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**The control of messenger RNA (mRNA) translation has been increasingly recognized as a key regulatory step for gene control, but clear examples in eukaryotes are still scarce. Nucleo-cytoplasmic male sterilities (CMS) represent ideal genetic models to dissect genetic interactions between the mitochondria and the nucleus in plants. This trait is determined by specific mitochondrial genes and is associated with a pollen sterility phenotype that can be suppressed by nuclear genes known as restorer-of-fertility (*Rf*). In this study, we focused on the Ogura CMS system in rapeseed and showed that reversion to male sterility by the PPR-B fertility restorer (also called Rfo) occurs through a specific translation inhibition of the mitochondria-encoded CMS-causing mRNA *orf138*. We also demonstrate that PPR-B binds within the coding sequence of *orf138* and acts as a ribosome blocker to specifically impede translation elongation along the *orf138* mRNA. Rfo is the first recognized fertility restorer shown to act this way. These observations will certainly facilitate the development of synthetic fertility restorers for CMS systems in which efficient natural Rfs are lacking.**

cytoplasmic male sterility | restorer-of-fertility | translation | mitochondria | pentatricopeptide repeat

Fine-tuning of gene expression provides cells with necessary proteins to function properly. Each step in the flow of information going from DNA to proteins offers cells with potential checkpoints to adjust the type and the activity of proteins they synthesize. Changes in transcriptional patterns play major roles in gene regulation in both prokaryotes and eukaryotes and are orchestrated by different molecular means. Posttranscriptional regulatory mechanisms allow for faster reshaping of cellular proteomes compared to purely transcriptional events. In particular, the control of messenger RNA (mRNA) translation has been increasingly recognized as a key regulatory step of gene control in most genetic systems. All phases of translation, including initiation, elongation, termination, and ribosome recycling, constitute potential checkpoints to modulate gene expression. Translational control can be mediated by mRNA structural features or through the action of proteinaceous or RNA transfactors (1–4).

In eukaryotic cells, the spread out of genetic information between the nuclear and cytoplasmic genomes adds an additional layer of complexity to gene regulation processes. Cytoplasmic genomes are extremely low in gene contents, and virtually all regulatory functions of organellar gene expression are nuclear encoded (5–7). Nucleo-cytoplasmic male sterilities (CMS) represent ideal genetic models to understand nucleo-mitochondrial coadaptation processes. CMS is a widely expanded trait of plants characterized by an inability to produce functional pollen. CMS traits are specified by poorly conserved mitochondrial genes and can be suppressed by nuclear-encoded restorer-of-fertility (*Rf*) genes that specifically act in most cases to down-regulate the expression of corresponding CMS-specifying mitochondrial genes (8). In recent years, several *Rf* genes were identified in various crop species, and most of them were found to encode proteins belonging to the large family of pentatricopeptide repeat (PPR) proteins (9). PPR proteins are highly specific RNA-binding proteins that widely diversified in eukaryotes, mainly in terrestrial plants (10). PPR proteins have been shown to play multifarious roles in mitochondrial and plastid RNA expression processes, going from gene transcription to mRNA translation (11). *Rf*-PPR–mediated suppressing activity most often alters mitochondrial CMS-causing mRNA levels (12–15). The Ogura CMS *Rf*-PPR from radish is an exception among fertility restorers as it was shown to not affect its cognate CMS-conferring mRNA, either in size or in abundance (16). The Ogura CMS, originally identified in radish (*Raphanus sativus*) and later transferred to rapeseed (*Brassica napus*), is controlled by the mitochondrial *orf138* locus (17, 18). We showed that the Ogura restorer-of-fertility protein named PPR-B (19–21) associates in vivo with the *orf138* mRNA and that this association leads to a strong decrease in Orf138 protein level, notably in tapetal cells and developing pollen grains (22). The Ogura *Rf*-PPR was thus suspected to impact the translation of *orf138* mRNA, but this hypothesis needed to be validated and the way by which PPR-B may interfere with the translation of the *orf138* transcript determined. In the present study, we demonstrate that PPR-B CMS-suppressing activity implies a specific down-regulation of *orf138* translation and, very interestingly, that this control likely operates through a blockade of ribosome progression along the *orf138* coding sequence. The Ogura fertility restorer is the first *Rf* protein demonstrated to act this way.

## Results

**The Orf138 Protein Is Not Produced In Mitochondria in the Presence of PPR-B.** The biochemical characterization of fertility restorers in planta has often been rendered difficult by the tissue specificity

### Significance

**Nucleo-cytoplasmic male sterilities (CMS) are two-component genetic systems in which mitochondria-encoded male sterility transcripts are controlled through the action of nuclear-encoded restorer-of-fertility proteins. Fertility restorers most often impact the accumulation of CMS transcripts. In this analysis, we demonstrate that fertility restoration in the Ogura system from radish operates through a specific blockade of ribosome progression along the *orf138* CMS transcript. Our analysis reveals that CMS transcripts can be controlled at the translational level, a discovery that will be instrumental to produce custom synthetic fertility restorers.**

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of associated molecular mechanisms (23). This is true for the Ogura CMS system, as we showed that fertility restoration correlates with a profound decrease in Orf138 in tapetal cells and microspores but not in other plant tissues (22). We could, however, produce a restored rapeseed transgenic line (named B1) containing four copies of *PPR-B* in which Orf138 reduction was nearly ubiquitous. This provided us with an ideal biological material to characterize the effect of PPR-B on Orf138 production since the observed decrease was homogeneous across all plant tissues in this line. Immunoblot analyses were first conducted to confirm the near-complete disappearance of Orf138 in mitochondrial extracts prepared from B1 plant inflorescences (Fig. 1*A*). In organello protein syntheses in the presence of [$^{35}$S] methionine were then carried out with mitochondria prepared from CMS and restored B1 plants. Virtually identical translation profiles were revealed in both lines, except for one protein, close to 20 kDa, which was clearly visible in the CMS but not in the B1 line (Fig. 1*B*). A CMS-specific protein of the exact same size was identified in previous in organello profiles and was demonstrated to correspond to the Orf138 protein, as it could be immunoprecipitated with an Orf138-specific antibody (24). The lack of Orf138 in B1 in organello translation products strongly favored an incapacity of mitochondria to produce the Orf138 protein under the action of PPR-B rather than an increased instability of Orf138 in the restoration context.

**The Translation of *orf138* mRNA Is Impaired in the Presence of PPR-B.** The in organello translation results strongly suggested a likely negative impact of PPR-B on the translation of the *orf138* mRNA. Therefore, the translation status of the *orf138* transcript was first evaluated by polysome sedimentation analysis in both CMS and restored plants. The cotranscription of *orf138* with the *atp8* gene first necessitated replacing the cytoplasm of the B1 line with that of the male-sterile 18S line, in which the *orf138* gene is not associated with *atp8* and transcribed as a monocistronic mRNA (24). The B1 line was then used to pollinate male-sterile 18S flowers, and several F1 descendants were tested by immunoblot analysis to evaluate their content in Orf138 protein accumulation. In control, a nontransgenic Pactol Fertile (PF) × 18S F1 hybrid line was also generated. Unlike male-sterile PF/18S plants, all B1/18S hybrid plants were found to accumulate barely detectable levels of Orf138 as in the original B1 line (*SI Appendix*, Fig. S1). Polysomes were then isolated from rapeseed B1/18S and PF/18S inflorescences and fractionated on continuous sucrose density gradients. Ten fractions were collected along the gradients after centrifugation and analyzed by subsequent RNA gel blot. Polysome integrity was verified by the distribution of ribosomal RNAs along the gradients in the presence of $MgCl_2$ (*SI Appendix*, Fig. S2). The disruption of polysomes with ethylenediaminetetraacetic acid (EDTA) indicated that polysomal RNAs migrated toward the center and the bottom of the gradients, whereas ribosome-free mRNAs accumulated in the upper fractions (*SI Appendix*, Fig. S2). Total RNAs were extracted from each fraction and subjected to RNA gel-blot analysis using probes specifically recognizing the *orf138* transcript as well as *atp9* and *atp1* as controls (Fig. 2*A*). Obtained hybridization signals were then quantified, and their relative distribution along the gradients was determined for each transcript (Fig. 2*B*). In the absence of PPR-B, we observed that the majority of the *orf138* signal accumulated in the central fractions, suggesting active translation of *orf138* in this genetic context. However, in the presence of PPR-B, the peak of *orf138* hybridization signal was clearly shifted toward upper fractions, revealing a negative impact of PPR-B on *orf138* mRNA and polysome association. The distributions of *atp1* and *atp9* were unaffected by the PPR-B status, indicating that PPR-B–mediated translational impairment was specific to *orf138*. In a next effort to better understand the origin of PPR-B translational repression, ribosome-sequencing (Ribo-Seq) analyses were developed to compare the translational status of all mitochondria-encoded mRNA in CMS and fertility-restored lines. Total ribosome footprints were prepared from both lines and then mapped to the rapeseed mitochondrial genome and the *orf138* locus. RNA-sequencing (RNA-Seq) experiments were also developed to quantify the steady-state levels of all mitochondrial transcripts in the two genetic backgrounds. RNA-Seq data revealed no major impact of PPR-B on mitochondrial mRNA abundance, except for several ribosomal protein transcripts whose steady-state levels were reduced by a factor of around 2 (*SI Appendix*, Fig. S3). Calculated translational efficiencies (see *Materials and Methods* for details) indicated a slight decrease of ribosome coverage for most mitochondrial transcripts by less than a factor of 2 in the restored B1 line compared to the CMS line, except for the *orf138* mRNA, which was found to be around 16-fold less translated under PPR-B action (Fig. 3*A*). Interestingly, a few ribosomal protein transcripts (e.g., *rpl16* or *rps14*) appeared to be slightly up-translated in the presence of PPR-B. The impact of measured translational differences on mitochondrial protein accumulation was next evaluated by immunoblot assays (Fig. 3*B*). Among the few tested proteins, no major differences in protein steady-state levels could be detected between CMS and B1 lines. The only reproducible differences concerned the Nad7 protein, which appeared to slightly overaccumulate in B1 plants compared to the CMS line, and, of course, the *orf138* protein, which was here again hardly detectable in the B1 line. Altogether, these results strongly supported that the lack of Orf138 production in the restored B1 line resulted from a potent impairment of *orf138* mRNA translation.

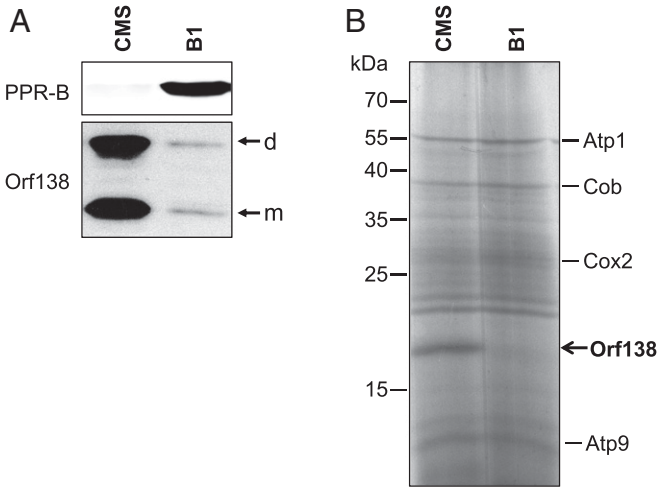



**Fig. 1.** The Orf138 protein is not produced in the presence of PPR-B. (*A*) Immunoblot assay showing the steady-state levels of Orf138 and PPR-B proteins in floral tissues of CMS and transgenic B1 lines. m: monomeric Orf138, d: dimeric Orf138. (*B*) Autoradiography of in organello [$^{35}$S]methionine-labeled translation products from mitochondria isolated from CMS and transgenic B1 rapeseed hypocotyls. Mitochondria translation products were separated on a 15% sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gel. The position of the Orf138 protein is indicated.

**The PPR-B Fertility Restorer Binds within the Coding Sequence of the *orf138* mRNA.** We previously demonstrated that PPR-B specifically associates with the *orf138* mRNA in vivo (22). To understand how this association could negatively impact *orf138* translation, we sought to identify the binding site of PPR-B within the *orf138* transcript. PPR proteins are known to associate with their RNA target via a one PPR motif-one nucleotide recognition rule and amino acid combinations at positions 5 and 35 of each repeat are major determinants for RNA base selection (25–27). We thus used the established PPR recognition code to predict the most likely binding regions of PPR-B within the

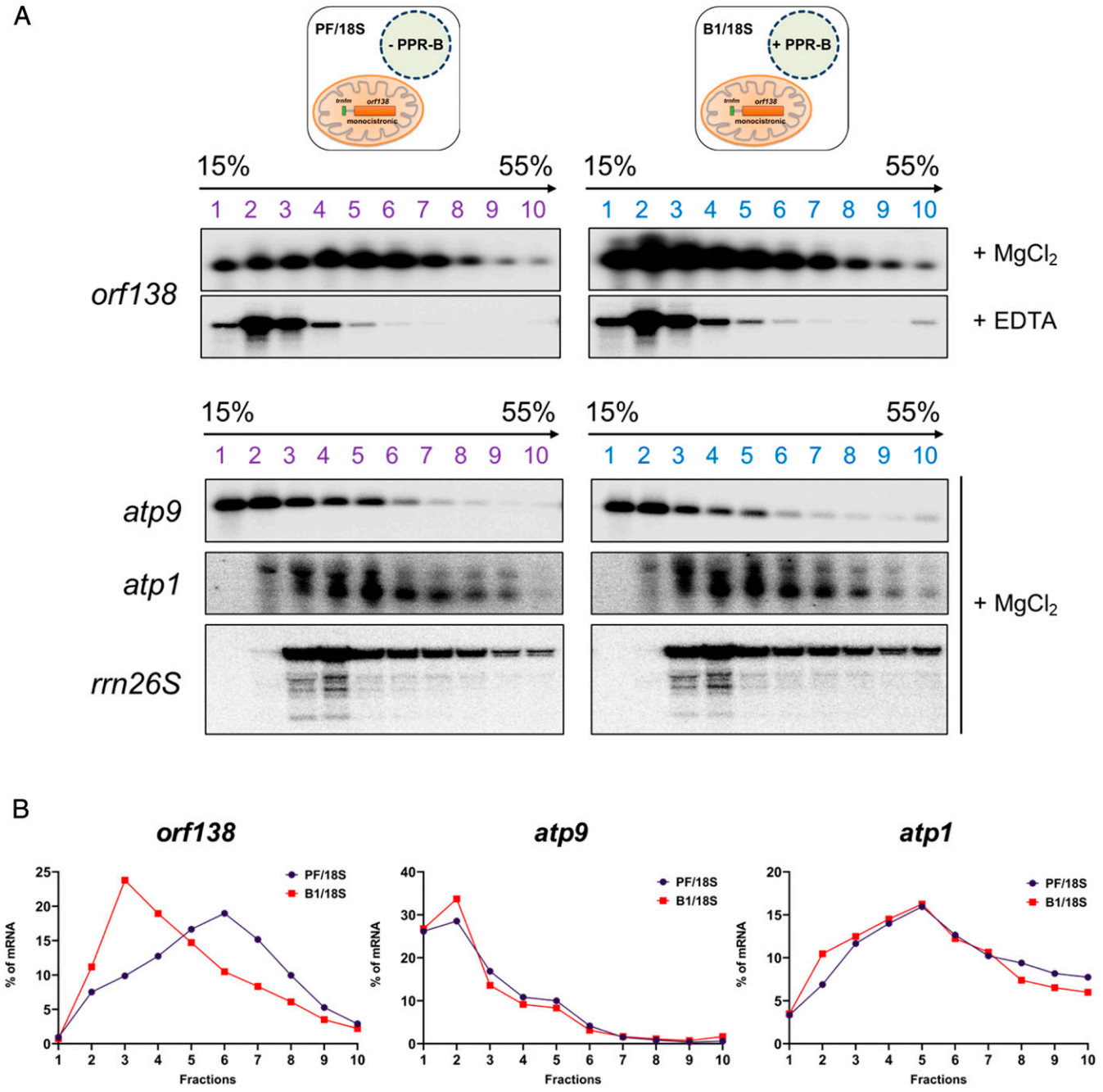



**Fig. 2.** The association of the *orf138* transcript with mitochondrial polysomes is perturbed by PPR-B. (*A*) Total polysomes extracted from flower buds of PF/18S and B1/18S plants were fractionated in 15 to 55% sucrose density gradients by ultracentrifugation and under conditions maintaining (+$MgCl_2$) or disrupting (+EDTA) polysome integrity and analyzed by RNA gel blot assays using the indicated gene probes. rrn26S corresponds to the mitochondrial 26S ribosomal RNA, and its hybridization profile attests for the integrity of polysomes along the gradients in the presence of $MgCl_2$. (*B*) Quantification of hybridization signals along the polysomal gradients. The hybridization signals corresponding to *orf138*, *atp9*, and *atp1* transcripts were quantified using the ImageQuant software (GE Healthcare Life Sciences) in each fraction and genotype. The indicated values correspond to the percentage of contribution of each fraction to the sum of all hybridization signals obtained over the entire gradients.

rapeseed mitochondrial genome and the *orf138* locus (Fig. 4). Interestingly, a highly-probable binding region corresponding to a short segment located 55 nucleotides downstream of *orf138* AUG codon could be identified (Fig. 4*B*). Other identified potential binding sites located in intergenic regions of the *B. napus* mitochondrial genome had virtually no chance to be associated with PPR-B–mediated restoration activity. The in vivo RNA-binding specificity of PPR-B was then assessed by RNA-immunoprecipitation and sequencing assay (RIP-Seq). Immunoprecipitations were conducted with the anti–PPR-B antibody from both male-sterile and restored mitochondrial extracts. To limit coenriched RNA fragments to the regions physically covered by PPR-B, RIP-Seq assays were done by digesting the extracts with RNase-I prior to immunoprecipitation. Coenriched RNA species were deep sequenced after complementary DNA synthesis, mapped to the rapeseed mitochondrial genome and the *orf138* locus. A single protected zone of 67 nucleotides was found to be highly and specifically enriched in PPR-B immunoprecipitation (Fig. 5*A*). Interestingly,

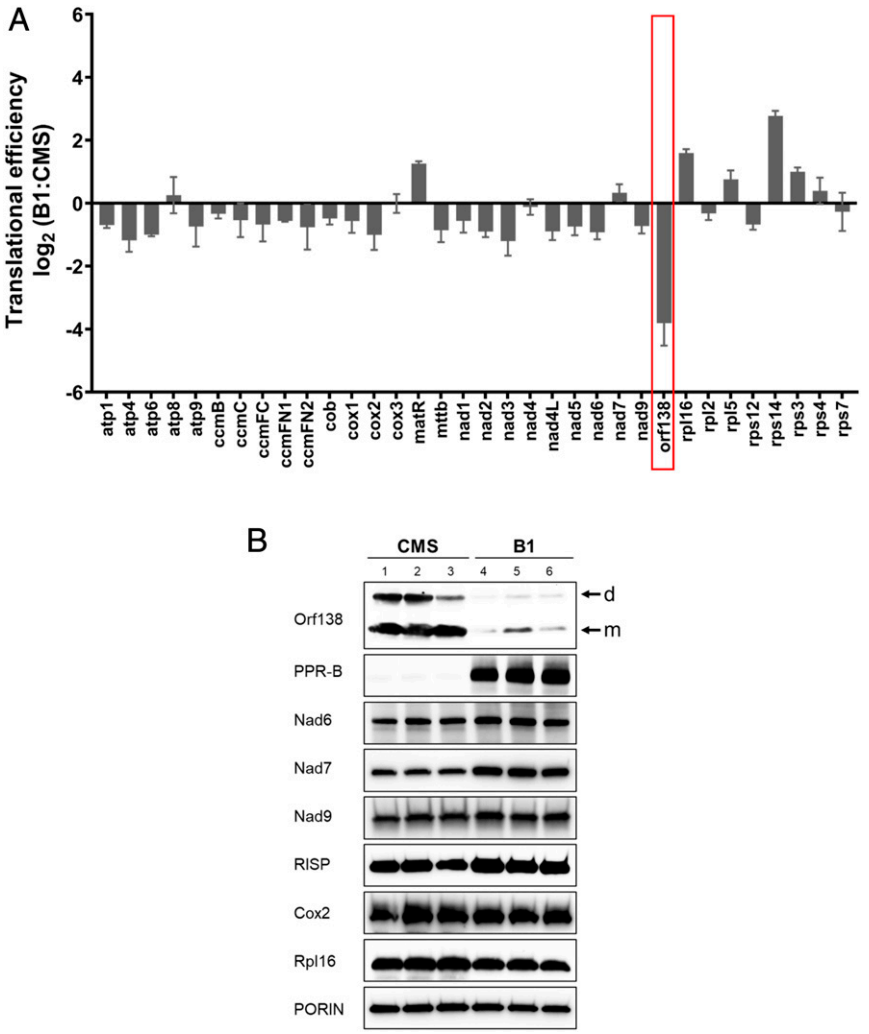
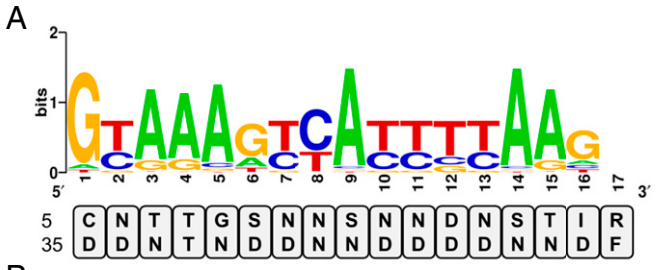



**Fig. 3.** Translation of the *orf138* mRNA is strongly reduced under the effect of PPR-B. (*A*) Compared Ribo-Seq analysis of mitochondrial mRNAs in flower buds of B1 and CMS lines. The bars depict $\log_2$ ratios of translational efficiencies of mitochondria-encoded mRNAs in B1 plants to the CMS line (see *Materials and Methods* for details). The reported values are means of two independent biological replicates (error bars indicate SD). (*B*) SDS-PAGE immunoblots performed on total mitochondrial protein extracts prepared from flower buds of B1 and CMS plants and probed with antibodies to Orf138 and PPR-B as well as subunits of respiratory complex I (Nad6, Nad7, and Nad9), complex III (RISP), complex IV (Cox2), and the ribosomal protein Rpl16. Porin was used as protein loading control. Results obtained on three independent protein preparations are shown for each line. m: monomeric Orf138, d: dimeric Orf138.

this region lies within the *orf138* coding sequence and includes the predicted PPR-B binding site (Fig. 4). To further demonstrate that PPR-B shows significant affinity to this region of the *orf138* transcript, gel shift assays were developed using a series of in vitro transcribed probes mapping to the 5′ region of the *orf138* mRNA (Fig. 5*B*) and a recombinant form of PPR-B fused to the maltose binding protein (Fig. 5*C*). Among the different RNA probes tested, PPR-B showed a clear and strong binding affinity for all the probes containing the predicted binding site (Fig. 5*D*). In contrast, PPR-B did not associate with nearby probes that did not contain the GTAAAGTTAGTGTAATA sequence, strongly supporting that this sequence is the PPR-B binding site in vivo.

**PPR-B Interferes with the Progression of Translating Ribosomes along the *orf138* mRNA.** The location of PPR-B binding site within *orf138* coding sequence led us to analyze in detail the distribution of ribosome footprints along the *orf138* coding sequence in the presence and in the absence of PPR-B. First, RNA-Seq read profiles along the di-cistronic *orf138* transcript but did not reveal any major differences between the CMS and B1 lines (Fig. 6*A*), except a slight decrease of read coverage for both *orf138* and *atp8* in the restored line, as shown in *SI Appendix*, Fig. S3. In contrast, Ribo-Seq read distributions that confirmed the strong decrease of ribosome coverage on the *orf138* open reading frame and a lack of major impact of PPR-B on *atp8* translation. The position of the PPR-B binding site within *orf138* suggested that it may block translation elongation along the transcript. To see whether this might be possible, we calculated the average number of ribosomes along the *orf138* transcript before and after the PPR-B binding site in both B1 and CMS lines. These data were normalized by both gene fragment length and abundance as estimated from RNA-Seq read coverage. A moderate reduction of about 25% in ribosome coverage could be detected in the B1 line before the PPR-B binding site (Fig. 6*B*), which is much less pronounced than the global translational decrease measured for *orf138* in the B1 line (Fig. 3*A*). In contrast, the shortage in ribosome density along the *orf138* segment located downstream of PPR-B binding site was found to be much more dramatic (Fig. 6*B*) and in the same magnitude as the observed translation reduction of the *orf138* gene in the B1 line (Fig. 3*A*). This observation does not support an impact of PPR-B on *orf138* translational initiation




| Rank | P-value | Strand | Location | Sequence |
|---|---|---|---|---|
| 1 | 1.15E-06 | - | intergenic | GTAAATCCACTTCAGGT |
| 2 | 2.32E-06 | + | intergenic | GTAGAATTATTCCAAGT |
| **3** | **6.52E-06** | **+** | ***orf138* CDS** | **GTAAAGTTAGTGTAATA** |
| 4 | 1.11E-05 | - | intergenic | ACAAAGCCACTTTCAGC |
| 5 | 1.21E-05 | + | intergenic | GCATAGTCATTCCAGGT |
| 6 | 1.38E-05 | - | intergenic | GTAAAGCTAGTTTACGG |

**Fig. 4.** The PPR-B protein is predicted to associate within the coding sequence of *orf138*. (*A*) Combinations of amino acids at position 5 and 35 of each PPR-B PPR repeat are listed from N to C terminus. The generated combinations were used to calculate probabilities of RNA base recognition by each PPR-B PPR repeat according to the PPR code (25–27). The sequence logo depicting these probabilities was obtained with http://weblogo.berkeley.edu/. (*B*) The nucleotide preference scores were then used to scan both strands of the *B. napus* mitochondrial genome (GenBank AP006444.1) and the *orf138* locus sequence (18) with the FIMO program (67). The sequence of the six most-probable PPR-B binding sequences are shown with their respective location. The *P* values were determined with the FIMO program.

but rather an incapacity of elongating ribosomes to cross the PPR-B binding site when the fertility restorer protein is present.

## Discussion

**The Ogura Fertility Restorer Protein Is an mRNA-Specific Translation Elongation Inhibitor.** Besides being essential for protein production, mRNA translation is an important control step in gene expression, and all phases of translation constitute potential control checkpoints. It has been shown that the initiation step, which consists of the loading of the small ribosomal subunit and the charged initiator transfer RNA on the start codon prior to full ribosome assembly, represents the major checkpoint of translational control (28–31). In eubacteria, such regulation mostly operates by outcompeting the binding of the initiation complex to the mRNA 5′ translation initiation region, most particularly to the Shine-Dalgarno sequence. This can be achieved in multiple ways, such as the association of regulatory proteins, small antisense RNAs, or metabolites or via the action of signals like temperature or pH (1, 2). In eukaryotes, the regulation of translation initiation occurs mostly via the binding of partially complementary antisense microRNAs to mRNA 5′ or 3′ untranslated regions (UTRs) (4). Our analysis strongly suggests that the translation control used to silence the CMS-causing *orf138* transcript of the Ogura system operates at a different level than the initiation step. Translational inhibition has long been suspected to be the molecular mechanism associated with fertility restoration in this CMS system since the decrease in Orf138 protein accumulation is not accompanied by any impact on the *orf138* mRNA accumulation, whereas PPR-B was found to associate with the *orf138* transcript in vivo (22). The in organello synthesis, polysome sedimentation, and Ribo-Seq analyses presented in this study are perfectly concordant and unambiguously support that PPR-B–mediated *orf138* silencing effectively involves a specific inhibition of *orf138* translation (Figs. 1–3). Moreover, the location of PPR-B binding sites within the *orf138* coding sequence (Figs. 4 and 5) and the barely altered ribosome density upstream of this site when PPR-B is present (Fig. 6) strongly support that PPR-B translational control occurs by hindering translation elongation along the *orf138* mRNA and not by affecting the initiation step. Thus, PPR-B seems to act by blocking ribosome translocation during translation elongation along *orf138* transcript, most likely by steric hindrance (Fig. 7). Interestingly, this control does not affect *atp8*—the second orf of the *orf138-atp8* di-cistronic transcript—indicating an independent translational control of *orf138* and *atp8*. This observation correlates with our recent characterization of the *Arabidopsis* mitochondrial translatome, which revealed that orfs contained in di-cistronic mRNAs are differentially translated, implying possible internal entry of ribosomes upstream of downstream orfs (32).

The translational inhibitory function that we reveal for the PPR-B fertility restorer (Rfo) in our study has not been previously described to control the expression of any organellar genes, including those associated with CMS (11, 33). On the contrary, some P-type PPR proteins have been shown to facilitate translation in organelles in an mRNA-specific manner (34, 35). The mode action of PPR-B is, however, reminiscent of the way organellar helical repeat proteins, including PPR proteins, act to stabilize mitochondrial and plastid RNAs by impeding the progression of exoribonucleases along mRNAs from their 5′ or 3′ extremities (36–40). PPR proteins thus have an inherent capacity to act as roadblocks to impede the progression of RNA-processing enzymes along transcripts, which in the case of ribosomes, implies to counteract the strong helicase activity of elongating ribosomes (41). The binding strength of PPR-B to its target site may be important to efficiently block translation elongation along the *orf138* mRNAs in vivo, as we previously showed that all PPR-B repeats are indispensable for complete fertility restoration in rapeseed (42). Similarly, a PPR-B allele comprising only four amino acid substitutions was also found to be incompetent for fertility restoration in radish (21). Recently, a natural single nucleotide substitution altering the third base of the Rfo binding site was shown to prevent fertility restoration, confirming not only the link between this region of *orf138* and Rfo for fertility restoration but also the need for a perfect association between Rfo and its RNA target site to prevent Orf138 protein production (43).

Other known examples involving the impairment of translation elongation are scarce. Codon usage, Shine and Dalgarno-like sequences, or mRNA secondary structures have been shown to influence translation elongation speed or rate along bacterial orfs but do not lead to a complete block of translation elongation (1). In plants, microRNAs have also been found to partially work as ribosome blocker, although the biological impact of such translational repression remains unclear (4, 44, 45). The analysis of PPR-B activity reveals a way of translational control that leads to an arrest of the translation elongation for a mitochondrial transcript. The recent deciphering of the PPR recognition code (25, 46) allows the recoding of PPR proteins to bind any sequence of interest (47–49). It will therefore be interesting to see if PPR-B translation inhibitory activity can be recreated using synthetic or recoded PPR proteins and then use such a factor to investigate whether the blockade of translational elongation requires a binding in the proximal region of *orf138* mRNA.

**The Ogura Fertility Restorer Protein Is Shown to Act as a Translation Elongation Blocker.** *Rf* genes have been cloned from various crop species, and most of them were found to encode PPR proteins (8, 50). Large-scale phylogenetic analyses have shown that identified *Rf*-PPR genes are evolutionarily related and have evolved from a distinct subgroup of PPR genes called *Rf*-like or RFL (51). However, molecular analyses have revealed that *Rf*-PPRs reduce the accumulation of their cognate CMS-inducing mitochondrial RNAs or proteins through different mechanisms.

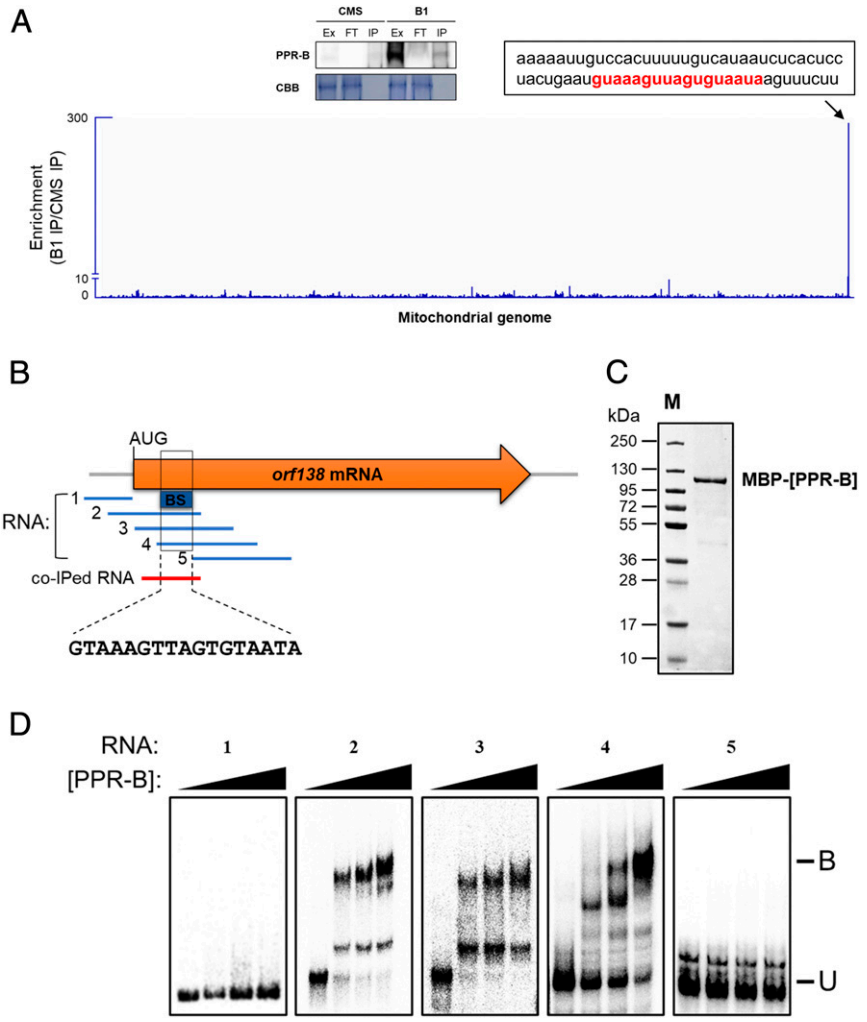



**Fig. 5.** PPR-B binds 55 nucleotides downstream of the *orf138* translational start codon. (*A*) RIP-Seq/RNase-I assay on mitochondrial extracts prepared from flower buds of male-sterile and restored plants. Coimmunoprecipitated RNA species were used for cDNA synthesis and deep sequenced. Sequencing reads were mapped to the mitochondrial genome, and a screenshot produced with Integrated Genome Viewer software illustrating the enrichment ratios along the rapeseed mitochondrial genome and the *orf138* gene is shown. The sequence of the most enriched region (with the predicted PPR-B binding site appearing in red) is shown in the upper right rectangle. Immunoblot validation of immunoprecipitations is also presented. (*B*) Schematic repression of the relative positions of the different in vitro transcribed RNA probes used in gel shift assays shown in *D*. The approximate position of the RNA segment enriched in RIP-Seq assay (see *A*) is shown as a red line. The location and sequence of the predicted PPR-B binding site are also displayed. (*C*) SDS-PAGE gel stained with Coomassie blue demonstrating the purity of the MBP-PPR-B fusion expressed and purified from *Escherichia coli*. Five micrograms of purified MBP-PPR-B were loaded on the shown gel. Protein molecular weight markers are also shown (M). (*D*) Gel mobility shift assays done with the MBP-PPR-B fusion and the RNA probes shown in *B*; 200 pM of radiolabeled RNA probes along with 0 to 800 nM of the fusion protein and 0.5 mg/mL heparin as negative binding competitor were added in each reaction. U corresponds to the unbound probes and B to the probes bound to MBP-PPR-B.

The vast majority of fertility restorer proteins, such as RF1A, RF1B, or RF6 in rice (12, 52), RFN and RFP in rapeseed (53–55), or RF1 and RF3 in wheat (56), induce specific cleavage within the coding sequence of their cognate CMS-inducing mitochondrial transcripts. Such *Rf*-PPRs, and several other RFL proteins involved in the processing of conserved mitochondrial transcripts (57–61), generally bind 20 to 100 bases upstream of the cleavage sites and induce subsequent endonucleolytic processing through a still unclear mechanism (62). The recruitment of an unidentified endonuclease and a potential influence of RNA secondary structure or sequence close to the processing sites have been proposed to explain why cleavage does not always occur at the same distance from PPR binding sites (49). In most cases, the RNA cleavage induced by *Rf*-PPRs results in a significant decrease in the accumulation of CMS transcripts, leading to a reduction in the production of corresponding CMS proteins and, hence, to fertility restoration. The reason Rfo binding does not induce any RNA cleavage within the *orf138* transcript as opposed to most other *Rf*-PPR proteins in their cognate CMS mRNA remains mysterious. The translational suppression activity that we reveal here for the Ogura fertility restorer is the very first example described for a PPR protein and thus for an *Rf*-PPR. It has been suggested that the rice RF1A may negatively impact the translation of its cognate CMS mRNA, *orf79*. However, the observed translational inhibitory effect is not directly imputable to RF1A but is secondary to an RF1A-induced RNA cleavage, liberating a nontranslatable monocistronic form of the *orf79* transcript (63). Similarly, the petunia *Rf*-PPR592 protein has been suggested to impact the translation of the CMS-associated mRNA *pcf*, although changes in *pcf* 5′ processing were also detected in restored plants (64). RNA coimmunoprecipitation assays with *Rf*-PPR592 showed greatest enrichment in a region of the *pcf* 5′ leader

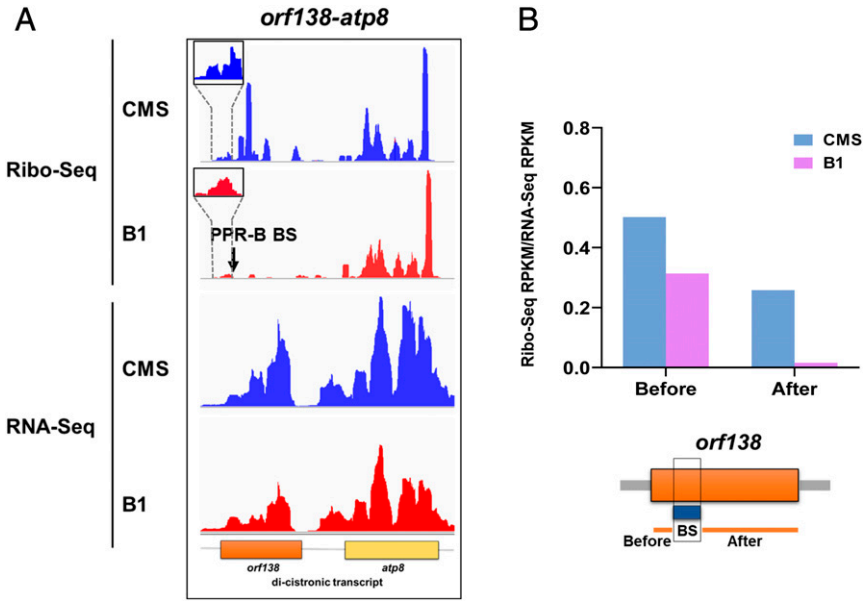



**Fig. 6.** The decrease in ribosome footprints due to PPR-B along the *orf138* mRNA is much more pronounced downstream than upstream of PPR-B binding site. (*A*) Screen captures from the Integrated Genome Viewer software showing the distribution of RNA-Seq reads and ribosome footprints (Ribo-Seq) along the *orf138* locus in B1 and CMS lines. The distributions were normalized to the number of reads mapping to the mitochondrial genome. Magnified views of the ribosome footprint distributions upstream of PPR-B biding site are shown in circles for both genotypes. (*B*) Normalized ribosome footprint densities calculated before and after of PPR-B binding site in the *orf138* transcript. Shown data are means of two biological repeats.

overlapping with *pcf* 5′ processing site (65). These results do not allow to firmly conclude on the molecular function of *Rf*-PPR592, but its preferential association with a region of *pcf* 5′ UTR favors a role in *pcf* transcript 5′-end processing (66). PPR-B sequence is highly similar to that of RFL proteins known to induce RNA endonucleolytic cleavage in *Arabidopsis* (51, 57). Minor sequence differences between PPR-B and these RFLs or the involvement of specific *cis*-elements or RNA secondary structures downstream of the binding sites may be responsible for their difference in activity. The molecular mode of action of PPR-B demonstrates that CMS genes could be silenced by simply targeting a PPR protein in their coding sequence to inhibit their translation, regardless of the presence of sequences or structural elements favorable for RNA cleavage. Our observations should thus facilitate the production of synthetic fertility restorers for CMS systems in which no efficient restorers have been identified yet.

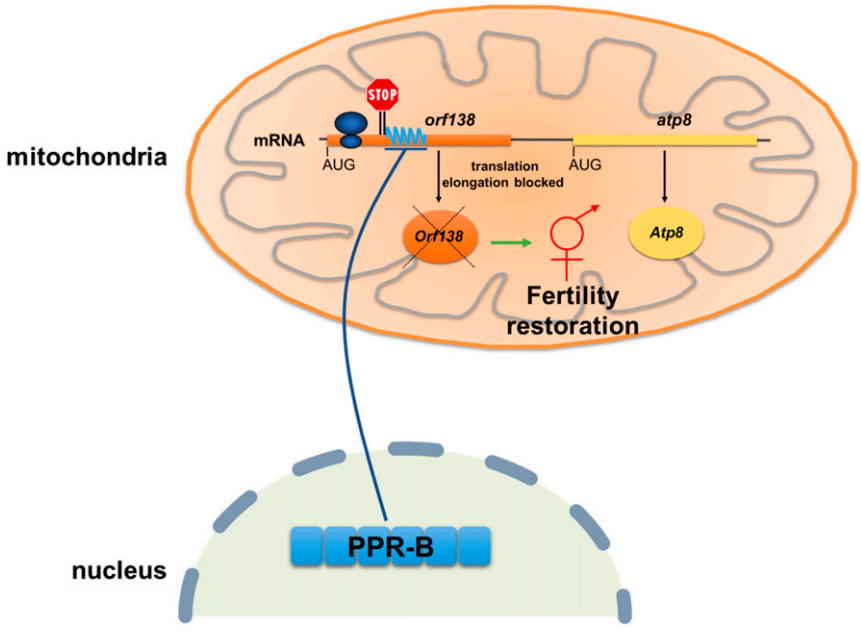



**Fig. 7.** Model of PPR-B mode of action to restore fertility in the Ogura CMS system. Drawing illustrating the molecular mode of action of the PPR-B which, after transfer into mitochondria, specifically inhibits translation elongation along the *orf138* mRNA, thereby inhibiting the production of the mitochondrial protein Orf138 and restoring male fertility.

## Materials and Methods

Plant materials, preparation of mitochondria, polysome association analysis, immunodetection of proteins, Ribo-Seq, RNA-Seq, high-resolution RIP-Seq, gel mobility shift assays, and in organello protein synthesis are described in *SI Appendix, SI Materials and Methods*.

**Data Availability.** Ribo-Seq and RNA-Seq sequencing data have been deposited in National Center for Biotechnology Information Gene Expression Omnibus (GSE178722 and GSE178543). All other study data are included in the article and/or *SI Appendix*.

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