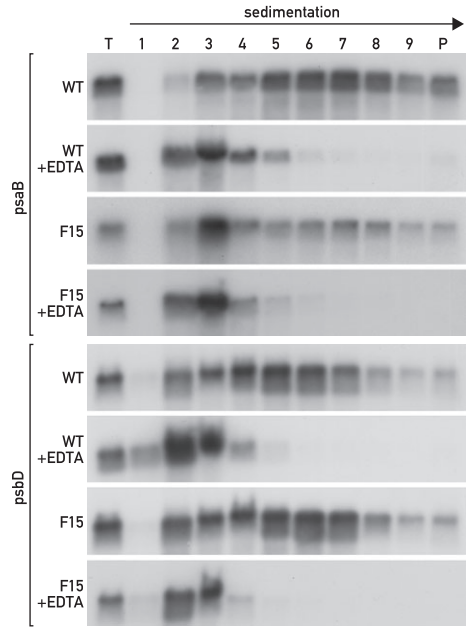

Figure 5. Comparison of polysome profiles in wild-type and *F15-Tab 1* cells. Soluble cell extracts were fractionated by sucrose density gradient centrifugation in the presence of Mg^{2+} or EDTA, as indicated. RNA was isolated from the different fractions shown, blotted on filters and hybridized with *psaB* and *psbD* probes. The direction of sedimentation is indicated with an arrow.

An unusual property of Tab 1 is its high sensitivity to proteolysis. The full-size protein could only be detected after SDS solubilization of total cell extracts. Several attempts to confirm the chloroplast localization of Tab 1 by cellular fractionation resulted in the complete loss of the protein in all cases.

Tab 1 binds *psaB* mRNA

One problem associated with TPR and PPR proteins is that when they are expressed as recombinant protein in *E. coli* they are often insoluble, making the analysis of their biochemical functions difficult. Although RNA binding was reported recently for the HCF107 protein that contains half-a-tetratricopeptide repeat motifs (HAT motifs), which are variants of TPR repeats (Hammani *et al.* 2012), in most cases chloroplast TPR proteins could not be produced in soluble form. A few PPR proteins have been expressed in soluble form, and their RNA binding properties could be examined. Examples include the PPR protein HCF152 from Arabidopsis involved in chloroplast RNA processing (Meierhoff *et al.*, 2003; Nakamura *et al.*, 2003) and PPR10 from maize, which binds to specific sites on chloroplast mRNAs, defines the position of their 5′ and 3′ termini and protects them against exonucleolytic degradation (Pfalz *et al.*, 2009). We did not succeed in expressing the full size Tab 1 protein in soluble form in *E. coli*. The presence of several long Ala stretches are expected to make the protein highly insoluble and prone to aggregation. Even the part of the protein containing the PPPEW/OPR repeats was insoluble when expressed as recombinant protein in *E. coli*. To test its RNA activity we therefore fused this domain to the maltose binding protein and could show that it has RNA binding activity with some specificity towards the *psaB* 5′-UTR, at least compared with an unrelated RNA. Competition experiments with other chloroplast RNAs, however, revealed that although Tab 1 does not bind to the *psbB* 5′-UTR, it also binds efficiently to the *psbC* 5′-UTR. Both the *psaB* and *psbC* 5′-UTRs are U-rich and have a similar base composition (76 and 80% AU, respectively). Although the Tab 3 protein appears to bind preferably U-rich RNA based on the competition with oligo U, this cannot explain its specificity towards the *psaB* mRNA because the *psbB* 5′-UTR is even more U-rich (92% AU). It is possible that its specific binding to the *psaB* 5′-UTR requires the cooperation of other proteins such as Tab 2, which is also necessary for translation of the same message (Dauvillée *et al.*, 2003). In the case of Nac2, a nucleus-encoded factor involved in the stability/processing of the *psbD* mRNA of *C. reinhardtii*, its association with RBP40 was required for the specific binding of the Nac2-RBP40 complex to the *psbD* 5′-UTR (Schwarz *et al.*, 2007).

Polysome profiles of *psaB* mRNA in *tab 1-F15*

Although the polysome profile of *psaB* mRNA in the *tab 1-F15* mutant displays a shift towards the monosome peak, compared with the wild type, a significant fraction of *psaB* mRNA remains associated with large complexes that are sensitive to EDTA (Figure 5). Eberhard *et al.* (2011) also found that in the case of the *tda1* mutant deficient in the initiation of translation of the *atpA* mRNA, this RNA remained associated with large complexes that are insensitive to EDTA. The question arises therefore whether the large complexes seen in Figure 5 for *psaB* mRNA represent polysomes or ribonucleoprotein complexes that could be involved in mRNA storage. It is also possible that Tab 1 could have a dual function in translation, and be involved in both translation initiation and elongation. A deficient initiation coupled to a slow-down in elongation would have a compensatory effect, and could partly stabilize polysomes. Because the mutation in Tab 1 creates a stop codon at residue 670, this would give rise to a truncated product of 69 000 kDa that may still demonstrate partial activity. Although such a protein was undetectable in *tab 1-F15* (Figure 3), we cannot exclude that trace quantities of this protein could give rise to some residual translational activity. Finally, we cannot exclude that Tab 1 is not absolutely required for the initiation of translation of the *psaB* mRNA, but that in its absence the residual level of translation is too low to support photoautotrophic growth.

Tab 1 belongs to the PPPEW/OPR protein family

A striking feature of Tab 1 is the presence of 10 PPPEW/OPR repeats, which constitute 30% of the protein. The remaining

part does not contain any known motif but is characterized by several long stretches of Ala residues. Amongst the nucleus-encoded proteins involved in chloroplast gene expression, many are repeat-containing proteins (Schmitz-Linneweber and Small, 2008). Proteins of this sort are widespread in nature and the atomic structure of several representatives have been determined: alpha-catenin, with 12 ARM repeats of 42 amino acids (Huber *et al.*, 1997); the A subunit of protein phosphatase 2A, with 15 HEAT repeats of 39 amino acids (Groves *et al.*, 1999); Pumilio, with eight Puf repeats of 36 amino acids (Edwards *et al.*, 2001); and protein phosphatase 5, with three TPR (tetratricopeptide repeats) of 38 amino acids (Das *et al.*, 1998). These structural studies have revealed that the repeats exist as tandem helical repeats, and that they form a superhelix with an extended surface that is believed to be involved in protein–protein interactions or in RNA recognition, as shown for *Pumilio*, a translational repressor of the *hunchback* mRNA in *Drosophila* (Edwards *et al.*, 2001). The best-characterized chloroplast repeat-containing proteins are the PPR (penta trico peptide repeats) proteins of land plants. These proteins are part of a large family that includes 450 members (Schmitz-Linneweber and Small, 2008; Small and Peeters, 2000). Although PPR proteins have not yet been crystallized, it is likely that they have a structure similar to the TPR proteins. These PPR proteins are involved in several aspects of chloroplast RNA metabolism, including translation, RNA processing and RNA editing. Some of the PPR proteins and at least one protein with HAT repeats have been shown to bind to specific RNA sequences (Nakamura *et al.*, 2003; Pfalz *et al.*, 2009; Hammani *et al.* 2011). With all these proteins, the superposition of helical domains could form an interaction surface with RNA, similar to the case of Pumilio. In contrast to plants *Chlamydomonas* contains only 11 PPR protein and ~80 TPR protein genes (Eberhard *et al.*, 2011). Instead, the PPPEW/OPR family has greatly expanded in this alga. In this respect the RNA binding activity of Tab 1 revealed in this work is important, as it shows that OPR proteins are directly involved in chloroplast RNA metabolism. Besides translation (Auchincloss *et al.*, 2002; Eberhard *et al.*, 2011), these proteins are also required for RNA processing and splicing (Merendino *et al.*, 2006). It is likely that amongst the other 39 PPPEW/OPR proteins of unknown function identified in this work, several of them participate in specific post-transcriptional steps of chloroplast, and perhaps mitochondrial, gene expression in *C. reinhardtii*, a possibility that can be tested through reverse genetics. This assigned function of OPR proteins is compatible with the large number of nucleus-encoded factors identified through genetic means, which are involved in the expression of specific chloroplast genes. An important problem is to elucidate the molecular basis of the sequence-specific RNA binding activities of the OPR proteins. The crystal structure of the analogous PUF repeats supports a model in which each repeat of the protein binds a single or several nucleotides of its target sequence (Edwards *et al.*, 2001; Wang *et al.*, 2001, 2002). A new challenge will be to elucidate the code of the OPR proteins that connect the succession and amino acid sequence of the repeats with the nucleotide sequence of the RNA target.

EXPERIMENTAL PROCEDURES

Strains and media

Wild-type *C. reinhardtii*, *tab 1-F15* (Stampacchia *et al.*, 1997) and UVM4 (Neupert *et al.*, 2009) mutant cells were grown in Tris-acetate-phosphate (TAP) medium, as described by Harris (1989). For some experiments minimal medium High salt minimum medium (HSM) was used.

Genomic complementation

The mutant strain *tab 1-F15* was rescued by transformation with an ordered cosmid library (Purton and Rochaix, 1994; Zhang *et al.*, 1994), and transformants were selected on either HSM or TAP medium under light, under conditions that block the growth of the *tab 1-F15* mutant. Cells were treated with autolysin to remove the cell wall, and were then transformed using the glass-bead method (Kindle, 1990).

Oligonucleotides and plasmid constructions

The oligonucleotides used in this work are listed in Table S1.

A Tab 1 cDNA *Not*I fragment of 4.2 kb was isolated from a *C. reinhardtii* cDNA library and cloned in Bluescript KS. The cDNA was inserted into pGenD between the *Nde*I and *Eco*RI sites downstream of the *PsaD* promoter and 5′-UTR (Fischer *et al.*, 1996). Because this cDNA was not complete, the authentic coding sequence was reconstituted from the adjacent genomic DNA that encodes the C-terminal 185 amino acids of Tab 1. The HA tag consisting of three copies of the HA peptide YPYDVPDYA was inserted in frame in the unique *Sac*I site of Tab 1, yielding p958. The paromomycin expression cassette (Sizova *et al.*, 1996) was inserted upstream of the *PsaD* promoter in p958, yielding p1001. The 5.4-kb genomic fragment of Tab 1 was inserted in pSL18 with the HA tag at the *Sac*I site, yielding p925.

To test whether the 10 PPPEW/OPR repeats are sufficient for rescuing the *tab 1-F15* mutant, the following plasmids were constructed. The unique *Nae*I site of the pGenD2 plasmid (Fischer *et al.*, 1996) was changed into a *Hpa*I site, and the paromomycin cassette was inserted into the *BamH*I site, yielding plasmid p909 that includes the transit peptide of PsaD. Upon digestion of this plasmid with *Hpa*I and *EcoR*I, a tag (Tag2c) containing His6, HA and StrpII was inserted, yielding plasmid p1034. The 1964-bp *Xba*I-*Pvu*I fragment from the Tab 1 cDNA containing all of the repeats was then inserted into the *Hpa*I site of p1034, yielding p1036.

To produce recombinant protein for RNA binding studies, the region containing the 10 PPPEW repeats was amplified by PCR using the oligonucleotides TAB 1-5′RVb and TAB 1-3′*Sal*I as primers, and the product was inserted in the pST4 plasmid cut with *EcoR*V and *Sal*I, downstream of the maltose binding protein gene driven by the T7 promoter (pST4-MBP-psaB). Moreover, a *Tab 1* gene fragment corresponding to amino acids 390–743 of Tab 1 was amplified with primers TAB 1-5′seq and TAB 1-3′seq (Table S1), and inserted into the *Nde*I site of the pet28a vector (Novagen, now EMD Millipore, http://www.emdmillipore.com). This yielded a recombinant protein of 44 kDa that was used for preparing antibodies against Tab 1.

RNA binding experiments

Because the intact Tab 1 protein is insoluble, a shorter version (amino acids 390–747) fused to the maltose binding protein was produced in *E. coli* with the pST4-MBP-psaB plasmid (Figure S1). The soluble cell extract was loaded on an amylose resin (New England Biolabs, http://www.neb.com), washed with 20 mM Tris-HCl, pH 7.4, 0.2 M NaCl, 10 mM beta-mercaptoethanol, 1 mM EDTA and eluted with washing buffer containing 10 mM maltose, giving rise to four fractions (Figure S1). Fraction 2 was used for the RNA binding experiments. Between 0.8 and 4.8 μg of purified recombinant Tab 1 protein (corresponding to a concentration of 6.6–200 nM) were incubated with ^{32}P-labeled *psaB* 5′-UTR transcript (3 fmol *psaB* RNA *in vitro*, transcribed from pet28-psaB) in 30 μl of 50 mM Tris-HCl, pH 8.0, 100 mM KCl, 6 mM $MgCl_2$, 15% glycerol (v/v), 1 μg yeast tRNA, 16 U RNasin for 20 min on ice (Barneche *et al.*, 2006). Competition studies were performed by pre-incubating the competitor RNA for 5 min before the addition of labeled RNA. In some experiments 10 U of T_1 RNase were added to the reaction. Samples were electrophoresed on a 5% polyacrylamide gel (29 : 1) overnight at 50 V using Tris-borate-EDTA buffer. Gels were dried and exposed to −70°C overnight and quantified with a phosphorimager.

Polysome purification

Preparation and size fractionation of polysomes were performed as described by Barkan (1988) and Yohn *et al.* (1996), with slight modifications. Chloramphenicol (100 μg ml^{-1}) was added to the culture 10 min prior to collecting cells at 2×10^6 ml^{-1} from a 400-ml culture that was split into two equal parts. Each culture was centrifuged, and the pellet was frozen and ground with a mortar and pestle in liquid nitrogen. One extract was resuspended in polysome extraction buffer [200 mM Tris-HCl, pH 9, 200 mM KCl, 35 mM $MgCl_2$ hexahydrate, 25 mM ethylene glycol-bis(b-aminoethylether)-*N*,*N*,*N*9,*N*9-tetraacetic acid (EGTA), 0.2 M sucrose, 1% Triton X-100, 2% polyoxyethelene-10-tridecyl-ether], with 0.5 mg ml^{-1} heparin, 0.7% beta-mercaptoethanol, 100 μg ml^{-1} chloramphenicol, 0.5% Na-deoxycholate and Protease Inhibitor Cocktail (Sigma-Aldrich, http://www.sigmaaldrich.com). In the other extract $MgCl_2$ was replaced by 20 mM EDTA. Following a 20-min centrifugation at 7700 *g* at 4°C, the supernatant was layered onto linear 15–60% sucrose density gradients (40 mM Tris–HCl, pH 9, 20 mM KCl, 10 mM $MgCl_2$, 0.5 mg ml^{-1} heparin, 0.7% beta-mercaptoethanol-mercaptoethanol, and 100 μg ml^{-1} chloramphenicol. In the EDTA gradients $MgCl_2$ was replaced with 1 mM EDTA. Centrifugation was at 4°C (Beckman SW 40; Beckman Coulter, https://www.beckmancoulter.com) for 3.5 h at 256 000 ***g***.

Bioinformatic analysis

The original repeats of Tab 1 were identified using MEME (http://meme.sdsc.edu/meme/intro.html). The same software was used for identifying repeats in the 15 Tab 1-related proteins identified by BLAST. A multiple sequence alignment of the identified repeats was built using MAFFT in mode G-INS-i (Katoh *et al.*, 2005). After manual curation of the multiple sequence alignments, a generalized profile was built using PFTOOLS (PFMAKE in mode semiglobal and substitution matrix BLOSUM45; Sigrist *et al.*, 2002), and calibrated on a shuffled version of UniProtKB/Swiss-Prot (release 34). The generalized profile was named PPPEW.

The PPPEW profile was used to search the full *Chlamydomonas* proteome (Merchant, 2007) (http://genome.jgi-psf.org/Chlre4/Chlre4.home.html), with PFSEARCH (PFTOOLS) combined with FTREP, a program using a signal processing technique (autocorrelation) to promote low-scoring matched repeats if in phase with proximal matches (L. Cerutti, A. Bohne, J.D. Rochaix and O. Vallon, unpublished data).

ACKNOWLEDGEMENTS

We thank Nicolas Roggli for drawings and photography, Silvia Ramundo for technical advice and Michel Goldschmidt-Clermont for his critical reading of the article. This work was supported by grant 31003A_133089/1 from the Swiss National Foundation.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the on-line version of this article:

Figure S1. Recombinant MBP-Tab 1 protein (amino acids 390–747).

Figure S2. Mutation of *tab 1-F15.*

Figure S3. Estimation of the quantity of Tab 1 protein.

Table S1. Oligonucleotides.

Table S2. Determination of the essential region of Tab 1.

Appendix S1. Determination of the quantity of Tab 1 protein.

Please note: As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

REFERENCES

Auchincloss, A.H., Zerges, W., Perron, K., Girard-Bascou, J. and Rochaix, J.D. (2002) Characterization of Tbc2, a nucleus-encoded factor specifically required for translation of the chloroplast psbC mRNA in *Chlamydomonas reinhardtii*. *J. Cell Biol.* **157**, 953–962.

Barkan, A. (1988) Proteins encoded by a complex chloroplast transcription unit are each translated from both monocistronic and polycistronic mRNAs. *EMBO J.* **7**, 2637–2644.

Barkan, A. and Goldschmidt-Clermont, M. (2000) Participation of nuclear genes in chloroplast gene expression. *Biochimie*, **82**, 559–572.

Barneche, F., Winter, V., Crevecoeur, M. and Rochaix, J.D. (2006) ATAB 2 is a novel factor in the signalling pathway of light-controlled synthesis of photosystem proteins. *EMBO J.* **25**, 5907–5918. Epub 2006 Nov 5930.

Choquet, Y. and Vallon, O. (2000) Synthesis, assembly and degradation of thylakoid membrane proteins. *Biochimie*, **82**, 615–634.

Choquet, Y., Stern, D.B., Wostrikoff, K., Kuras, R., Girard-Bascou, J. and Wollman, F.A. (1998) Translation of cytochrome *f* is autoregulated through the 5′ untranslated region of *petA* mRNA in Chlamydomonas chloroplasts. *Proc. Natl. Acad. Sci. USA,* **95**, 4380–4385.

Das, A.K., Cohen, P.W. and Barford, D. (1998) The structure of the tetratricopeptide repeats of protein phosphatase 5: implications for TPR-mediated protein-protein interactions. *EMBO J.* **17**, 1192–1199.

Dauvillée, D., Stampacchia, O., Girard-Bascou, J. and Rochaix, J.D. (2003) Tab 2 is a novel conserved RNA binding protein required for translation of the chloroplast *psaB* mRNA. *EMBO J.* **22**, 6378–6388.

Eberhard, S., Finazzi, G. and Wollman, F.A. (2008) The dynamics of photosynthesis. *Annu. Rev. Genet.* **42**, 463–515.

Eberhard, S., Loiselay, C., Drapier, D., Bujaldon, S., Girard-Bascou, J., Kuras, R., Choquet, Y. and Wollman, F.A. (2011) Dual functions of the nucleus-encoded factor TDA1 in trapping and translation activation of *atpA* transcripts in Chlamydomonas reinhardtii chloroplasts. *Plant J.* **67**, 1055–1066.

Edwards, T.A., Pyle, S.E., Wharton, R.P. and Aggarwal, A.K. (2001) Structure of Pumilio reveals similarity between RNA and peptide binding motifs. *Cell*, **105**, 281–289.

Fischer, N., Stampacchia, O., Redding, K. and Rochaix, J.D. (1996) Selectable marker recycling in the chloroplast. *Mol. Gen. Genet.* **251**, 373–380.

Girard-Bascou, J., Choquet, Y., Schneider, M., Delosme, M. and Dron, M. (1987) Characterization of a chloroplast mutation in the *psaA2* gene of *Chlamydomonas reinhardtii*. *Curr. Genet.* **12**, 489–495.

Goldschmidt-Clermont, M., Girard-Bascou, J., Choquet, Y. and Rochaix, J.D. (1990) Trans-splicing mutants of Chlamydomonas reinhardtii. *Mol. Gen. Genet.* **223**, 417–425.

Groves, M.R., Hanlon, N., Turowski, P., Hemmings, B.A. and Barford, D. (1999) The structure of the protein phosphatase 2A PR65/A subunit reveals the conformation of its 15 tandemly repeated HEAT motifs. *Cell*, **96**, 99–110.

Harris, E.H. (1989) *The Chlamydomonas Source Book: A Comprehensive Guide to Biology and Laboratory Use*. San Diego, CA: Academic Press, Inc.

Hammani, K., Cook, W.B. and Barkan, A. (2012) RNA binding and RNA remodeling activities of the half-a-tetratricopeptide (HAT) protein HCF107 underlie its effects on gene expression. *Proc. Nat. Acad. Sci. USA.* **109**, 5651–5656.

Huber, A.H., Nelson, W.J. and Weis, W.I. (1997) Three-dimensional structure of the armadillo repeat region of beta-catenin. *Cell*, **90**, 871–882.

Katoh, K., Kuma, K., Toh, H. and Miyata, T. (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res.* **33**, 511–518.

Kindle, K.L. (1990) High-frequency transformation of *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci. USA* **87**, 1228–1232.

Meierhoff, K., Felder, S., Nakamura, T., Bechtold, N. and Schuster, G. (2003) HCF152, an Arabidopsis RNA binding pentatricopeptide repeat protein involved in the processing of chloroplast psbB-psbT-psbH-petB-petD RNAs. *Plant Cell* **15**, 1480–1495.

Merchant, S. (2007) The Chlamydomonas genome reveals the evolution of key animal and plant functions. *Science*, **318**, 245–250.

Merendino, L., Perron, K., Rahire, M., Howald, I., Rochaix, J.D. and Goldschmidt-Clermont, M. (2006) A novel multifunctional factor involved in trans-splicing of chloroplast introns in Chlamydomonas. *Nucleic Acids Res.* **34**, 262–274. Print 2006.

Nakamura, T., Meierhoff, K., Westhoff, P. and Schuster, G. (2003) RNA-binding properties of HCF152, an Arabidopsis PPR protein involved in the processing of chloroplast RNA. *Eur. J. Biochem.* **270**, 4070–4081.

Nelson, N. and Yocum, C.F. (2006) Structure and function of photosystems I and II. *Annu. Rev. Plant Biol.* **57**, 521–565.

Neupert, J., Karcher, D. and Bock, R. (2009) Generation of Chlamydomonas strains that efficiently express nuclear transgenes. *Plant J.* **57**, 1140–1150.

Pfalz, J., Bayraktar, O.A., Prikryl, J. and Barkan, A. (2009) Site-specific binding of a PPR protein defines and stabilizes 5′ and 3′ mRNA termini in chloroplasts. *EMBO J.* **28**, 2042–2052.

Purton, S. and Rochaix, J. (1994) Complementation of a *Chlamydomonas reinhardtii* mutant using a genomic cosmid library. *Plant Mol. Biol.* **24**, 533–537.

Schmitz-Linneweber, C. and Small, I. (2008) Pentatricopeptide repeat proteins: a socket set for organelle gene expression. *Trends Plant Sci.* **13**, 663–670.

Schwarz, C., Elles, I., Kortmann, J., Piotrowski, M. and Nickelsen, J. (2007) Synthesis of the D2 protein of photosystem II in Chlamydomonas is controlled by a high molecular mass complex containing the RNA stabilization factor Nac2 and the translational activator RBP40. *Plant Cell* **19**, 3627–3639.

Sigrist, C.J., Cerutti, L., Hulo, N., Gattiker, A., Falquet, L., Pagni, M., Bairoch, A. and Bucher, P. (2002) PROSITE: a documented database using patterns and profiles as motif descriptors. *Brief. Bioinform.* **3**, 265–274.

Sizova, I.A., Lapina, T.V., Frolova, O.N., Alexandrova, N.N., Akopiants, K.E. and Danilenko, V.N. (1996) Stable nuclear transformation of *Chlamydomonas reinhardtii* with a Streptomyces rimosus gene as the selective marker. *Gene*, **181**, 13–18.

Small, I.D. and Peeters, N. (2000) The PPR motif - a TPR-related motif prevalent in plant organellar proteins. *Trends Biochem. Sci.* **25**, 46–47.

Stampacchia, O., Girard-Bascou, J., Zanasco, J.L., Zerges, W., Bennoun, P. and Rochaix, J.D. (1997) A nuclear-encoded function essential for translation of the chloroplast *psaB* mRNA in chlamydomonas. *Plant Cell* **9**, 773–782.

Stern, D.B., Goldschmidt-Clermont, M. and Hanson, M.R. (2010) Chloroplast RNA metabolism. *Annu. Rev. Plant Biol.* **61**, 125–155.

Wang, X., Zamore, P.D. and Hall, T.M. (2001) Crystal structure of a Pumilio homology domain. *Mol. Cell* **7**, 855–865.

Wang, X., McLachlan, J., Zamore, P.D. and Hall, T.M. (2002) Modular recognition of RNA by a human pumilio-homology domain. *Cell*, **110**, 501–512.

Wostrikoff, K. and Stern, D. (2007) Rubisco large-subunit translation is autoregulated in response to its assembly state in tobacco chloroplasts. *Proc. Natl. Acad. Sci. USA* **104**, 6466–6471.

Wostrikoff, K., Girard-Bascou, J., Wollman, F.A. and Choquet, Y. (2004) Biogenesis of PSI involves a cascade of translational autoregulation in the chloroplast of Chlamydomonas. *EMBO J.* **23**, 2696–2705.

Yohn, C.B., Cohen, A., Danon, A. and Mayfield, S.P. (1996) Altered mRNA binding activity and decreased translational initiation in a nuclear mutant lacking translation of the chloroplast *psbA* mRNA. *Mol. Cell. Biol.* **16**, 3560–3566.

Zhang, H., Herman, P.L. and Weeks, D.P. (1994) Gene isolation through genomic complementation using an indexed library of Chlamydomonas reinhardtii DNA. *Plant Mol. Biol.* **24**, 663–672.

