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Translation of the Chloroplast *psbC* mRNA Is Controlled by Interactions between Its 5′ Leader and the Nuclear Loci *TBC1* and *TBC3* in *Chlamydomonas reinhardtii*

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Translation of the chloroplast *psbC* mRNA in *Chlamydomonas reinhardtii* has been shown previously to require interactions between its 5′ untranslated region (5′ UTR) and the functions encoded by two nuclear loci, which we name here *TBC1* and *TBC2*. We show that a 97-nucleotide (nt) region located in the middle of the *psbC* 5′ UTR is required for translation initiation. Unlike most procaryotic *cis*-acting translational control elements, this region has a translational activation function and is located 236 nt upstream from the GUG translation initiation codon. In vivo pulse-labeling of chloroplast-encoded proteins and analyses of the expression of chimeric reporter genes in vivo reveal that a mutation of a newly described locus, *TBC3*, restores translation from the *psbC* 5′ UTR in the absence of either this *cis*-acting element or the wild-type *trans*-acting *TBC1* function. These data demonstrate that sequences located in the middle of the *psbC* 5′ UTR, *TBC1*, and *TBC3* functionally interact to control the translation of the *psbC* mRNA.

Plastids are a developmentally related class of organelles that carry out diverse cellular functions for plants and eucaryotic algae, e.g., photosynthesis, the assimilation of nitrogen and sulfur, and the synthesis of carbohydrates, amino acids, and fatty acids. During their evolution from procaryotic photosynthetic endosymbionts, plastids have become tightly integrated within the cells they inhabit. For example, most of the genes of the ancestral endosymbiont have been either lost or translocated to the nuclear genome. Nucleus-encoded plastid proteins are synthesized on cytosolic ribosomes and targeted to the appropriate plastid compartment (35). Most of the 100 to 200 genes that remain in plastid genomes encode components of the photosynthetic apparatus and the plastid gene expression system. This partitioning of the genes encoding plastid functions necessitates a coordination of the expression of the nuclear and plastid genomes (30, 31).

The coordinated expression of the nuclear and chloroplast genomes is critical for the biogenesis of photosystem II (PSII) during the differentiation of chloroplasts, one of the developmental fates of the plastids. PSII is one of four large multisubunit complexes present in the thylakoid membranes of chloroplasts. This complex extracts electrons from water by using light energy and transfers them across the thylakoid membrane to plastoquinone, the first reaction in the photosynthetic electron transport chain (32). Many PSII subunits are encoded by chloroplast genes, including the four reaction center polypeptides (D1, D2, P5, and P6). Other peripheral subunits are nucleus encoded. While little is known about how PSII is assembled, considerable progress has been made recently towards an understanding of the regulation of the expression of the four PSII reaction core subunit polypeptides (7, 10, 12, 19–21, 23, 26, 37, 39). Nuclear mutants of the eucaryotic green alga *Chlamydomonas reinhardtii* that are affected in the translation of specific chloroplast mRNAs encoding PSII reaction core subunits have been identified (12, 21, 29, 39, 40; reviewed in references 11 and 30). Each of these mutants identifies a nuclear locus that is required for the translation of a specific mRNA encoding a single PSII subunit. The basis for the specificity of these requirements is an enigma, as it seems that a single regulatory system should suffice for the expression of all the chloroplast-encoded PSII subunits.

A particularly well-studied example of this intergenomic coordination regulates the translation of the chloroplast *psbC* mRNA in *C. reinhardtii*, which encodes the 43-kDa chlorophyll-binding PSII reaction center subunit named P6 or, in higher plants, CP43. Genetic analyses have identified *cis*-acting sequences and *trans*-acting functions required for *psbC* mRNA translation initiation (29, 40). The nuclear mutants F34 and F64 each carry a mutation in a different nuclear locus that is specifically required for translation of the *psbC* mRNA. We have named these loci *TBC1* and *TBC2*, respectively, to indicate that they are required for translation (T) of the mRNA encoding the PSII (B) subunit P6 (C). A chloroplast suppressor point mutation of *tbc1-F34* revealed that position +226 (with respect to the 5′ end of the mRNA) is critical for the interaction with the *TBC1*-dependent *trans*-acting factor(s). This position is located 321 nucleotides (nt) from the GUG initiation codon, which begins at position 547. This suppressor mutation, *psbC-F34suI*, does not overcome the requirement for *TBC2*. Another chloroplast mutant, FuD34, is defective in translation of the *psbC* mRNA in a wild-type nuclear background. FuD34 has three closely spaced sequence alterations in the *psbC* 5′ untranslated region (5′ UTR): the insertion of two T residues (between positions +308 and +309) and the deletion of a C residue (position +314) (see Fig. 1). The sequence alterations in *F34suI* and *FuD34* affect two complementary sequences in the *psbC* 5′ UTR, which have the ability to form a stable stem-loop structure including the sequences between positions +222 and +319 (29). This sequence will be referred to hereafter in this work as the 222-319 region. The *psbC-FuD34* and *psbC-F34suI* mutations reveal that sequences in the 222-319 region are critical for translation of the *psbC* mRNA and for the role of *TBC1* in this process.

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TABLE 1. Strains used in this study

Strain	Phenotype (%)[a]	Nuclear genotype[b]	Chloroplast genotype	Source or reference(s)
WT	PSII (100)	*mt*$^+$ *CW15*	*psbC-WT*	This study
FuD34	PSII (0)	*mt*$^+$	*psbC-FuD34*	1, 29
RB1.1	PSII (ND)	*tbc3-rb1*	*psbC-FuD34*	This study
RB1.4A	PSII (10)	*tbc3-rb1 CW15*	*psbC-FuD34*	This study
RB1.12	PSII (10)	*tbc3-rb1 CW15*	*psbC-FuD34*	This study
RB1.2	ND	*mt*$^-$ *tbc3-rb1*	*psbC-FuD34*	This study
RB1.2A	PSII (50)	*tbc1-F34 tbc3-rb1*	*psbC-WT*	This study
RB1.2B	PSII (50)	*tbc1-F34 tbc3-rb1*	*psbC-WT*	This study
RB1.5D	PSII (50)	*mt*$^+$ *tbc3-rb1*	*psbC-WT psbC*(*Δ222-319*)*-aadA*	This study
DP.RB1C, DP.RB1D	PSII (10)	*mt*$^-$/*mt*$^+$ *TBC3/tbc3-rb1 arg7-2/arg7-3*	*psbC-FuD34*	This study
FuD50::psbC(FuD34)-aadA	WT, Sp^S	*mt*$^+$	*psbC*(*FuD34*)*-aadA*	This study
FuD50::psbC(Δ222-319)-aadA	WT, Sp^S	*mt*$^+$	*psbC*(*Δ222-319*)*-aadA*	This study
FuD50::psbC-aadA	WT, Sp^R	*mt*$^+$	*psbC-aadA*	40
F34.2A	PSII (0)	*mt*$^-$ *tbc1-F34*	*WT*	1, 3, 9, 29, 40
F64.2	PSII (0)	*mt*$^-$ *tbc2-F64*	*WT*	1, 3, 29, 40

[a] Values indicate the percent wild-type PSII level as determined by Western blot analysis. Abbreviations: PSII, PSII deficient; ND, not determined; Sp^R, spectinomycin resistant; Sp^S, spectinomycin sensitive; WT, wild type.
[b] *mt*, mating type.

In this study, we have taken genetic approaches to understand better the roles of *TBC1* and the 222-319 region of the *psbC* 5′ UTR in controlling *psbC* mRNA translation. Specifically, we have tested our previous hypothesis that the 222-319 region functions to repress the initiation step of *psbC* mRNA translation (29). We also report the isolation and characterization of a mutation in a newly described locus, *TBC3*, which we propose is also involved in the regulation of *psbC* mRNA translation.

MATERIALS AND METHODS

Culture conditions and genetic analyses. Strains (Table 1) were grown in Tris-acetate-phosphate (TAP) medium (16). Cultures were grown under light at an intensity of 100 microeinsteins m^{-2} s^{-1}, with the exception of the strains derived from crosses 8 and 9, which were grown under light at an intensity of 20 microeinsteins m^{-2} s^{-1}. The induction of gametes, crosses, maturation of zygotes, and dissection of tetrads were carried out as described by Levine and Ebersold (22). Complementation tests were carried out according to the method of Bennoun et al. (3) as modified by Goldschmidt-Clermont et al. (14). Viability tests were performed by spotting 10 μl of each culture onto petri plates containing the various media solidified with 2% agar (Difco). Individual clones were characterized by their fluorescence transients after a dark-light transition (1, 2). RB1.5D (Table 1) is a spectinomycin resistant *mt*$^+$ strain obtained from cross 7 (Table 2). The spectinomycin resistance of RB1.5D, reflecting aminoglycoside adenyltransferase (AAD) expression from *psbC*(*Δ222-319*)*-aadA*, and the partial reduction of PSII determined from its fluorescence transient (data not shown) show that this strain carries *tbc3-rb1*. RB1.5D did not inherit *psbC-FuD34* from its parental *mt*$^-$ strain RB1.2, as ascertained from its fluorescence transient, which is much closer to that of the wild type than that of the strains carrying *tbc3-rb1* and *psbC-FuD34*, and from the observation that many of its progeny (i.e., from crosses 8 and 9) were wild type for photosynthesis.

To characterize the genetic basis of the partial phenotypic reversion of RB1.1 (*mt*$^+$), this strain was crossed with a wild-type strain (*mt*$^-$ [Table 2, cross 1]). If the partial phenotypic reversion results from an alteration of the chloroplast genome, e.g., a reversion of *psbC-FuD34* or a chloroplast suppressor mutation, then all the progeny should express the partial PSII deficiency of RB1.1 (because the chloroplast genome of *C. reinhardtii* is inherited uniparentally from the *mt*$^+$ parent). Alternatively, if the suppression phenotype is caused by a nuclear mutation, then the suppression phenotype should segregate in a Mendelian fashion,

TABLE 2. Crosses performed in this study

Cross no.	Cross (*mt*$^+$ × *mt*$^-$)	Phenotype(s) of tetrads[a]	No. (type) of tetrads	Total no. of tetrads
1	RB1.1 × WT	2(PSII):2(PSII*)	5	5
2	FuD34 × RB1.2	2(PSII):2(PSII*)	21	21
3	RB1.1 × arg7.8	2(PSII*):2(PSII, Arg) 1(PSII):1(PSII*):1(PSII, Arg):1(PSII*, Arg)	24 (PD) 1 (T)	25
4	RB1.1 × arg7	2(PSII*):2(PSII, Arg) 1(PSII):1(PSII*):1(PSII, Arg):1(PSII*, Arg)	5 (PD) 1 (T)	6
5	MA16 × RB1.2	4(PSII):0(WT)	15	15
6	FuD50::psbC(FuD34)-aadA × RB1.2	2(Sp^R):2(Sp^S)	5	5
7	FuD50::psbC(Δ222-319)-aadA × RB1.2	2(Sp^R):2(Sp^S)	7	7
8	RB1.5D × F34.2A	2(PSII):2(WT) 2(PSII*):2(WT) 1(PSII):1(PSII*):2(WT)	3 (PD) 5 (NPD) 2 (T)	10
9	RB1.5D × F64.2	2(PSII):2(WT)	14	14

[a] Abbreviations: WT, wild type; Arg, arginine requiring; PSII, PSII deficient; PSII*, partial PSII activity; Sp^R, spectinomycin resistant; Sp^S, spectinomycin sensitive.

resulting in two partially PSII-deficient (i.e., suppressed) and two PSII-deficient members in each tetrad. In this case, the unaltered chloroplast genome harboring the *psbC-FuD34* mutation should be transmitted to all progeny from the mt^+ parent, RB1.1. Consistent with the latter hypothesis, each of the five tetrads obtained from this cross showed a 2:2 segregation of the weak and severe PSII fluorescence phenotypes (Table 2, cross 1). To confirm that the suppression phenotype is caused by a nuclear mutation, a partially PSII-deficient mt^- progeny from this cross (carrying the putative suppressor mutation) was backcrossed with FuD34 (mt^+). In this cross, all progeny inherited the *psbC-FuD34* mutation from FuD34. The 21 tetrads obtained showed a 2:2 segregation of the partial and severe PSII deficiencies, based on their fluorescence transients (Table 2, cross 2). These results demonstrate that the partial suppression of *psbC-FuD34* is caused by a nuclear mutation, which we name *tbc3-rb1*.

To determine whether *tbc3-rb1* can restore expression of the *aadA* reporter gene from the *psbC-FuD34* 5′ UTR, the chloroplast transformant FuD50::psbC(FuD34)-aadA (mt^+ *TBC3*) was crossed with *RB1.2* (mt^- *tbc3-rb1*) (Table 2, cross 6). In this cross, the psbC(FuD34)-aadA chimeric reporter gene (in the chloroplast genome of the mt^+ parent) was transmitted uniparentally to all the progeny, while the wild-type and mutant *TBC3* alleles were transmitted in a Mendelian fashion (i.e., 2:2). Although neither of the parental strains are resistant to spectinomycin, in each of the five tetrads from this cross, two members were spectinomycin resistant (Table 2, cross 6; see Fig. 2). The other two members were sensitive to the drug. This inheritance pattern suggests that *aadA* expression from the mutant *psbC-FuD34* 5′ UTR is restored by the *tbc3-rb1* mutation. Test crosses of the 10 mt^- progeny with FuD34 (mt^+) confirmed that the spectinomycin-resistant members had inherited *tbc3-rb1* (data not shown). Moreover, all the spectinomycin-resistant progeny of cross 6 produced fluorescent transients that were similar to the transient produced by *tbc3-rb1* in the presence of a wild-type *psbC* allele, which indicates a weak PSII deficiency (described in Results). When test crossed to *FuD34* (mt^+), the spectinomycin sensitive mt^- progeny produced only severely PSII-deficient progeny (data not shown), confirming that they are wild type for *TBC3*.

To determine whether the suppression of *psbC-FuD34* is mediated through the 222-319 region of the *psbC* 5′ UTR, RB1.2 (mt^- *tbc3-rb1*) (Table 1) was crossed with the FuD50::psbC(Δ222-319)-aadA chloroplast transformant (mt^+) (Table 2, cross 7). The design of this experiment and the analysis of its results were similar to those in the experiment described above. Two members of each of the seven tetrads from this cross were resistant to spectinomycin (see Fig. 2A), suggesting that *tbc3-rb1* can restore translation of the mutant chimeric mRNA. Analysis of the fluorescence transients of 100 to 200 random progeny from test crosses of the mt^- progeny with FuD34 (mt^-) confirmed that the spectinomycin resistance and sensitivity correlated with the inheritance of *tbc3-rb1* and *TBC3*, respectively (data not shown).

To determine whether the PSII deficiency produced by *tbc1-F34* is suppressed by *tbc3-rb1*, RB1.5D (mt^+ *TBC1 tbc3-rb1* [*psbC-WT*]) was crossed with F34.2A (mt^- *tbc1-F34 TBC3* [*psbC-WT*]) (Table 2, cross 8). Because *TBC1* and *TBC3* are unlinked (see Results), similar numbers of parental ditype (PD) and nonparental ditype (NPD) tetrads should be obtained. NPDs contain two double mutant members (*tbc3-rb1*; *tbc1-F34*) and two members that are wild type for both loci. Suppression would be indicated by PSII activity in these double mutants; i.e., approximately half of the PD and NPD tetrads would have four members with PSII activity. Tetratype (T) tetrads would contain three members with PSII activity and one PSII-deficient member. Alternatively, if *tbc3-rb1* does not suppress *tbc1-F34*, then the two progeny bearing *tbc1-F34* in the NPDs and Ts would be PSII deficient and all tetrads would show the PD phenotype for photosynthesis. (For simplicity, we scored the very weak PSII deficiency produced by *tbc3-rb1* as wild type.) Fluorescence transient analysis of the 10 tetrads obtained from this cross supported the argument for a suppression interaction (Table 2); three tetrads contained two wild-type and two PSII-deficient members (PDs), five tetrads contained two partially PSII-deficient members and two wild-type members (NPDs), and two tetrads (Ts) contained two wild-type members, one partially PSII-deficient member, and one completely PSII-deficient member. The partially PSII-deficient progeny were presumed to be the double mutants in which *tbc1-F34* was partially suppressed by *tbc3-rb1*. To test this hypothesis, all four members from three NPDs were crossed with the wild type and 100 to 200 progeny from each test cross were scored for PSII activity based on fluorescence transients. The two partially PSII-deficient members in each of these tetrads generated approximately 25% completely PSII-deficient progeny, consistent with their genotype being *tbc1-F34 tbc3-rb1*, while the two wild-type members gave rise to only wild-type progeny (data not shown).

To test whether the PSII deficiency produced by *tbc2-F64* is suppressed by *tbc3-rb1*, RB1.5D (*TBC2 tbc3-rb1* [*psbC-WT*]) was crossed with F64.2 (mt^- *tbc2-F64 TBC3* [*psbC-WT*]) (Table 2, cross 9) and the resulting tetrads were scored for PSII activity based on fluorescence transient analysis. The 14 tetrads showed a 2:2 segregation of photosynthesis and complete PSII deficiency (Table 2). Following the rationale described above, and knowing that *TBC2* and *TBC3* are unlinked (see Discussion), we conclude that *tbc3-rb1* does not suppress *tbc2-F64*.

Pulse-labeling and gel electrophoresis. Pulse-labeling of whole cells was carried out in minimal medium with [^{14}C]acetate for 5 min under an illumination of 20 microeinsteins m^{-2} s^{-1} in the presence of cycloheximide (8 μg/ml) (8). Lysates of the labeled cells were fractionated by electrophoresis on a sodium dodecyl sulfate (SDS)-polyacrylamide gel (7.5 to 12% polyacrylamide) as described previously (4). To standardize the amount of lysate analyzed for each strain, 15 μg of chlorophyll was loaded in each lane, except in lanes 2 and 3, which contained 10 and 5 μg, respectively. The gels were then subjected to autoradiography.

Nucleic acid manipulations. Standard techniques were used to manipulate and analyze nucleic acids. For Northern blot experiments, 5-μg samples of total RNA were electrophoresed through a 1% agarose gel containing formaldehyde and MOPS (morpholinepropanesulfonic acid) buffer, transferred to Hybond-C+ membranes (Amersham), and probed with double-stranded DNA probes, which had been random primer labeled with [α-^{32}P]dATP. The *aadA* probe was a 0.81-kb *Nco*I-*Pst*I cloned DNA fragment corresponding to the *aadA* structural gene (15). The *psbC* probe was a 0.95-kb *Hin*dIII fragment derived from the chloroplast DNA fragment R9 (28). The relative amounts of the RNA in the samples were standardized by probing the blots with either an *Eco*RI-*Dde*I fragment probe derived from the *psbB* locus (26) or a 1.1-kb *Bam*HI fragment (Bam01) containing *psaB*.

Construction of chimeric genes. For the construction of *psbC(FuD34)-aadA*, two oligonucleotide primers were used to amplify by PCR a DNA fragment corresponding to the *psbC* promoter and 5′ UTR (from −67 to +549) from a cloned 780-bp chloroplast *Taq*I DNA fragment from *FuD34* (29). The oligonucleotides used were *psbC* (*Cla*I, −67/−52), 5′ GATCGATGGATCCTCGATA AACTTTAGTT 3′), and *psbC* (*Nco*I, 549/532), 5′ ACGAGCCATGGACACT TTGCATTACCTCCG 3′. To construct the *psbC* 5′ UTR with a deletion of the region between positions +222 and +319, DNA fragments flanking this region were amplified by PCR; the 5′ fragment was derived from positions −67 to +221 by using the primers *psbC* (*Cla*I, −67/−52) and *psbC* (*Bcl*I, 227/204), 5′ TGAT CAGATTTAAAACAACTTAAT 3′, and the 3′ fragment was derived from positions +320 to +549 with primers *psbC* (*Bcl*I, 320/341), 5′ CTGATCATAA AATTCTATTCTTAAAAAC 3′, and *psbC* (*Nco*I, 549/532). The PCR-amplified DNA fragments were phosphorylated with T4 polynucleotide kinase, cloned into the dephosphorylated *Hin*cII site of pBluescribe (Stratagene), and sequenced from a double-stranded template with the Sequenase system (U.S. Biochemical). The fragments flanking the deletion were ligated by using the *Bcl*I site. The fragments containing the mutated UTRs were excised by digestion with *Cla*I and *Nco*I and translationally fused to the *aadA* coding sequence in the cg20-atpB-INT chloroplast transformation vector (for details of the transformation strategy, see reference 33). These constructs were transformed into the chloroplast genome of FuD50 with a microprojectile gun as described previously (42). Transformants were confirmed by genomic Southern blot analyses as described previously (40).

AAD assays. AAD assays were performed as described previously (15) with the following modifications. Cultures (20 ml) were grown in TAP medium under light at an intensity of 100 microeinsteins m^{-2} s^{-1} in TAP medium (16) to 2×10^6 to 5×10^6 cells ml^{-1}. Cells were concentrated by centrifugation, washed once in distilled H_2O (cell pellets can be stored at this point at −70°C with no detectable loss of activity), resuspended in 0.5 ml AAD-RB (4 mM $MgCl_2$, 100 mM NH_4Cl, 25 mM Tris-HCl [pH 8], 20% glycerol, 0.5 mM dithiothreitol), sonicated at 60 W for 1.0 min on ice, and centrifuged at 100,000 × *g*. The protein concentration of the supernatant was determined by the assay of Smith et al. (36). AAD reactions and quantification of adenylated streptomycin were carried out as described previously (15), except that 1.0 μCi of [α-^{32}P]ATP was used per reaction.

Western blot analyses. For the Western blot analyses of the P6 and D1 polypeptide levels (see Fig. 4), extracts were prepared by resuspension of the cells in 50 mM Tris-HCl (pH 6.8)–2% SDS, incubation at 100°C for 1.0 min, and then centrifugation for 10 min in a microcentrifuge. Supernatants were used as protein extracts. For the Western blot analysis of AAD (see Fig. 1 and 2), protein extracts were prepared as described above for the AAD assays. The protein concentration of the supernatant was determined by the assay of Smith et al. (36). Protein samples (30 μg) were made with 50 mM Tris-HCl (pH 6.8)–2% SDS–100 mM DTT–0.25% bromophenol blue, electrophoresed through 15% acrylamide gels, and transferred to Protran nitrocellulose membranes (Schleicher and Schuell). Protein transfer was verified by staining the filter with Ponceau S (34). Filters were blocked in 5% nonfat dry milk–0.02% Tween 20–phosphate-buffered saline (PBS) (34) and incubated with primary antisera raised against D1 or the recombinant AAD polypeptide (40). For the anti-AAD antisera, this incubation was carried out for at least 12 h at 4°C. Filters were washed 3 times in PBS, reacted with peroxidase-linked anti-rabbit immunoglobulin (Amersham) for 1 h, and washed 3 times with PBS. Signals were revealed with a chemiluminescence detection system (Supersignal; Pierce).

RESULTS

Translation from the *psbC* 5′ UTR requires a 97-nt region. The *psbC* 5′ UTR is able to promote the expression of the bacterial *aadA* gene in vivo (40). To determine whether the three position changes in the *psbC* 5′ UTR of *FuD34* (Fig. 1) (29), and not another unidentified mutation, abolish the translation of the *psbC* mRNA in this strain, we investigated whether this 5′ UTR is unable to drive the expression of the

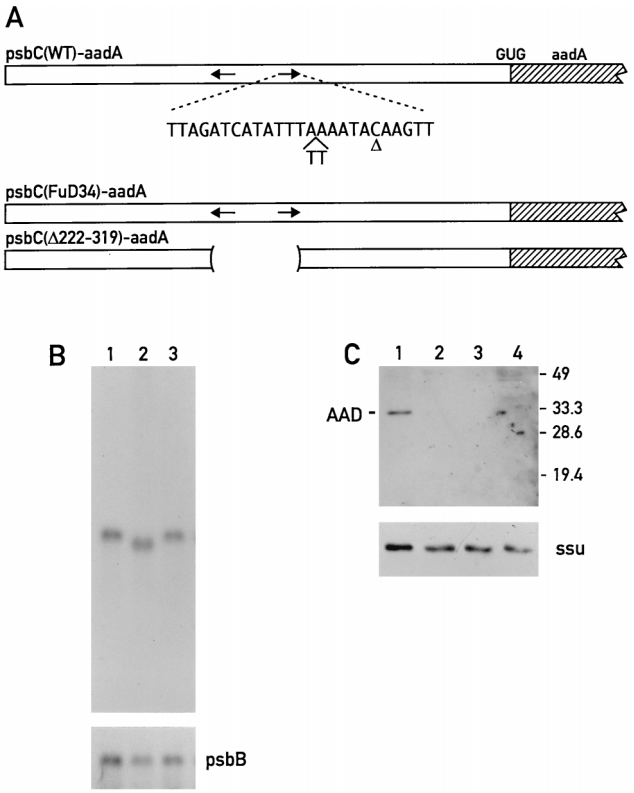

FIG. 1. The 222-319 region is required for translation from the *psbC* 5′ UTR. (A) The 5′ UTRs used in the chimeric genes are illustrated. The arrows indicate the complementary sequences in the 222-319 region. The *psbC-FuD34* mutation, i.e., an insertion of two T residues and the deletion of a C residue, is shown relative to the wild-type sequence. (B) Northern blot analysis of total RNA preparations from the chloroplast transformants. Lane 1, FuD50::psbC(WT)-aadA; lane 2, FuD50::psbC(Δ222-319)-aadA; and lane 3, FuD50::psbC(FuD34)-aadA. The level of the *psbB* mRNA was used to standardize for the amount of RNA in each lane. (C) Western blot analysis of the AAD polypeptide expressed in the chimeric reporter gene chloroplast transformants. Lane 1, FuD50::psbC(WT)-aadA; lane 2, FuD50::psbC(FuD34)-aadA; lane 3, FuD50::psbC(Δ222-319)-aadA; and lane 4, a nontransformed wild-type strain. The level of the small subunit of the RUBISCO (ssu) detected with an antiserum raised against the holoenzyme was used to standardize for the amount of protein in each lane. Molecular masses (in kilodaltons) are indicated to the right of the gel.

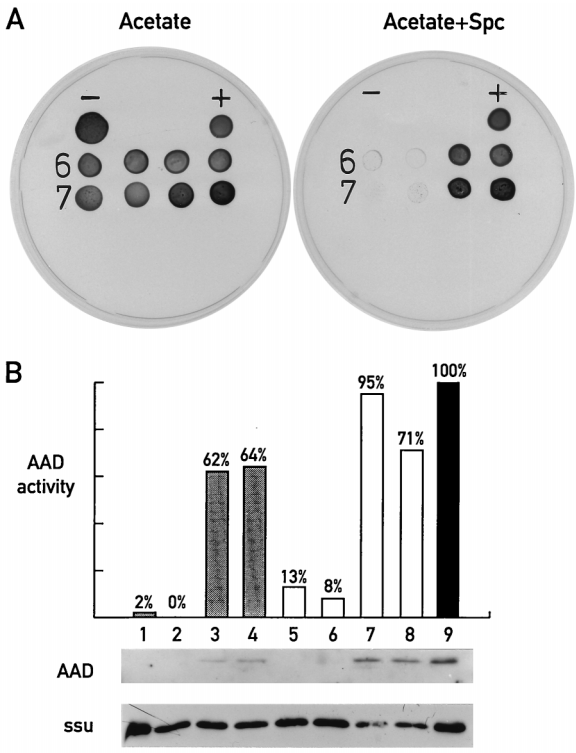

FIG. 2. Levels of AAD polypeptide and its enzymatic activity. (A) Tests for *aadA*-mediated spectinomycin resistance of a representative tetrad from cross 6 (Table 2), FuD50::psbC(FuD34)-aadA × RB1.2 (Table 1), and cross 7, FuD50::psbC(Δ222-319)-aadA × RB1.2. A minus sign indicates a nontransformed wild-type strain. A plus sign indicates a transformant of the FuD50::psbC(wt)-aadA chimeric gene. Acetate indicates nonselective TAP medium. Acetate+Spc indicates TAP medium with 250 μg of spectinomycin ml^{-1}. (B) Bar heights depict the level of AAD enzyme activity detected in extracts of a tetrad from cross 6 [FuD50::psbC(FuD34)-aadA] (bars 1 to 4) and cross 7 [FuD50::psbC(Δ222-320)-aadA] (bars 5 to 8) relative to the level detected in an extract of the FuD50::psbC(WT)-aadA transformant (bar 9). The AAD polypeptide detected by Western blot analysis in the same extracts is shown below the graph. The level of the small subunit of the RUBISCO (ssu), detected with an antiserum raised against the holoenzyme, was used to standardize for the amount of protein in each lane.

aadA reporter gene. The *psbC* 5′ UTR from *FuD34* was translationally fused to the coding region of *aadA*, which confers spectinomycin and streptomycin resistance to *C. reinhardtii* when expressed in the chloroplast (15). The resulting chimeric gene, *psbC*(*FuD34*)-*aadA*, was integrated into the chloroplast genome of FuD50 as described previously (40). Although strains carrying the *psbC*(*FuD34*)-*aadA* integration accumulate the chimeric mRNA (Fig. 1B, lane 3), they do not express the *aadA* reporter gene, as evidenced by their spectinomycin sensitivity (Fig. 2A, left-hand two strains in the tetrad from cross 6; Table 2). Moreover, the AAD polypeptide was not detected in an extract of this transformant with antisera raised against the recombinant protein (Fig. 1C, lane 2). Nor did we detect the enzymatic activity encoded by *aadA* in extracts from strains carrying this chimeric gene (Fig. 2B, bars 1 and 2). In contrast, the chimeric gene with the wild-type 5′ UTR and otherwise identical to *psbC*(*FuD34*)-*aadA* confers spectinomycin resistance (Fig. 2A) (40) and expresses the AAD polypeptide (Fig. 1C, lane 1) and AAD enzymatic activity (Fig. 2B, bar 9). Thus, we conclude that the three changes in the *psbC* 5′ UTR of *FuD34* (Fig. 1A) correspond to the *psbC-FuD34* mutation that abolishes the translation of the *psbC* mRNA.

It was postulated previously that *psbC-FuD34* blocks translation of the *psbC* mRNA by stabilizing a repressive stem-loop secondary structure in the 222-319 region (29). Thus, we expected that the removal of this region would derepress translation from the *psbC* 5′ UTR. To test this prediction, a mutant 5′ UTR with a deletion in the 222-319 region was translationally fused to the *aadA* structural gene and the resulting chimeric gene [*psbC*(*Δ222-319*)-*aadA*] was introduced into the chloroplast genome of *FuD50*. Contrary to the hypothesis, the 222-319 deletion drastically impaired the translation of the chimeric reporter mRNA. Even though strains carrying *psbC*(*Δ222-319*)-*aadA* accumulate the chimeric mRNA (Fig. 1B, lane 2), they were sensitive to spectinomycin (Fig. 2A, left-hand two strains in the tetrad from cross 7; Table 2). In addition, extracts prepared from them lacked detectable AAD polypeptide (Fig. 1C, lane 3; Fig. 2B, lanes 5 and 6) and contained approximately 10-fold less AAD enzyme activity than did extracts of the control transformant carrying the *psbC*(*WT*)-*aadA* chimeric gene (Fig. 2B, compare bars 5 and 6 with bar 9). This reduction does not result from an indirect effect of the deletion, as the replacement of the 222-319 region

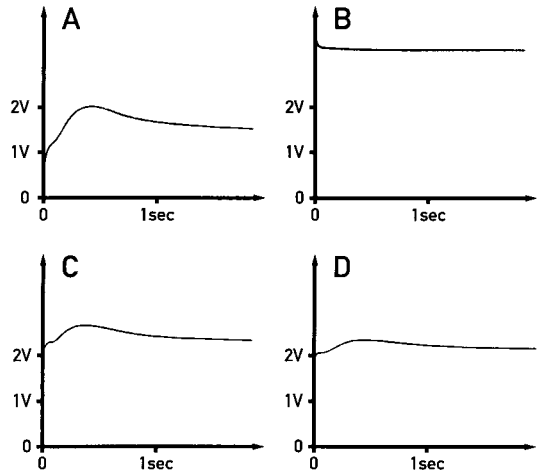

FIG. 3. Fluorescence transients produced by wild-type and mutant strains. (A) The initial rise in the fluorescence transient reflects PSII activity in the wild type (*TBC3 psbC-WT*). (B) The fluorescence transient of FuD34 (*TBC3 psbC-FuD34*) is flat due to its lack of PSII. (C) The fluorescence transient of the partial phenotypic revertant of FuD34, RB1.1 (*tbc3-rb1 psbC-FuD34*), is intermediate between those of the wild type and FuD34. (D) The diploid, which is heterozygous for the *TBC3* locus, DP.RB1C (*tbc3-rb1/TBC3 psbC-FuD34*), gives a fluorescence transient similar to that of RB1.1 (C).

with a heterologous sequence—which is similarly A and U rich, has the potential to form a stem-loop secondary structure, and is identical in size to the deleted region—did not restore *aadA* expression (41). Therefore, and contrary to the previous hypothesis, the 222-319 region, or some element within it, is required for translation from the *psbC* 5′ UTR. The *psbC-FuD34* mutation appears to abolish the ability of this element to promote the initiation of *psbC* mRNA translation.

A nuclear mutation partially suppresses the PSII-deficient phenotype of FuD34. FuD34 completely lacks PSII activity due to its inability to translate the *psbC* mRNA (29). Consequently, this mutant gives a flat fluorescence transient (Fig. 3B) and is inviable on minimal medium lacking a reduced carbon source (data not shown). We isolated a spontaneous partial phenotypic revertant of FuD34 and named it RB1.1. RB1.1 is viable on minimal media (data not shown) and gives a fluorescence induction transient (Fig. 3C) that is intermediate between those of FuD34 (Fig. 3B) and the wild type (Fig. 3A). The observation that this reversion phenotype segregates in a Mendelian fashion in crosses demonstrates that it is produced by a nuclear suppressor mutation (see Materials and Methods for experimental details). We have named this suppressor mutation *tbc3-rb1* and the affected locus *TBC3*. (It will be shown below that *TBC3* is genetically distinct from *TBC1* and *TBC2*.)

To determine whether the suppression phenotype of *tbc3-rb1* is recessive or dominant, we generated two diploid strains that are heterozygous at the *TBC3* locus and carry the suppressible chloroplast *psbC-FuD34* mutation (DP.RB1C and DP.RB1D) (Table 1). Both diploid strains produced fluorescence transients (Fig. 3D) that were similar to those produced by the haploid strain RB1.1 (*tbc3-rb1 psbC-FuD34*) (Fig. 3C) and indicative of partial PSII activity. Thus, the observation that *psbC-FuD34* can be suppressed by *tbc3-rb1* in the presence of a wild-type *TBC3* allele demonstrates that this suppression phenotype is dominant.

tbc3-rb1 produces a weak PSII deficiency, which is independent of *psbC-FuD34*. Strains that carry *tbc3-rb1* and a wild-type *psbC* allele generate a fluorescence transient that is indicative of a slight diminution of PSII activity (data not shown).

While constructing double-mutant strains carrying *tbc3-rb1* and *arg7-8* or *arg7*, we found that the *TBC3* and *ARG7* loci are linked and approximately 3 centimorgans apart (Table 2, crosses 3 and 4). *TBC1* and *TBC2* have been shown previously to be genetically distinct (3). Neither *TBC1* nor *TBC2* is linked to *ARG7* (41), and thus neither locus is linked to *TBC3*. Therefore, *TBC3* is a newly described locus, which we propose to be involved in the regulation of *psbC* mRNA translation (see below).

***tbc3-rb1* partially restores the PSII complex in a strain carrying *psbC-FuD34*.** As another measure of the restoration of PSII by *tbc3-rb1*, we determined the level of the PSII subunits P6 (encoded by *psbC*) and D1 (encoded by *psbA*) in two strains (RB1.4A and RB1.12) (Table 1) that carry this suppressor mutation and the *psbC* mutation it suppresses, *psbC-FuD34*. The stability of both polypeptides requires the synthesis of the other reaction center subunits and the assembly of PSII (29). Therefore, the steady-state levels of these subunits are accurate measures of the amount of PSII in a strain. Examination of the Western blots in Fig. 4 revealed that P6 and D1 accumulate in wild-type strains (*TBC1 TBC3* [*psbC-WT*]) (lane 1 in both panels) but not in extracts of FuD34 (*TBC1 TBC3* [*psbC-FuD34*]) (lane 4 in both panels). In *FuD34*, D1 is rapidly degraded in the absence of P6 synthesis (29). In the strains in which the PSII deficiency produced by *psbC-FuD34* is partially suppressed by *tbc3-rb1* (RB1.4A and RB1.12 [Table 1]), both P6 (Fig. 4A, lane 5) (data not shown for RB1.12) and D1 (Fig. 4B, lanes 5 and 6) were detected, although at levels that were considerably lower than in the wild type. To quantify the level of PSII in these strains, the intensities of the P6 and D1 signals detected in protein extracts prepared from them were compared with the signals obtained from wild-type extract at various dilutions (supplemented with extract from FuD34 so that the lanes contained equal amounts of protein). These comparisons revealed that both P6 and D1 in RB1.4A and RB1.12 accumulate to approximately 10% of their respective levels in the wild-type control strain (Fig. 4A, compare lanes 3 and 5; Fig. 4B, compare lane 3 with lanes 6 and 7). From these data, we conclude that *tbc3-rb1* can partially restore the accumulation of the PSII complex in strains carrying the *psbC-FuD34* mutation.

Similarly, Western blot analyses of the levels of P6 and D1 confirmed that the suppression of *psbC-FuD34* by *tbc3-rb1* is dominant; the level of the PSII reaction center in DP.RB1C (Fig. 4, lanes 7) was determined to be ca. 10% of the wild-type level (lanes 3) and similar to the level in haploid strains carrying *psbC-FuD34* and one copy of *tbc3-rb1* (Fig. 4A, lane 5, and Fig. 4B, lanes 6 and 7).

In addition, the level of PSII is reduced approximately twofold by the *tbc3-rb1* mutation in the presence of a wild-type *psbC* allele, based on the level of the PSII core subunits P6 and D1 in RB1.5D (*tbc3-rb1 psbC-WT*) (Table 1) as determined by the Western blot analysis in Fig. 4 (Fig. 4A, compare lanes 2 and 6; Fig. 4B, compare lanes 2 and 8). Thus, *tbc3-rb1* produces a slight PSII deficiency, which is independent of *psbC-FuD34* and consistent with the fluorescence transients produced by *tbc3-rb1* in the presence of a wild-type *psbC* allele (described above).

The *tbc3-rb1* suppressor mutation restores the synthesis of the P6 polypeptide from the *psbC* mRNA bearing the *psbC-FuD34* mutation. To test directly whether *tbc3-rb1* restores the synthesis of P6 from the *psbC-FuD34* mutant allele, we compared the chloroplast-encoded proteins synthesized in the wild type (*TBC3* [*psbC-WT*]), FuD34 (*TBC3* [*psbC-FuD34I*]), RB1.4A and RB1.12 (*tbc3-rb1* [*psbC-FuD34*]), and RB1.5D (*tbc3-rb1* [*psbC-WT*]) following a 5-min pulse with [^{14}C]acetate

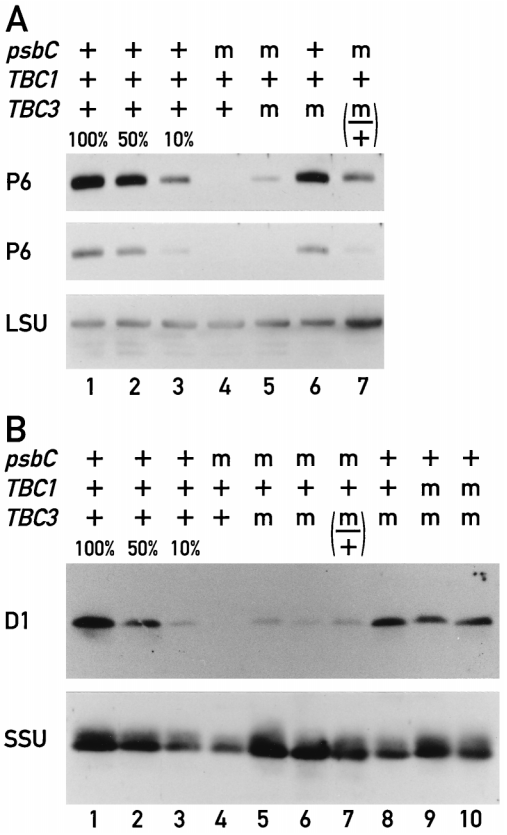

FIG. 4. PSII levels determined by Western blot analysis. (A) The Western blot was reacted with antisera raised against P6 (the PSII subunit encoded by *psbC*). Lane 1, 100%, or 20 μg of wild type (*TBC1 TBC3 psbC-WT*); lane 2, 50%, or 10 μg of wild type; lane 3, 10%, or 2 μg of wild type; lane 4, 20 μg of FuD34 (*TBC1 TBC3 psbC-FuD34*); lane 5, 20 μg of RB1.4A (*TBC1 tbc3-rb1 psbC-FuD34*); lane 6, 20 μg of RB1.5D (*TBC1 tbc3-rb1 psbC*); lane 7, 20 μg of DP.RB1C (*TBC1 TBC3/tbc3-rb1 psbC-FuD34*). To facilitate comparisons among all lanes, two different exposures are shown. LSU, large subunit of RUBISCO. The Western blot was reacted with antisera raised against D1 (the PSII subunit encoded by *psbA*). Lane 1, 30 μg of wild type; lane 2, 15 μg of wild type (plus 15 μg of FuD34); lane 3, 3 μg of wild type (plus 27 μg of FuD34); lane 4, 30 μg of FuD34; lane 5, 30 μg of RB1.4A; lane 6, 30 μg of RB1.12 (*TBC1 tbc3-rb1 psbC-FuD34*); lane 7, 30 μg of DP.RB1C; lanes 8 and 9, RB1.2A and RB1.2B (*tbc1-F34 tbc3-rb1 psbC*), respectively. The level of the small subunit of RUBISCO (SSU), detected with an antiserum raised against the holoenzyme, was used to control for the total amount of protein in each lane. For each strain, the presence of wild-type (+) and mutant (m) alleles of *psbC*, *TBC1*, and *TBC3* is indicated. The presence of both types of alleles is indicated by m and + in parentheses.

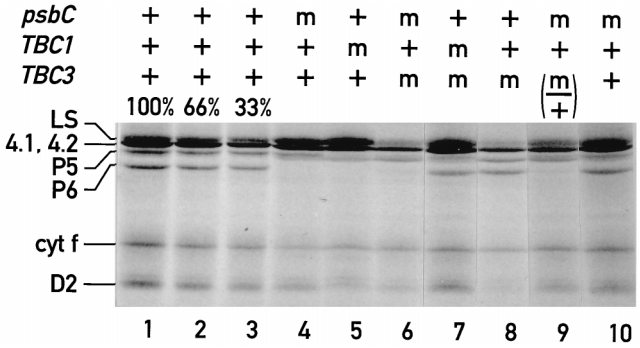

FIG. 5. Chloroplast-encoded proteins pulse-labeled with [^{14}C]acetate. Lanes 1 to 3, wild type (*TBC1 TBC3 psbC*-WT); lane 4, FuD34 (*TBC1 TBC3 psbC-FuD34*); lane 5, F34 (*tbc1-F34 TBC3 psbC-WT*); lane 6, RB1.4A (*TBC1 tbc3-rb1 psbC-FuD34*); lane 7, RB1.2A (*tbc1-F34 tbc3-rb1 psbC*-WT); lane 8, RB1.1C (*TBC1 tbc3-rb1 psbC-WT*); lane 9, DP.RB1C (diploid; *TBC1/TBC1 tbc3-rb1/TBC3 psbC-FuD34*); and lane 10, DP.WT (diploid; *TBC1/TBC1* and *TBC3/TBC3 psbC-WT*). For each strain, the presence of wild-type (+) and mutant (m) alleles of *psbC*, *TBC1*, and *TBC3* is indicated. The presence of both types of alleles is indicated by m and + in parentheses. The polypeptides indicated (and genes encoding them) are LS (*rbcL*), 4.1 (*atpA*), 4.2 (*atpB*), P5 (*psbB*), P6 (*psbC*), cytochrome *f* (cyt f) (*petA*), and D2 (*psbD*). The percentages above lanes 1 to 3 indicate the amounts of wild-type extract loaded relative to the amount in the experimental lanes, lanes 4 to 10, based on chlorophyll concentration.

in vivo. Cycloheximide was used to block cytosolic protein synthesis, thereby facilitating the detection of the chloroplast genome-encoded proteins. As shown previously (29), P6 synthesis was detected from a wild-type *psbC* allele (Fig. 5, lanes 1 to 3) but not from the mutant *psbC-FuD34* allele (lane 4) in FuD34. The levels of P6 synthesis from the mutant *psbC-FuD34* allele in two strains containing the *tbc3-rb1* suppressor mutation (lane 6 and data not shown) were approximately 10% of that of the wild-type strain. The level of P6 synthesized from a wild-type *psbC* allele may be slightly reduced in the presence of the *tbc3-rb1* mutation and a wild-type *psbC* allele (compare lanes 1 and 2 with lane 8). The levels of the *psbC* mRNA were similar in total RNA preparations of the wild type (*TBC3* [*psbC-WT*]) (Fig. 6A, lane 1), FuD34 (*TBC3* [*psbC-FuD34*]) (lane 2) and RB1.1 (*tbc3-rb1* [*psbC-FuD34*]) (Fig. 6A, lane 3), thus excluding any significant effects of *tbc3-rb1* or *psbC-FuD34* on the transcription or stability of the *psbC* mRNA. From these data, we conclude that the *tbc3-rb1* suppressor mutation restores approximately 10% of the wild-type level of P6 synthesis from the *psbC* mRNA bearing the *psbC-FuD34* 5′ UTR mutation.

The level of P6 synthesis in DP.RB1C (*tbc3-rb1/TBC3* [*psbC-FuD34*]) that was detected during the 5-min pulse-labeling experiment shown in Fig. 5 (lane 9) was similar to the levels in the haploid strains RB1.4A and RB1.12 (*tbc3-rb1* [*psbC-FuD34*]) carrying one copy of the *tbc3-rb1* suppressor mutation (lane 6). This result confirms further that the suppression of *psbC-FuD34* is dominant.

In three of the four strains carrying *tbc3-rb1*, the synthesis of the ribulose bisphosphate carboxylase (RUBISCO) large subunit (lanes 6, 8, and 9) appears to be reduced relative to the wild type (lanes 1 to 3). These differences may reflect a pleotropic effect of *tbc3-rb1* on the synthesis of this polypeptide. However, no significant differences in the steady-state levels of RUBISCO were detected in strains carrying this mutation (Fig. 4A, lanes 5 to 7; Fig. 4B, lanes 5 to 10).

The *tbc3-rb1* suppressor mutation restores the expression of a reporter gene from the *psbC* 5′ UTR bearing the *psbC-FuD34* mutation. The *tbc3-rb1* mutation does not suppress *psbC-MA16* (Table 2, cross 5), a mutation that introduces a duplication of two codons in the *psbC* structural gene and abolishes the synthesis of functional P6 polypeptide (23). This allele specificity suggests that *tbc3-rb1* restores *psbC* mRNA translation through an effect on the 5′ UTR in *FuD34*. To determine unambiguously whether the suppression of *psbC-FuD34* by *tbc3-rb1* results from an effect on the *psbC* 5′ UTR or whether other sequences of the *psbC* mRNA are involved in this effect, we asked whether the *tbc3-rb1* mutation can restore expression of the *aadA* from the *psbC-FuD34* 5′ UTR (see Materials and Methods for experimental details). Strains that carry the *psbC-(FuD34)-aadA* chimeric reporter gene and *tbc3-rb1* are spectinomycin resistant, while sibling strains that inherit the wild-type *TBC3* allele are sensitive to the drug (Fig. 2A). This spectinomycin resistance phenotype reflects increased levels of *aadA* reporter gene expression in the strains that inherited *tbc3-rb1*.

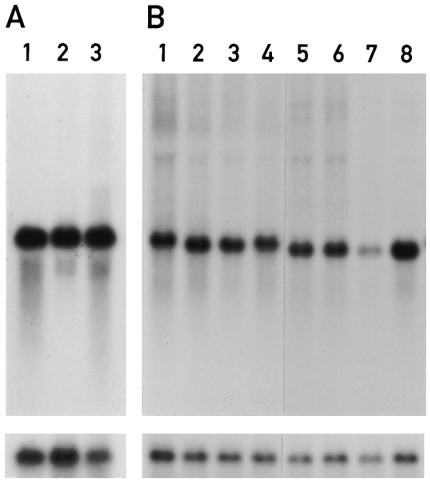

FIG. 6. Northern blot analysis. (A) The *psbC* mRNA was detected in RNA from the following strains: wild type (*TBC3 psbC-WT*) (lane 1), FuD34 (*TBC3 psbC-FuD34*) (lane 2), and RB1.1 (*tbc3-rb1 psbC-FuD34*) (lane 3). (B) Levels of *aadA* RNA in chloroplast transformants expressed from the chimeric genes *psbC*(*FuD34*)-*aadA* (lanes 1 to 4) and *psbC*(Δ*222-319*)-*aadA* (lanes 5 to 8) in RNA preparations from a tetrad from crosses 6 and 7 (Table 2). RNA was from the *TBC3*-bearing progeny (lanes 1, 2, 5, and 6) or from the *tbc3-rb1*-bearing progeny (lanes 3, 4, 7, and 8). The level of the *psaB* mRNA was used to standardize for the amount of RNA in each lane.

Western blot analyses of the AAD polypeptide levels and in vitro assays for the AAD enzymatic activity in protein extracts prepared from a representative tetrad (Fig. 2B, bars and lanes 3 and 4) revealed that the restoration of *aadA* expression by *tbc3-rb1* (ca. 65% of the level from the wild-type 5′ UTR) was more pronounced than the restoration of P6 synthesis or PSII accumulation (ca. 10% of the wild-type levels). Although the basis for this difference is unknown, it may indicate that the AAD polypeptide has a greater half-life than P6 and, therefore, can accumulate to a greater extent. The accumulation of the *psbC*(*FuD34*)-*aadA* mRNA was unaffected by *tbc3-rb1* (Fig. 6B, lanes 1 to 4). From these data we conclude that the mechanism(s) underlying the suppression by *tbc3-rb1* operates entirely through the *psbC* 5′ UTR.

The *tbc3-rb1* suppressor mutation alleviates the requirement for the 222-319 region of the *psbC* 5′ UTR. To determine whether the suppression of *psbC-FuD34* is mediated through the 222-319 region of the *psbC* 5′ UTR, we asked whether *tbc3-rb1* is able to suppress the deletion of this region (see Materials and Methods). The results were similar to those of the experiment described above; although strains carrying the *psbC*(Δ*222-319*)-*aadA* chimeric reporter gene and a wild-type *TBC3* allele are spectinomycin sensitive due to their inability to express *aadA* from the mutant *psbC* 5′ UTR, sibling strains carrying the reporter gene and the *tbc3-rb1* suppressor mutation were spectinomycin resistant (Fig. 2A). In a representative tetrad, spectinomycin resistance correlated with the accumulation of the AAD polypeptide (Fig. 2B, lanes 7 and 8) and AAD activity (bars 7 and 8). Northern blot analyses revealed that the *psbC*(Δ*222-319*)-*aadA* chimeric mRNA is present at comparable levels in the members of this tetrad (Fig. 6B, lanes 5 to 8). From these data, we conclude that *tbc3-rb1* can alleviate the requirement for the 222-319 region for translation from the *psbC* 5′ UTR. The wild-type *TBC3* function, therefore, either prevents or is unable to activate translation of the *psbC* mRNA in the absence of this *cis*-acting regulatory region.

***tbc3-rb1* suppresses a mutation of the *TBC1* locus.** The location of the *psbC-F34sul* suppressor mutation of *tbc1-F34*, at position +226 of the *psbC* 5′ UTR (29, 40), suggests an interaction between the 222-319 region and a *TBC1*-dependent *trans*-acting factor. As *tbc3-rb1* alleviates the requirement for this region, it might also alleviate the requirement for *TBC1*. This hypothesis predicts that *tbc3-rb1* would suppress the severe PSII deficiency produced by *tbc1-F34*. Consistent with this prediction, we found that strains carrying *tbc1-F34* and *tbc3-rb1* have PSII activity (see Materials and Methods for experimental details). Western blot analyses revealed that two strains carrying both *tbc1-F34* and *tbc3-rb1* accumulate approximately 50% of the wild-type level of PSII (Fig. 4, compare lane 2 with lanes 9 and 10). As shown previously, strains carrying *tbc1-F34* (and a wild-type *TBC3* allele) do not accumulate PSII, due to their inability to express P6 (29) (Fig. 5, lane 5). In the two strains in which the PSII deficiency produced by *tbc1-F34* is suppressed by *tbc3-rb1* (RB1.2A and RB1.2B) the synthesis of P6 was detected at approximately 50% of the wild-type level in the pulse-labeling experiments shown in Fig. 5 (lane 7 for RB1.2A; data not shown for RB1.2B). Thus, we conclude that *tbc3-rb1* can partially suppress the defect in *psbC* mRNA translation produced by *tbc1-F34.*

Because *TBC2* is also required for translation from the *psbC* 5′ UTR (40), we tested whether *tbc3-rb1* can suppress the PSII deficiency produced by *tbc2-F64* (see Materials and Methods for details). The observation that strains carrying *tbc2-F64* and *tbc3-rb1* are PSII deficient revealed that the requirement for wild-type *TBC2* function is not alleviated by the *tbc3-rb1* suppressor mutation.

DISCUSSION

Although the chloroplast translational apparatus is homologous to those of procaryotes (reviewed in reference 17), the 222-319 region of the *psbC* 5′ UTR differs in two respects from most procaryotic *cis*-acting controlling elements of mRNA translation. While the latter are repressive and situated close to the translation initiation codon (reviewed in reference 13), the 222-319 region of the *psbC* 5′ UTR has a translational activation function and is located 236 nt upstream from the GUG translation initiation codon. Such translational regulatory elements may be general for chloroplast mRNAs, as the 5′ UTR of *petD* in *C. reinhardtii* contains two regions that are required for translation of this mRNA and are located more than 160 nt upstream from the translation initiation codon (33).

Some similarities exist between this system and translation of *Saccharomyces cerevisiae* mitochondrial mRNAs (reviewed in reference 18). In both systems, translation of specific mRNAs requires genetic interactions between 5′ UTR sequences and gene-specific nucleus-encoded functions. Furthermore, the 5′ UTR sequences that are required for translation of yeast mitochondrial mRNAs, like the 222-319 region in the *psbC* 5′ UTR, are typically not immediately adjacent to the translation initiation codon (5, 6, 25, 27, 38). The nucleus-encoded translational activators in yeast mitochondria have been postulated to be involved in the assembly of the cytochrome *c* oxidase complex, which is located in the inner mitochondrial membrane. This is supported by the finding that three translational activator proteins of *COX3* mRNA translation (PET54, PET122, and PET494) are located at the inner mitochondrial membrane (24). From these observations it was postulated that these proteins function to direct the *COX3* mRNA to the inner membrane for translation and membrane insertion of its product (24).

TBC1 and *TBC3* operate through the *psbC* 5′ UTR. This is evidenced by the ability of this 5′ UTR to confer the require-

ment for *TBC1* on the expression of a heterologous reporter gene, i.e., in the absence of the P6 coding sequence and the *psbC* 3′ UTR (40). Similarly, the *tbc3-rb1* mutation can restore the expression of the *aadA* reporter gene from the 5′ UTR bearing the *psbC-FuD34* mutation or the 222-319 region deletion. The ability of *tbc3-rb1* to suppress the deletion of the 222-319 region demonstrates that 5′ UTR sequences outside of this region are sufficient for the restoration of translation underlying this suppression. That *tbc3-rb1* also suppresses *tbc1-F34* shows that it alleviates the requirement for the functional interaction between *TBC1* and the 222-319 region. From these data, two alternative models can be made. In the first model, a *TBC1*-dependent factor interacts with the 222-319 region within the *psbC* 5′ UTR to inhibit a *TBC3*-dependent translational repressor from acting on a site located outside this region. In the absence of either the 222-319 region or wild-type *TBC1* function, the *TBC3*-dependent repressor would cause a constitutive inhibition of translation initiation. The *tbc3-rb1* mutation would eliminate or diminish this repression (through a dominant-negative effect) and thereby restore *psbC* mRNA translation. In the second model, *TBC1* and the 222-319 region function with *TBC3* to activate *psbC* mRNA translation. In the absence of *TBC1* or the 222-319 region, wild-type *TBC3* would be insufficient to promote translation. The dominant *tbc3-rb1* mutation might alter the *TBC3* gene product in such a way that it is sufficient to promote *psbC* mRNA translation in the absence of functional *TBC1* or 222-319 region. The twofold reduction of P6 synthesis and PSII accumulation in strains carrying *tbc3-rb1* and a wild-type *psbC* allele indicates that this mutant allele does not provide optimal *TBC3* function. Although the molecular mechanisms involved cannot be precisely determined from the genetic data presented here, these data demonstrate that the 222-319 region of the *psbC* 5′ UTR, *TBC1*, and the newly described locus *TBC3* functionally interact to control the translation of the *psbC* mRNA.

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ADDENDUM IN PROOF

The suppression of *tbc1-F34* by *tbc3-rb1* does not result from an alteration of the transcription or stability of the *psbC* mRNA, because Northern blot analysis (similar to that shown in Fig. 6) showed that this mRNA accumulates to similar levels in four members of an NPD tetrad derived from cross 8.

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