Characterization of Mbb1, a nucleus-encoded tetratricopeptide-like repeat protein required for expression of the chloroplast *psbB/psbT/psbH* gene cluster in *Chlamydomonas reinhardtii*

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Genetic analysis has revealed that the accumulation of several chloroplast mRNAs of the green alga *Chlamydomonas reinhardtii* requires specific nucleus-encoded functions. To gain insight into this process, we have cloned the nuclear gene encoding the Mbb1 factor by genomic rescue of a mutant specifically deficient in the accumulation of the mRNAs of the *psbB/psbT/psbH* chloroplast transcription unit. Mbb1 is a soluble protein in the stromal phase of the chloroplast. It consists of 662 amino acids with a putative chloroplast-transit peptide at its N-terminal end. A striking feature is the presence of 10 tandemly arranged tetratricopeptide-like repeats that account for half of the protein sequence and are thought to be involved in protein–protein interactions. The Mbb1 protein seems to have a homologue in higher plants and is part of a 300-kDa complex that is associated with RNA. This complex is most likely involved in *psbB* mRNA processing, stability, and/or translation.

The expression of chloroplast genes coding for components of the photosynthetic apparatus largely depends on nucleus-encoded factors that are synthesized in the cytoplasm and subsequently imported into the organelle. Some of these factors are general components of the chloroplast protein-synthesizing system, whereas others are required only for the expression of specific chloroplast genes or small sets of genes (for review see refs. 1 and 2). Both genetic (3–13) and biochemical data (14–16) indicate that these factors are involved in various posttranscriptional steps such as RNA stability, RNA processing, splicing, and translation.

In *Chlamydomonas reinhardtii*, these nucleus-encoded factors seem to act in a gene-specific manner and interact with specific regions of chloroplast mRNAs. We have shown that the mRNAs of the *psbB/T/H* transcription unit that encode polypeptides of the photosystem II core complex fail to accumulate in the nuclear mutant *222E* (10), which is unable to grow photoautotrophically. Another mutant with a similar phenotype has been characterized by Sieburth *et al.* (17). The target site of the function encoded by the *MBB1* locus that is altered in the *222E* mutant seems to be the *psbB* 5′ untranslated region (UTR; ref. 18). Similarly, determinants for *psbD* and *petD* mRNA instability in the *nac2–26* and *mcd1–1* nuclear mutants of *C. reinhardtii* are located in the 5′ UTR (11, 19). Several chloroplast mRNAs of *C. reinhardtii*, in particular those of *psbA*, *psbB*, and *psbD*, exist in two forms with a long and short 5′ UTR (11, 18, 20). The long form, which is present in low amounts, seems to be an RNA precursor that is processed into the mature abundant mRNA. In the case of the *psbB* and *psbD* mRNAs, mutations that drastically destabilize the short mature mRNA do not markedly affect the stability of the long RNA (11, 18). It has been shown that the *psbD* precursor transcript can be cross-linked to a 47-kDa protein only with wild-type but not with *nac2–26* mutant extracts (11). Extensive site-directed mutagenesis of the 5′ UTR of *psbD* and *petD* has revealed the existence of short cis-elements in these regions, which are important for RNA stability (21, 22). These studies have been extended recently by stabilizing the target *psbB*, *psbD*, and *petD* RNAs in the corresponding mutant background through the insertion of a track of 18 G residues within the 5′ UTR (18, 21, 23). The polyG blocked a processive 5′ to 3′ exonuclease activity. However, although the transcript was stabilized, it was not translated, raising the possibility that the nucleus-encoded functions that are altered in these mutants may also play a role in translation. The polyG did not affect translation in the presence of the wild-type *Mbb1* allele.

Genetic analysis in the yeast *Saccharomyces cerevisiae* has demonstrated that the expression of specific mitochondrial genes coding for membrane proteins of the respiratory complexes also depends on proteins that are nucleus-encoded. In particular, expression of the mitochondrial cytochrome *b* gene requires several factors for splicing, at least two for translation, and one, Cbp1p, for the stabilization of the *COB* mRNA (24). Genetic evidence strongly suggests that Cbp1p interacts with the *COB* 5′ UTR to protect the mRNA from degradation and suggests that Cbp1p could either bind directly to the RNA or that it is part of a complex that recognizes specifically the *COB* 5′ UTR (24). The analysis of mutation suppressors in the *COB* 5′ UTR has identified several components involved in mitochondrial mRNA processing and decay. These components include Cbp1p itself, Pet127p, which is required for 5′ end-processing of several mitochondrial mRNAs, and, surprisingly, a subunit of a mitochondrial 3′ to 5′ exonuclease (25).

Chloroplast RNA stability determinants are also present in the 3′ UTRs of the mRNAs. These regions often contain inverted repeats that can potentially fold into stem–loop structures that seem to be important in impeding a processive 3′ to 5′ exonuclease (26–28). Several proteins that have been found to bind specifically to the region of the inverted repeat are part of a high-molecular weight complex that resembles the *Escherichia coli* degradosome (29, 30).

Although several nucleus-encoded functions involved in the stability of specific chloroplast mRNAs have been identified by

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Abbreviations: HA, hemagglutinin; TPR, tetratricopeptide-like repeat; UTR, untranslated region.

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genetic means, little is known of their molecular identity and how they act at the molecular level, in particular whether they interact directly or indirectly with their target chloroplast RNAs. We have cloned the genes of several of these nucleus-encoded factors recently from *C. reinhardtii* by mutant rescue with an indexed library or by gene tagging. One of them is involved in *psaA trans*-splicing (31), and another is required for *psbD* mRNA accumulation (32). In this work, we have cloned the *Mbb1* gene, which is specifically required for the accumulation of the transcripts of the *psbB* gene cluster (10, 18), and we have shown that the Mbb1 protein is localized in the stromal phase of the chloroplast and is part of a 300-kDa complex that is associated with RNA.

Materials and Methods

Strains, Media, and Genetic Crosses. The *C. reinhardtii* mutant strains *222E* and *cw15* have been described (10, 33). Tris-acetate medium and high-salt medium were prepared as described by Rochaix *et al.* (34). For the phenotypic analysis of strains (spot test), 2 ml of culture were grown overnight in Tris-acetate medium under dim light, and then 15 μl was aliquoted onto agar plates containing the appropriate media.

Nuclear Transformation. For the nuclear rescue of *222E,* we used a *222E-cw15* double mutant (cell-wall deficient) to increase the rate of transformation. Preparation of the cosmid DNA from the indexed library and nuclear transformation were performed according to methods described in refs. 35 and 36, respectively. Selection for growth on high-salt medium in the light identified two rescuing cosmids. When a 10-kb *Sal*I/*Eco*RI fragment from these cosmids, cloned in the pKS vector, was used for transformation, ≈200 colonies were obtained per plate; with the 4.3-kb *Sal*I/*Xba*I fragment cloned into pKS (pKS-AB), 50 colonies per plate were obtained; and with the corresponding cDNA cloned in pBluescript KS(+), 1–4 colonies were recovered on 8 of 30 plates.

DNA Manipulation and Nucleic Acid Analysis. Standard techniques were used to manipulate and analyze nucleic acids (37). Both strands of the cDNA were sequenced (ABI PRISM 377 DNA Sequencer, Perkin–Elmer) from subclones obtained by *Exo*3 digestion. The sequence data have been submitted to the GenBank databases under accession no. AJ296291.

Total *Chlamydomonas* DNA extractions were performed according to the method of Rochaix *et al.* (34). After appropriate digestion with restriction endonucleases, DNA fragments were separated by standard agarose gel electrophoresis (38). RNA extraction and RNA blot analysis were performed as described (32, 39).

The *Mbb1* cDNA probe corresponds to the 1.13-kb *Acc*I DNA fragment containing the tetratricopeptide-like repeat (TPR) domain from pKS-Mbb1 cDNA (a plasmid containing the *Mbb1* cDNA inserted at the *Eco*RV site of pKS). The *rbcS* probe corresponds to the 0.7-kb *Taq*I/*Sal*I DNA fragment containing part of the coding sequence of *rbcS2* gene (40).

An *Xba*I site was engineered just upstream of the stop codon in the genomic DNA of *Mbb1* by PCR with oligonucleotides Oligo-3′ *Xba*I (5′-AGGTCCATGGGATCTAGATGAGGCGGTGGAGGCAC-3′) and Reverse Primer. The product of this PCR amplification was cloned into pKS opened with *Eco*RV to produce a plasmid called pKS-3′ *Xba*I. The *Xba*I site of pKS was removed by *Eco*RI/*Not*I digestion, Klenow blunting, and religation to produce pKS-3′ *Xba*I(R/N). The triple hemagglutinin (HA) tag was inserted into pKS-3′ *Xba*I(R/N) opened with *Xba*I. Then, the *Mbb1* 3′ UTR fragment harboring the sequence encoding the HA epitopes was excised by *Nco*I/*Xho*I digestion and inserted in pKS-AB opened by *Xho*I and partially digested with *Nco*I. Correct insertion and orientation were verified by sequencing.

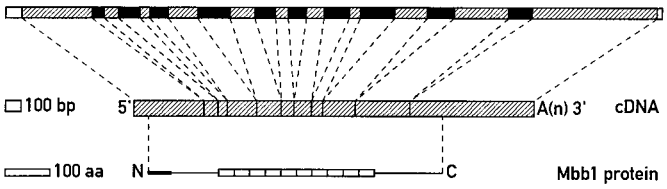

Fig. 1. Structure of the *Mbb1* gene. Schematic view of the *Mbb1* gene with its 10 introns (black boxes) on the genomic 4282 bp *Xba*I–*Sal*I fragment, the corresponding 2,680-bp cDNA and the 662-amino acid Mbb1 protein with its putative transit peptide (thick line at N-terminal end), and the 10 tandemly arranged TPR domains (open boxes).

Cell Fractionation and Polysome Preparation. Chloroplast isolation and preparation of polysomes were performed as described (32, 41, 42, 43). RNA derived from the polysome fractions were hybridized with the ^{32}P-labeled chloroplast *Eco*RI DNA fragment R07 specific for the 16S ribosomal RNA and the *Nco*I-*Eco*RI fragment of the p38.A.*Nco*I plasmid (18) specific for *psbB.*

Immunoblot Analysis. Samples were electrophoresed on SDS/10% PAGE (37) and electroblotted to a Protran 0.45-μm nitrocellulose membrane (Schleicher and Schuell). Membranes were incubated with specific antibodies as described (31, 44).

Results

Cloning of the *Mbb1* Gene. To elucidate the role of the nucleus-encoded function required for *psbB* mRNA accumulation, we cloned the *Mbb1* gene affected in the *222E* nuclear mutant of *C. reinhardtii*. Assuming that the original mutation resulted in a loss of function, the *222E* mutant was rescued by transformation with an indexed genomic cosmid library constructed by Zhang *et al.* (35), selecting for growth on minimal medium (high-salt medium). Two cosmids were isolated after a large-scale screen (see *Materials and Methods*). After subcloning, a 4.3-kb *Xba*I/*Sal*I (AB fragment) common to both of these cosmids was isolated that still rescued the mutant. This fragment was used to screen a *C. reinhardtii* cDNA library. A 2.7-kb cDNA was identified that was able to restore phototrophic growth in *222E*, albeit at very low frequency (see *Materials and Methods*). Sequencing of the cDNA and the AB genomic fragment revealed that the gene contains 10 introns (Fig. 1).

To check whether the cloned genomic fragment corresponds to the gene altered in *222E*, we performed a Southern blot analysis (Fig. 2*A*). Total DNA from wild type, *222E*, and *222E* rescued with the genomic AB fragment (222E$^+$) was digested with *Pst*I and hybridized with the cloned cDNA. It can be seen that a 3.0-kb band in wild type is replaced by a new band of 3.2 kb in *222E* (Fig. 2*A Left*). The genomic and spot-test analysis of three tetrads from the cross between wild type and *222E* showed that the 3.0-kb and 3.2-kb bands cosegregated with the growth and nongrowth phenotype on minimal medium, respectively (Fig. 2*A Center*). This finding indicates that a genomic rearrangement occurred at the *MBB1* locus in the original *222E* mutant, resulting in the loss of photoautrophophic growth. In the rescued *222E*$^+$ strain, both the wild-type and the *222E* genomic bands were detected (Fig. 2*A Right*). Thus, the rescuing AB DNA integrated by nonhomologous recombination in the *222E* mutant as expected for nuclear transformation in *C. reinhardtii*.

RNA blot analysis with the *Mbb1* cDNA probe revealed a band of 2.7 kb in the wild-type poly(A) RNA preparation (Fig. 2*B*) strongly suggesting that the full-length cDNA has been cloned.

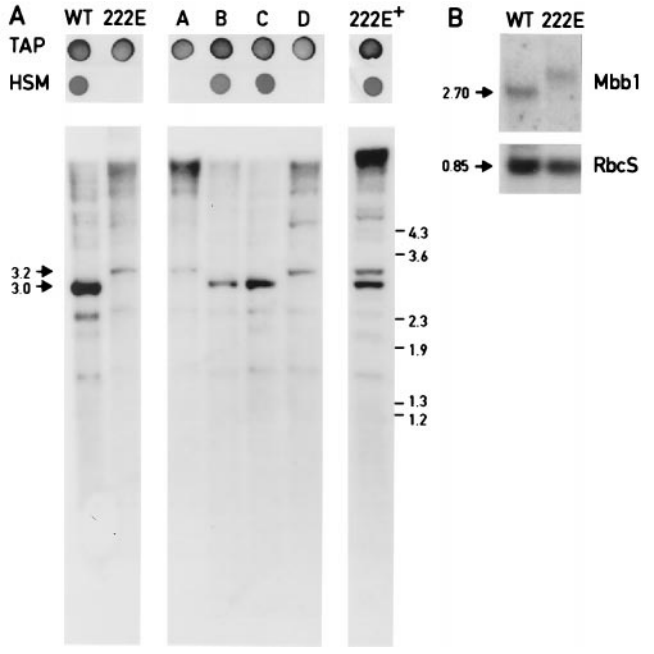

Fig. 2. (*A*) Structural changes at the *Mbb1* locus of the *222E* mutant. (*Top*) Growth test on acetate (Tris-acetate-medium) and minimal medium (high-salt) of wild type (WT), *222E* (*Left*) and the four progeny A, B, C, and D from one of the three tetrads analyzed from the cross between wild type and *222E* (*Center*). *222E*$^+$ refers to the *222E* mutant strain rescued with the genomic *Mbb1* DNA (*Right*). (*Bottom*) Southern blot analysis of DNA of the wild type, *222E*, the progeny A, B, C, and D, and *222E*$^+$. The DNAs were digested with *Pst*I and hybridized with the labeled cDNA. Fragment sizes of the marker are indicated in kilobases. (*B*) *Mbb1* transcript. RNA blot with ≈2 μg of polyadenylated RNA from wild type and *222E* hybridized with the *Mbb1* cDNA and an *RbcS* probe. Sizes are indicated in kilobases.

A larger transcript was found in the *222E* mutant (Fig. 2*B*) indicating that the genomic rearrangement in *222E* leads to the synthesis of an aberrant RNA. As expected, both the wild-type and *222E* aberrant *Mbb1* RNA forms are present in the rescued *222E*$^+$ strain (data not shown).

Sequence Analysis of the Mbb1 cDNA. The cloned cDNA is 2,680 bp long and contains an ORF with the first ATG codon at position 95 and a TGA stop codon at position 2,082. This configuration corresponds to a 5′ UTR of at least 95 bases and a 3′ UTR of 596 bases. The sequence TGTAC is found in the cDNA 12 bp upstream of the poly(A) tail, closely matching the putative polyadenylation recognition motif (45).

The predicted protein consists of 662 amino acids with a molecular weight of 72,500. The N-terminal region of the protein is rich in R, S, and V residues (Fig. 3*A*), a hallmark of chloroplast-transit sequences (46). A putative V-X-A cleavage site is present that could give rise to a mature protein of 62.7 kDa. A BLAST search of the database revealed that the Mbb1 protein shows significant sequence identity with TPR proteins, in particular with a TPR protein from *Arabidopsis* of unknown function (BAA94982, 34% identity, 40% similarity; Fig. 3*B*). TPRs are loosely conserved motifs of 34 amino acids which have been found in a wide range of proteins with different biological function and are believed to be involved in protein–protein interactions (47). Seven hydrophobic residues, Trp-4, Leu-7, Gly-8, Tyr-11, Ala-20, Phe-24, and Ala-27, as well as Pro-32, are usually highly conserved amongst TPRs (48). The Mbb1 protein contains 10 tandemly arranged TPR-like domains (Figs. 1 and 3) that generally follow the consensus with one striking exception: Tyr-11 is replaced by Glu. Beside the TPRs no other known

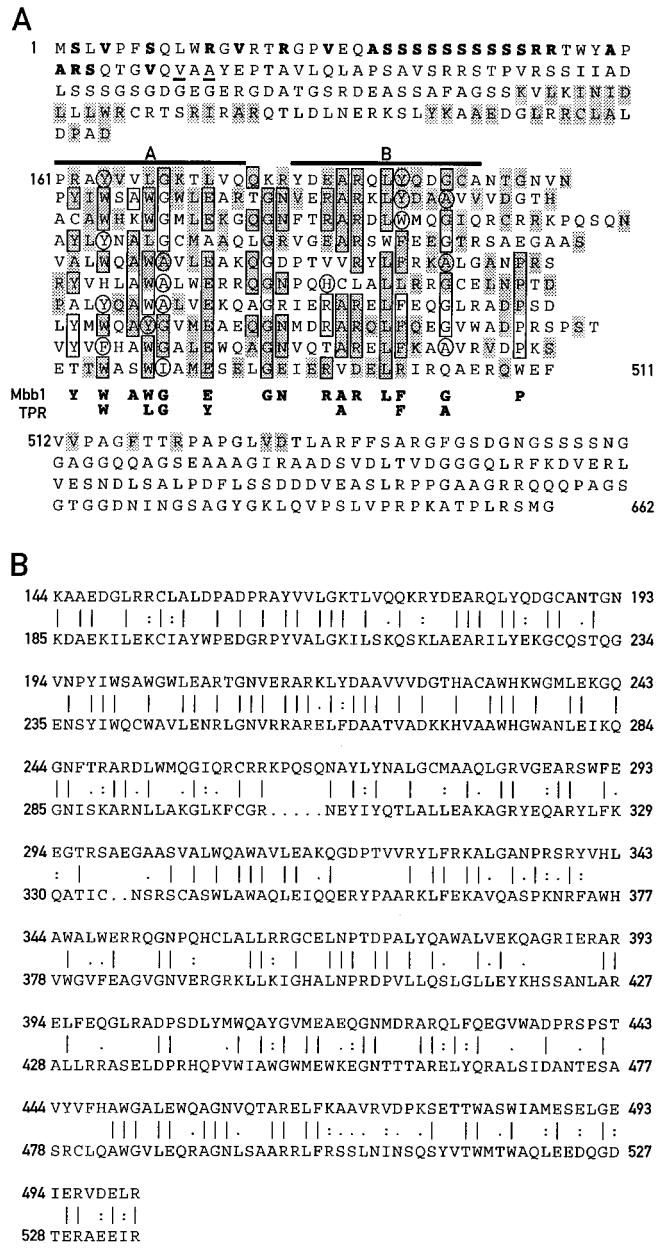

Fig. 3. (*A*) Predicted Mbb1 protein sequence. S, V, R, and A residues are marked in bold in the putative transit peptide. Consensus residues V and A of the potential cleavage site are underlined. The 10 aligned TPR domains are shown on top of each other together with the Mbb1 consensus and the general TPR consensus. The domains A and B are indicated by lines above the TPRs (see *Discussion*). Residues present at least five times among the 10 TPRs of Mbb1 are framed, and similar amino acids are circled. Identical and similar residues in Mbb1 and the *Arabidopsis* protein BAA9482 are shaded. (*B*) Alignment of the Mbb1 and *Arabidopsis* BAA9482 protein sequences.

protein motifs could be identified in the Mbb1 protein; in particular, no known RNA-binding domains could be detected.

Localization of the Mbb1 Protein. To characterize the product of the *Mbb1* gene, a triple HA-epitope tag was inserted near the 3′ end of the coding sequence in the genomic DNA (see *Materials and Methods*), and the resulting construct was introduced in the nuclear genome of the *222E* mutant strain by transformation. Whole-cell proteins from four transformants obtained independently were probed by immunoblotting with the anti-HA mono-

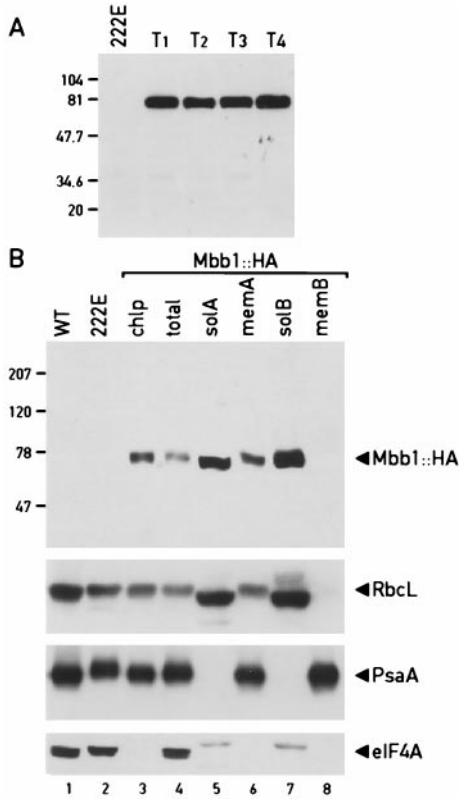

Fig. 4. Localization of the Mbb1 protein. (*A*) Immunoblot analysis of proteins separated by PAGE from *222E* and four independent Mbb1::HA transformants (T_{1-4})reacted with HA monoclonal antibody. (*B*) Immunoblot with proteins from different cellular fractions separated by SDS/PAGE. Wild-type (WT) cell extract (lane 1); cell extract from 222E (lane 2); chloroplast (chlp; lane 3); total cell (lane 4); soluble chloroplast fraction (solA; lane 5); chloroplast membrane fraction I (memA; lane 6); solB (lane 7); and memB (lane 8) are the same as lanes 5 and 6 except the chloroplast fractionation was performed in the presence of 0.5 M ammonium sulfate. Cell extracts and fractions shown in lanes 3–8 are derived from the strain containing Mbb1::HA. The immunoblot was revealed sequentially with monoclonal anti-HA antibodies and with polyclonal sera against RbcL, PsaA, and eIF4A. The minor signal in the soluble chloroplast fraction reacting with eIF4A antibodies is not known.

clonal antibody. A 70-kDa protein was detected readily in the extracts of each transformant, whereas no protein was recognized by the antibody in the mutant strain (Fig. 4*A*). An enrichment of the Mbb1::HA protein was found when the immunoblot was performed with isolated chloroplasts (Fig. 4*B*, lanes 3 and 4). The isolated chloroplasts were not significantly contaminated with cytosolic proteins, because the cytosolic eIF4A translation-initiation factor was detected only in whole-cell extracts but not in the chloroplast extracts (Fig. 4*B*, lanes 3 and 4). Separation of extracts from isolated chloroplasts into soluble and insoluble fractions revealed that the 70-kDa protein is mainly found in the soluble fraction, although a smaller amount is also associated within the membrane fraction (Fig. 4*B*, lanes 5 and 6). A similar distribution was found for the large subunit of Rubisco (RbcL), which is known to be a soluble protein. When the chloroplasts were fractionated in the presence of 0.5 M ammonium sulfate, which is known to strip off loosely bound proteins from membranes, Mbb1::HA and RbcL were found entirely in the soluble fraction (Fig. 4*B*, lanes 7 and 8). The soluble chloroplast fraction was not significantly contaminated with thylakoid membranes as indicated by the absence of the hydrophobic PsaA protein.

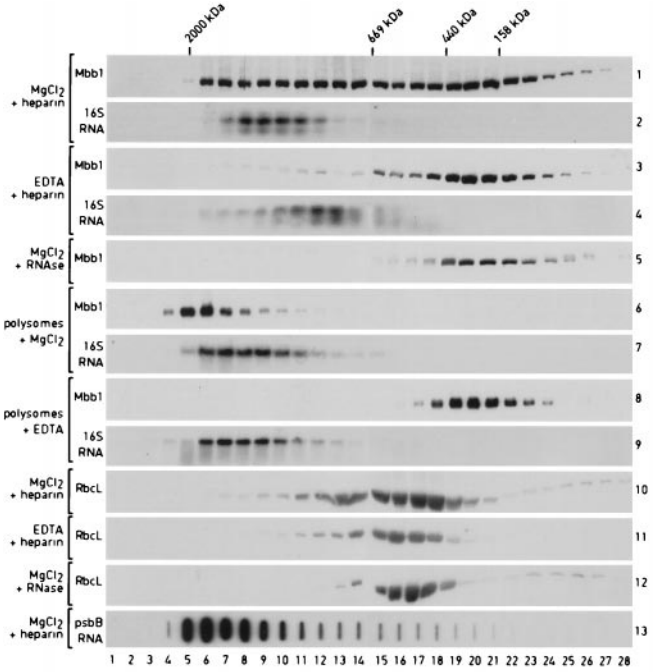

Fig. 5. The Mbb1 protein is part of a 300-kDa complex associated with RNA. Soluble cell extracts or polysome extracts from the *Mbb1*::*HA* strain, prepared as described (*Materials and Methods*), were fractionated by size-exclusion chromatography. The 28 50-μl fractions were collected, and each fraction was analyzed by SDS/PAGE and immunoblotting with anti-HA antibodies (rows 1, 3, 5, 6, and 8), by antibodies against RbcL (rows 10–12), or by RNA blotting with probes specific for the 16S rRNA (rows 2, 4, 7, and 9) and *psbB* genes (row 13). Because the *psbB* RNA was slightly degraded under the conditions used, a slot blot hybridization was performed. Size standards were used to calibrate the column as indicated.

The Mbb1 Protein Is Part of a 300-kDa Complex That Is Associated with RNA. The fact that the Mbb1 protein contains 10 TPR domains suggests that this protein might be part of a multiprotein complex. To test this possibility, soluble extracts from the *Mbb1*::*HA* strain were fractionated further by size-exclusion chromatography. In the presence of magnesium and heparin, the Mbb1::HA protein was found to be distributed over many fractions, although the signal seemed slightly higher in fractions 18–22 (Fig. 5, row 1). When the samples were prepared in the presence of EDTA or treated with RNase, the Mbb1::HA protein was found in a single peak corresponding to a size of ≈300 kDa (Fig. 5, rows 3 and 5). Thus, the size distribution observed in the presence of magnesium indicates that Mbb1::HA exists in two forms, one around 300 kDa and another in the heavier fractions which is EDTA- and RNase-sensitive. These results have been confirmed with extracts from three independent *Mbb1*::*HA* transformants (data not shown). Under similar conditions, RbcL, which is known to form a complex of 560 kDa, was found in the same fractions in the presence or absence of EDTA or RNase (Fig. 5, rows 10–12). The distribution of the heavy Mbb1::HA complex was very similar to that of 16S rRNA, a marker for chloroplast ribosomes (Fig. 5, rows 1 and 2). The latter was displaced as expected toward smaller sizes on addition of EDTA (Fig. 5, row 4), because this treatment is known to dissociate polysomes or monosomes into ribosomal subunits. To compare the properties of the Mbb1 complex with those of polysomes further, the latter were prepared from soluble extracts (see *Materials and Methods*) and fractionated by size-exclusion chromatography. In the presence of magnesium, the Mbb1::HA protein was found in high-molecular-mass fractions that coincided in part, although not completely, with those containing 16S

rRNA (Fig. 5, rows 6 and 7). Moreover, the Mbb1::HA signal from polysome extracts shifted slightly toward heavier fractions compared with total extracts (Fig. 5, rows 1 and 6). This shift may be caused by a decreased RNase activity in the polysome preparation compared with the soluble cell extract. The distribution of Mbb1::HA remained the same in the presence or absence of chloramphenicol, a drug known to stabilize polysomes, in the extraction buffers (data not shown). When the polysome pellet was resuspended in the presence of EDTA, the Mbb1::HA signal shifted to a size of 300 kDa, corresponding to the peak observed with soluble extracts treated with EDTA or RNase (Fig. 5, row 8). This result indicates that the high-molecular-mass complex contains Mbb1 as part of the 300-kDa complex and not as a single protein. However, under the same conditions, the 16S rRNA failed to shift toward lighter fractions (Fig. 5, row 9), possibly because of some protein aggregation that is not observed in total extracts (Fig. 5, row 4). Although we cannot completely rule out an EDTA-sensitive association of the Mbb1 complex with polysomes, it is more likely that Mbb1 is associated with a high-molecular-mass complex, with the same size distribution as polysomes or ribosomes and that is sensitive to both EDTA and RNase. Considering the function of Mbb1, the high-molecular-mass complex is likely to be associated with *psbB* mRNA. To test this hypothesis, the distribution of *psbB* mRNA after size-exclusion chromatography was determined by slot blot hybridization. Standard RNA blot analysis was not possible, because the RNA was partially degraded under the conditions used (Fig. 5, row 13). The distribution of *psbB* mRNA is compatible with an association of this RNA with the high-molecular-mass Mbb1 complex. However, because the distribution of polysomes/ribosomes and the heavy Mbb1 complex overlap, it is not possible to distinguish whether the signals observed are caused by *psbB* mRNA associated with polysomes or with the heavy Mbb1 complex. A clear answer will be possible only after separation of the heavy Mbb1 complex from the ribosomal fraction.

Discussion

Several nuclear mutants of *C. reinhardtii* deficient in the accumulation of a specific mRNA have been isolated and characterized. In all cases studied, the target site of the nucleus-encoded function involved in this process seems to be the 5′ UTR of the mRNA. Interactions between 5′ UTRs and specific nucleus-encoded factors play an important role not only in chloroplast but also in mitochondrial posttranscriptional steps of gene expression (49). Previous work has indicated that the Mbb1 function is required for the stable accumulation of the mRNAs of the *psbB/T/H* transcription unit of *C. reinhardtii* (10) and that it interacts either directly or indirectly with the *psbB* 5′ UTR for the stabilization, the processing, and/or the translation of the psbB mRNA (18).

We have used a genomic complementation strategy to isolate the *Mbb1* gene. This gene is transcribed into a 2.7-kb mRNA, which encodes a 662-amino acid protein with a putative chloroplast-transit peptide at its N-terminal end. To demonstrate that the Mbb1 protein is indeed a chloroplast protein, we have epitope-tagged the protein and found that it is localized in the soluble phase of the chloroplast compartment.

A striking feature of the Mbb1 protein is the presence of 10 tandemly arranged TPR-like motifs that comprise half of the protein sequence. This motif has been observed in a wide variety of organisms, ranging from bacteria to eukarya (47), in proteins involved in various biological functions, such as transcriptional repression, signal transduction, stress response, mitochondrial and peroxisomal protein transport, protein secretion, DNA replication, and cell division (50). The TPRs consist of motifs of 34 amino acids that are often arranged as tandem arrays of 3–16 degenerate repeats (47). The atomic structure of TPR motifs of the PP5 Ser/Thr phosphatase has revealed that each TPR motif consists of a pair of antiparallel α-helices (helix A and helix B) of equivalent length (51). Although the TPR-containing proteins do not seem to have a common biochemical function, the repeats themselves seem to mediate specific intermolecular or intramolecular protein–protein interactions (48).

It has been proposed that the highly conserved hydrophobic residues Trp-4, Leu-7, Gly-8, and Tyr-11 in helix A form a hydrophobic hole into which a knob formed by the conserved hydrophobic residues Ala-20, Phe-24, and Ala-27, of helix B could fit (52). Whereas these residues of helix B are well conserved in the TPRs of Mbb1, the conserved Tyr-11 residue in domain A is replaced by an acidic Glu residue in most TPRs (Fig. 3). Nine TPR-like domains have been found in another nucleus-encoded chloroplast protein, Nac2, which is required for the stable accumulation of the *psbD* mRNA in *C. reinhardtii*. Here, too, an acidic residue is present at position 11 (32). These specific TPR features are thus shared between the Mbb1 and Nac2 proteins, which are both involved in a similar function in chloroplast RNA metabolism. The observation that a protein with 10 TPR domains from higher plants is significantly related in sequence to Mbb1 raises the interesting possibility that it may perform a similar function (Fig. 3*B*). It is noteworthy that the TPR consensus Tyr-11 in domain A is also replaced by Glu in the *Arabidopsis* protein.

It seems that several chloroplast proteins involved in post-transcriptional steps of chloroplast gene expression contain TPR or TPR-like motifs. The maize Crp1 protein that is involved in processing the *petA-petD* transcript contains several TPR-related repeats, called PPR repeats (53, 55). These PPR repeats are degenerate 35-amino acid repeats that are usually tandemly arranged and are characteristic for a large-gene family in *Arabidopsis* (55). In contrast to the Nac2 and Mbb1 TPR-like domains, the plant PPR repeats contain the conserved Tyr-11 residue and are thus clearly distinct from the *Chlamydomonas* repeats. Another chloroplast protein that is required for the stable accumulation of PSI, Ycf3, also contains TPRs (54). Thus, several chloroplast proteins involved in posttranscriptional steps of chloroplast gene expression contain TPR motifs. Because of their peculiar structure, TPRs have been proposed to mediate intraprotein and interprotein interactions, and several TPR proteins are part of multiprotein complexes (48). These data raise the question whether some of these TPR proteins could be part of the same complex, in particular Mbb1 and Nac2, which are both required for the stability of specific chloroplast mRNAs. We have shown, however, that Mbb1 is associated with a complex of 300 kDa, whereas Nac2 is part of a complex of 600 kDa (32). Thus, both factors belong to different complexes and seem to act in an independent way. In agreement with this finding, the analysis of Nac2-Mbb1 double mutants revealed no synergistic effect but only additive effects on chloroplast RNA accumulation (F.E.V. and J.-D.R., unpublished results). It is also possible that the TPRs of Mbb1 and Nac2 are required to recruit a common component to these two distinct gene-specific complexes. Both complexes seem to associate with RNA, most likely *psbB* and *psbD* RNA for Mbb1 and Nac2, respectively. In the case of the Nac2 complex, it has been clearly shown that the RNA is nonpolysomal (32), and it is likely that the same holds for Mbb1; although based on the results of Fig. 5, we cannot completely rule out an association between the Mbb1 complex and polysomes.

The Mbb1 protein does not display any known RNA-binding motif, and attempts to demonstrate RNA binding with the recombinant Mbb1 protein have been inconclusive. It is thus likely that the interaction with the *psbB* 5′ UTR is mediated through another protein. The *psbB* mRNA exists in two forms with long and short 5′ UTRs, which accumulate to low and high levels, respectively, in wild-type cells (18). It is probable that the long *psbB* RNA is a precursor of *psbB* mRNA. Only the short

mature *psbB* mRNA, but not the long *psbB* RNA, is destabilized in the *222E* mutant. The long *psbB* RNA seems to be degraded by a 5′ to 3′ exonuclease in both 222E mutant and wild-type cells (18). A similar exonuclease activity has been detected during *psbD* and *petD* RNA decay (21, 19). One possibility is that the Mbb1 complex is involved in the processing of the *psbB* precursor RNA and that this maturation stabilizes the mature *psbB* mRNA either through a modification of its 5′ end or through the binding of additional factors. Processing could prevent the 5′ to 3′ exonuclease from gaining access to the body of the RNA. Alternatively, the Mbb1 complex could bind downstream of the processing site and thereby impart protection against the 5′ exonucleolytic activity. Finally, it is also possible that Mbb1 is a specific translation factor for *psbB* mRNA and that it is the failure to initiate translation that leads to the degradation of this RNA in the *222E* mutant. When a polyG tract was inserted in the 5′ UTR of a *psbB-aadA* chimeric mRNA to block exoribonuclease activity, the transcript seemed stable in both wild-type and *222E* strains, but it did not confer spectinomycin resistance in the *222E* background (18). Our finding that Mbb1 contains TPR repeats and is part of a large complex associated with RNA, and the striking parallel with Nac2, are important steps toward the elucidation of the exact function of Mbb1 and its interacting partners.

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