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Translational coupling of the maize chloroplast *atpB* and *atpE* genes

(photosynthesis/protein synthesis/ribosome function/cyanobacteria/ATP synthase)

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ABSTRACT **The genes for the β and ε subunits of maize chloroplast ATP synthase are encoded by the organelle genome, are cotranscribed, and have overlapping translation initiation and termination codons. To determine whether the *atpB* and *atpE* genes are translationally coupled, they were transformed into *Escherichia coli* on a multicopy plasmid. Synthesis of full-length β and ε polypeptides demonstrated correct initiation of translation by the bacterial ribosomes. To assay for translational coupling, the promoter-distal *atpE* gene was fused to *lacZ*, resulting in the synthesis of an active hybrid β-galactosidase. A frameshift mutation was introduced into the promoter-proximal *atpB* gene, and its effect on the transcription and translation of the *atpE*::*lacZ* fusion was measured. The mutation resulted in a 1000- to 2000-fold reduction in β-galactosidase activity, but only a 2-fold decrease in LacZ mRNA synthesis rates or galactoside transacetylase levels. Similar results were obtained when the *atpB*/*atpE*::*lacZ* fusion and the *atpB* frameshift mutation were introduced into the photosynthetic cyanobacterium *Synechocystis* sp. PCC6803. We show that >99% of *atpE* translation depends on successful translation of *atpB* and, thus, conclude that the two genes are translationally coupled.**

The energy-transducing ATP synthase (ATPase) has a highly conserved structure that couples proton translocation across membranes with the synthesis of ATP. The complex present in chloroplasts, mitochondria, and prokaryotes consists of an F_1, or CF_1, extrinsic membrane component composed of five subunits (α–ε) that catalyzes the final step in oxidative phosphorylation or photophosphorylation (1, 2). The integral membrane F_0, or CF_0, sector forms the proton channel. Genes for the α, β, and ε subunits of CF_1 and subunits I, III, and IV of CF_0 are present in the chloroplast genome (3–6). The remaining three subunits (γ, δ, and II) are nuclear encoded and are imported into chloroplasts for assembly into an active enzyme. The chloroplast *atp* genes are organized into two transcriptional units that have retained a similar positional conservation to the arrangement of genes in the *atp* operon of *Escherichia coli* (3, 4). One cluster of genes has the order *atpI*/*atpH*/*atpF*/*atpA*, and there is evidence that they are cotranscribed (3, 4). The other chloroplast-encoded *atp* genes are located many kilobases from the *atpIHFA* cluster (4–6), have the order *atpB*/*atpE*, and encode the β and ε subunits, respectively, of the membrane component CF_1 (7, 8). The *atpBE* genes are cotranscribed as a dicistronic message that does not appear to be processed (7, 8).

An unusual feature of the *atpBE* genes from several plant species, which is not seen for the other chloroplast *atp* genes, is overlapping of the termination codon of *atpB* with the initiation codon of *atpE* in the sequence ATGA (7–11). The close proximity of the termination and initiation codons of two genes, or the overlapping of parts of their sequences, indicates that translation of the genes may be coupled. In *E. coli* translational coupling has been identified in the *trp* (12, 13), *gal* (14), *ilv* (15), and several ribosomal protein (16–19) operons, where it presumably serves as a mechanism to ensure the appropriate level of synthesis of these proteins. Reports on translational coupling in other organisms are scarce, and no translation coupling in chloroplast genes has been reported, although the existence of coupling was invoked to explain the overlapping β and ε genes (7, 8).

In this paper we have examined whether translation of the maize ε gene is coupled to translation of the upstream β gene when expressed either in *E. coli* or in the photosynthetic cyanobacterium *Synechocystis* sp. PCC6803. An *atpE*::*lacZ* fusion has been used to study the effect of a frameshift mutation in *atpB* on transcription and translation of the *atpE*::*lacZ* fusion. The translational frameshift results in slight transcriptional, but significant translational polarity, which shows that synthesis of the β and ε subunits of chloroplast ATP synthase is translationally coupled. Identification of overlapping reading frames in the chloroplast genes encoding proteins of other multisubunit complexes (5, 6) suggests that translational coupling may be a general mechanism in the organelle to ensure controlled synthesis of some of these subunits.

MATERIALS AND METHODS

DNA Manipulations. The maize β and ε genes were isolated by screening a library of chloroplast DNA, cloned in the λ insertion vector NM641 (20), by use of a "nick-translated" (Amersham kit) spinach β gene as probe (7). A plasmid for expression of β and ε genes in *E. coli* was constructed by subcloning the appropriate 3.8-kilobase (kb) *Eco*RI fragment (8) from a positive phage into the *Eco*RI site of the P_L expression plasmid pLc236 (21). The resulting plasmid, in which *atpB* and *atpE* are transcribed from P_L, is named pPBI33 (Fig. 1). A plasmid in which the genes are incorrectly orientated for transcription from P_L is named pPBI34. A translational fusion between *atpE* and *lacZ* was made by isolating a 4.1-kb *Pvu* II fragment from pPBI34, where one *Pvu* II site is located in the pLc236 vector (21) and the other is located at codon 43 in the *atpE* gene (8). The *Pvu* II fragment was cloned into the *Hin*cII sites of pUC7 (22) to give an intermediary plasmid named p491. By using an *Eco*RI site in the polylinker of pUC7 and an *Eco*RI site in the chloroplast DNA upstream of β (8), the β and partial ε genes were transferred from p491 into the *Eco*RI site of pMC1403 (23) to

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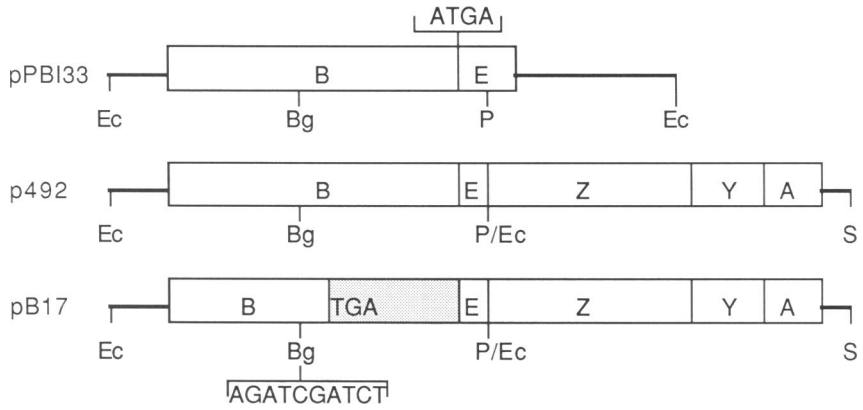

FIG. 1. Plasmids for expression of maize ATPase β and ϵ subunits and β-galactosidase fusion proteins in *E. coli*. The letters B, E, Z, Y, and A identify the *atpB*, *atpE*, *lacZ*, *lacY*, and *lacA* genes, respectively. For pPBI33 the sequence for the overlapping termination codon of the β gene and the initiation codon of the ϵ gene are shown. The *atpBE* genes are transcribed from a P_L promoter in pPBI33. For p492 and pβ17 the junction between ϵ and Z is shown by a line marked P/Ec, which indicates a polylinker region fusing the *Pvu* II (P) and *Eco*RI (Ec) sites. In pβ17 the sequence generated by repairing the *Bgl* II (Bg) site with Klenow fragment and the position of the frameshifted TGA termination sequence is shown. The shaded area in pβ17 represents nontranslated sequences between the β and ϵ genes. A *Sal* I (S) site is also marked. Derivatives of these plasmids were used for expression in *Synechocystis* 6803 and are described in the text.

give p492 (Fig. 1). Plasmid p492 encodes an *atpE*::*lacZ* gene fusion. To introduce a frameshift mutation into *atpB*, plasmid p492 was cut at a unique *Bgl* II site located at codon 134 of *atpB* (8), repaired with the Klenow fragment of DNA polymerase, and recircularized with T4 DNA ligase. The DNA was transformed into MC1061 (23), and colonies were identified that produced reduced levels of β-galactosidase on indicator plates containing 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside. Correct repair of a *Bgl* II site by DNA polymerase creates a *Cla* I site; putative frameshift mutants were screened for the presence of this new *Cla* I site. One of these mutants was designated pβ17 (Fig. 1), and the frameshift was confirmed by subcloning an appropriate *Sma* I fragment into M13mp18 and sequencing the DNA.

To examine translational coupling in *Synechocystis* 6803, the *lac* operon fusions encoded by p492 and pβ17 were transferred to the integration vector pKW1313, which is a derivative of pKW1188 (24). Plasmids p492 and pβ17 were cut at their unique *Sal* I sites distal to the 3′ end of *lacA* in the vector pMC1403 (23). They were then subjected to partial *Eco*RI digestion, and a *Sal* I/partial *Eco*RI fragment encoding the *lac* operon fusion was isolated. This fragment was cloned into pKW1313 linearized with *Sal* I and *Eco*RI to give either p492KW, where p492 was the fragment donor, or pβ17KW, where pβ17 was the donor. Plasmids were transformed into *Synechocystis* 6803 as described (24), except that cells were allowed to express the kanamycin gene for 48 hr before selection with the antibiotic. Integration of the *lac* operon fusions into kanamycin-resistant transformants was confirmed by DNA blotting and hybridization of isolated chromosomal DNA (24) digested with *Eco*RI and *Eco*RI plus *Sal* I. The probe used was ^{32}P-labeled RNA synthesized *in vitro* by transcription of the *Sal* I/partial *Eco*RI *lac* fusion fragment cloned into pGEM1 (Promega Biotec).

Expression in *E. coli*. β-Galactosidase were measured in *E. coli* cells after disruption of the membrane of intact cells with chloroform and SDS (25); galactoside transacetylase was measured by using sonicated extracts of *E. coli* as described (25). LacZ mRNA synthesis rates in *E. coli* were determined by pulse-labeling cells with [^{3}H]uridine, after which the extracted RNA was hybridized to *lacZ* DNA on filters. At 40 Klett units cells were labeled with 100 μCi of [^{3}H]uridine per ml (1 Ci = 37 GBq) for 1 min. RNA extraction and hybridization to DNA on filters was done by standard methods (26). The DNA attached to nitrocellulose filters was either single-stranded M13mp8 (22) used as a control or M13mp7 (M13NO7004) containing a 1.8-kb *Hin*dII fragment of *lacZ* orientated to hybridize to LacZ mRNA. Several concentrations of labeled RNA were used for hybridization to ensure that excess DNA was present on the filters. After being washed and treated with RNase A, radioactivity on the dried filters was counted in liquid scintillation fluid. The results obtained were corrected for background hybridization to M13mp8 filters.

Expression in *Synechocystis*. To extract *Synechocystis* 6803 proteins, cells were collected by centrifugation and resuspended in 0.1 culture vol of 50 mM Tris·Cl, pH 8.0/10 mM EDTA. Glass beads of 0.1-mm diameter were added and mixed rapidly three times for 3 min each with cooling between each cycle. The extract was filtered through Miracloth and centrifuged at 20,000 × *g* for 30 min to obtain a supernatant fraction. Samples were analyzed by PAGE using a 6% gel containing SDS. Other details are described in the legend for Fig. 2. To assay for β-galactosidase in *Synechocystis* 6803, cells were first broken with glass beads as described above. Aliquots of the supernatant fraction were assayed for β-galactosidase (25), and the protein concentration was measured with the Bio-Rad protein assay.

lac operon mRNA present in *Synechocystis* 6803 was measured by hybridization of unlabeled RNA to ^{32}P-labeled *lac* DNA. RNA was extracted from *Synechocystis* 6803 cells by rapidly mixing the cells in a detergent solution with glass beads and chloroform/phenol, after which the RNA was centrifuged through a CsCl solution (27). The RNA was added to Schleicher & Schuell BA85 nitrocellulose that had been previously washed with 20× SSC (1× SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0) and dried. Filters were baked at 80°C *in vacuo* and prehybridized at 42°C for 4 hr in 20 mM Tris·Cl, pH 8.0/600 mM NaCl/20 mM EDTA/0.1% SDS/0.02% polyvinylpyrollidone/50% (vol/vol) deionized formamide/0.02% Ficoll containing yeast tRNA at 50 μg/ml and denatured salmon sperm DNA at 50 μg/ml. The probe was an isolated *Eco*RI/*Sal* I fragment of pMC1403 (23) encoding *lacZ*, *lacY*, and *lacA*, that was "nick-translated" in the presence of [^{32}P]dCTP. RNA filters were hybridized with the probe in the above solution at 42°C for 18 hr, washed four times with 2× SSC/0.1% SDS, and two times with 0.1× SSC/0.1% SDS at 25°C. Radioactivity of the dried filters was counted in liquid scintillation fluid.

RESULTS

Synthesis of Maize ATP Synthase Subunits and Fusion Proteins. The β and ϵ genes encoded by plasmid pPBI33 are

under the transcriptional control of the P_L promoter of bacteriophage λ. Plasmid pPBI33 was transformed into an *E. coli* strain (K-12ΔH1) encoding a temperature-sensitive *c*I repressor, and cultures in logarithmic growth were either maintained at 30°C or induced at 41°C. Fig. 2 shows the results of immunoblots of SDS/polyacrylamide gels on which maize leaf or *E. coli* extracts have been electrophoresed. In lane 1 leaf β subunits of ATP synthase have been identified by using antiserum raised against purified chloroplast β subunits. In lane 2 trace amounts of chloroplast β subunits can be detected in uninduced *E. coli* cells containing pPBI33, but when induced at 41°C (lane 3), the synthesis of chloroplast β subunits is substantially increased. The maize β subunits synthesized in *E. coli* are the same M_r (56,000) as that present in chloroplasts, indicating that bacterial ribosomes are initiating translation of chloroplast *atpB* with fidelity, as has been seen for several other chloroplast genes in *E. coli* (28, 30, 31). Smaller cross-reacting polypeptides of M_r 51,000 are found in *E. coli* extracts (lanes 2 and 3). These are the bacterial β subunits of ATP synthase, which can be identified with antiserum raised against the chloroplast subunits (28).

Synthesis of chloroplast ε subunits of ATP synthase can also be seen in *E. coli* after induction of pPBI33 (Fig. 2). Cross-reacting material cannot be detected in uninduced *E. coli* extracts with immunoblots incubated by using antiserum raised against chloroplast ε subunits (lane 4). However, when expression of the ε gene is induced by a temperature shift of cells containing pPBI33, then chloroplast ε subunits are synthesized (lane 5). The maize ε subunits synthesized in *E. coli* are the same M_r (16,000) as those synthesized in leaves (lane 6), suggesting that the ε gene is also translated correctly in bacteria.

To develop a convenient assay for examination of translational coupling between *atpB* and *atpE*, an *atpE*::*lacZ* gene fusion was constructed to direct the synthesis of a hybrid

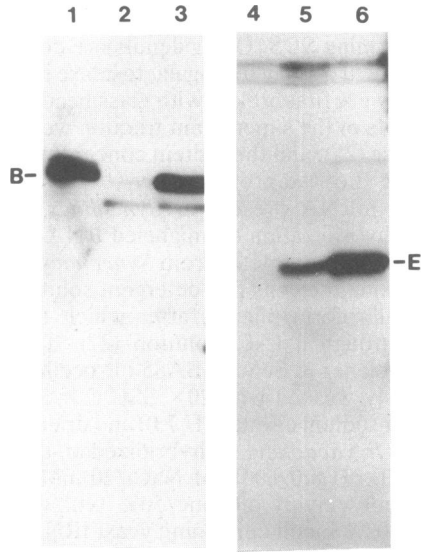

FIG. 2. Immunodetection of maize β (B) and ε (E) subunits, either after induction of *E. coli* strain K-12ΔH1 (21, 28) containing pPBI33 (lanes 2–5) or in leaves (lanes 1 and 6). Maize proteins were extracted by grinding young leaves in liquid N_2, homogenizing 1 g of the resulting leaf powder with 2 ml of 2% SDS, centrifuging at 20,000 × *g* for 30 min, and retaining the supernatant. Detergent-solubilized proteins were analyzed by PAGE on 10% (lanes 1–3) or 15% (lanes 4–6) gels containing SDS, transferred to nitrocellulose, incubated with the appropriate antiserum and ^{125}I-labeled protein A (29). The *E. coli* samples containing pPBI33 were grown in either noninducing conditions (30°C, lanes 2 and 4) or induced (42°C, lanes 3 and 5). The β subunit has a M_r of 56,000, and the ε subunit has a M_r of 16,000. Autoradiograms are shown.

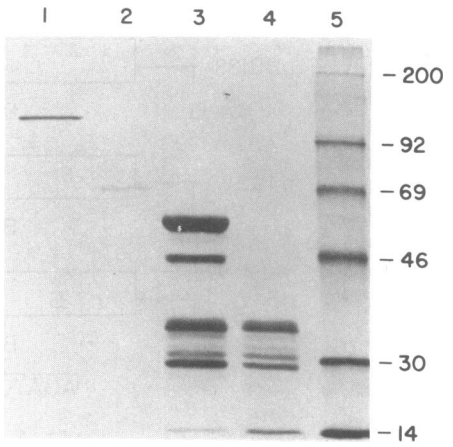

FIG. 3. Synthesis of hybrid β-galactosidase and ATP synthase β subunits in *E. coli* minicells labeled with [^{35}S]methionine. Minicells containing p492 (lanes 1 and 3) or pβ17 (lanes 2 and 4) were labeled, disrupted (28–30), and incubated with antiserum raised against β-galactosidase (lanes 1 and 2) or the ATP synthase β subunit (lanes 3 and 4). After treatment with protein A–Sepharose (30), the samples were analyzed by PAGE with a 10% gel containing SDS, and then autoradiographed. Lane 5 contains $M_r \times 10^{-3}$ markers.

β-galactosidase polypeptide. In plasmid p492 the first 42 codons of *atpE* have been fused in the correct translational reading frame with *lacZ*. Transformed cells containing the gene fusion plasmid could be readily identified as blue *lac*$^+$ colonies on indicator plates. The resulting hybrid *lac* operon is transcribed from the chloroplast *atpB* promoter that functions in *E. coli* (32) and contains the gene order *atpB/atpE*::*lacZ/lacY/lacA* (Fig. 1). The synthesis of a hybrid β-galactosidase was confirmed by transforming p492 into a minicell-producing *E. coli* strain and labeling isolated minicells with [^{35}S]methionine. Labeled polypeptides of the anticipated size were immunoprecipitated by using antiserum against β-galactosidase (Fig. 3, lane 1). The β subunits of ATP synthase were also immunoprecipitated from the labeled minicells with a specific antiserum (lane 3), showing that the first two proteins in the hybrid *lac* operon are expressed correctly. Active β-galactosidase could also be demonstrated by enzyme assay (Table 1).

β-Galactosidase fusion proteins were also synthesized in cyanobacteria when the hybrid *lac* operon was transferred from p492 to *Synechocystis* 6803. Assay of soluble proteins obtained by disruption of *Synechocystis* 6803 cells revealed β-galactosidase activity (Table 2). In addition, β-galactosidase polypeptides could be identified by immunoblotting of an SDS/polyacrylamide gel and incubation with antiserum against the enzyme (Fig. 4, lane 3).

Effect of an *atpB* Frameshift Mutation on *atpE* Expression in *E. coli*. The introduction of a frameshift mutation in pβ17 at codon 134 of *atpB* causes ribosomes to terminate translation prematurely at a UGA triplet spanning codons 149–150 of the known sequence (8). The effect of this can be seen in Fig. 3, in which synthesis of full-length β subunits encoded by p492

Table 1. Hybrid *lac* operon expression from plasmids p492 and pβ17 in *E. coli*

Plasmid	β-Galactosidase	LacZ mRNA,* cpm	Transacetylase
p492	2280 (100)	777 (100)	10.4 (100)
pβ17	1.4 (0.06)	395 (51)	8.4 (81)

Numbers within parentheses indicate percentage of values obtained for p492 in strain MC1061. Table values are the means of three different RNA concentrations hybridized at saturating DNA levels, each done in triplicate. All enzyme assays were done in triplicate, and the mean values are presented.
*Cpm hybridizing to filter per added 10^6 cpm.

Table 2. Hybrid *lac* operon expression in *Synechocystis* 6803 after integration of plasmids p492KW and pβ17KW

Plasmid	β-Galactosidase,* nmol	Lac mRNA,† cpm
p492KW	2130 (100)	808 (100)
pβ17KW	0.05 (0.04)	679 (84)

Numbers within parentheses indicate percentage of values obtained for p492KW. All enzyme assays were done in triplicate, and the mean values are presented. mRNA values presented were obtained from quadruplicate samples and are corrected for nonspecific hybridization to RNA extracted from *Synechocystis* 6803 transformed with the vector pKW1313.

*β-Galactosidase activity expressed as nmol of *o*-nitrophenylgalactosidase hydrolyzed per min per mg of protein.

†Cpm hybridizing per 100 μg of RNA on filter.

(lane 3) was not seen for pβ17 (lane 4). Instead, only the synthesis of several small, uncharacterized polypeptides was observed for pβ17, which was also observed for p492 (lanes 3 and 4). The consequence of premature termination of β translation was an almost complete prevention of expression of the distal *atpE*::*lacZ* gene fusion. This result has been demonstrated in two ways. Direct assay for β-galactosidase in permeabilized cells showed that when the frameshift plasmid pβ17 was present, the level of enzyme activity was reduced >99% compared with p492 (Table 1). The absence of β-galactosidase was confirmed by radioactive labeling of the products of p492 and pβ17 in minicells. The *atpE*::*lacZ* fusion protein is synthesized when p492 is present (Fig. 3, lane 1) but is not detectable when pβ17 products are examined (Fig. 3, lane 2). The polypeptides of M_r 69,000 present in lane 2 that cross-react with anti-β-galactosidase are probably LacZ fragments resulting from re-initiation at one of the known internal initiation sites (33).

To exclude the possibility that reduction of *atpE*::*lacZ* expression by a frameshift in *atpB* was due to the introduction of transcriptional polarity, LacZ mRNA synthesis rates and *lacA* expression were examined. Pulse-labeled RNA from cells containing p492 or pβ17 was hybridized to *lacZ* DNA on filters, and the amount of LacZ mRNA was measured. Table 1 shows that cells containing pβ17 had a 49% reduction of pulse-labeled LacZ mRNA when compared with LacZ mRNA in cells containing p492. The *lacA* gene is the final gene of the hybrid *lac* operon, and assay of its product, galactoside transacetylase, should provide another estimate on the degree of polarity introduced by the frameshift in *atpB*. Table 1 shows a 19% reduction in *lacA* expression due to the *atpB* frameshift. Data from the *lacZ* hybridization and *lacA* expression experiments thus show that some transcriptional polarity was introduced by premature termination of translation of *atpB*. However, there is only ≈2-fold reduction in expression from transcriptional effects, and this change does not fully account for the 1000- to 2000-fold reduction of β-galactosidase synthesized in cells containing pβ17. This difference appears to be primarily due to a reduction in translation of *atpE* when ribosomes terminate prematurely in *atpB*. Additional evidence that *atpE*::*lacZ* encoded by pβ17 is transcribed is the detection of the LacZ internal initiation fragment seen in Fig. 3 (lane 2).

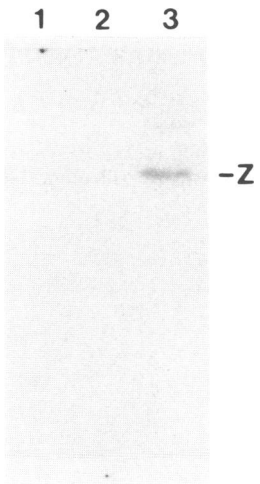

FIG. 4. Immunodetection of the *atpE*::*lacZ* encoded β-galactosidase fusion protein in *Synechocystis* 6803 in cells transformed with either pKW1313 (lane 1), pβ17KW (lane 2), or p492KW (lane 3). The hybrid β-galactosidase synthesized in cells transformed by p492KW is marked by Z.

Translational Coupling of Maize *atpB* and *atpE* in Cyanobacteria. Although translational coupling of the chloroplast β and ε genes could be seen in *E. coli*, examining this mechanism in a homologous translational system would be preferable. Unfortunately, reliable transformation of large fragments of DNA into higher plant chloroplasts has not been demonstrated, and a suitable *in vitro* translation system has not been developed from chloroplasts. For these reasons, we used the transformable photosynthetic cyanobacterium *Synechocystis* 6803, which is closely related in structure and function to chloroplasts. Synthesis of the *atpE*::*lacZ* gene fusion product could be detected by assays for β-galactosidase in soluble extracts from cells transformed with p492KW (Table 2). The β-galactosidase polypeptides were also seen when these extracts were used for electrophoresis and immunoblotting (Fig. 4, lane 3). In contrast, neither β-galactosidase activity nor the polypeptides could be detected in cells transformed with the *atpB* frameshift plasmid pβ17KW (Table 2; Fig. 4. lane 2).

Technical difficulties prevented us from examining polarity effects in *Synechocystis* 6803 by LacZ mRNA synthesis rates or galactoside transacetylase assays. Instead, *lac* operon expression in transformed *Synechocystis* 6803 was measured by hybridization of a labeled *lac* DNA probe to cyanobacterial RNA on filters. Table 2 shows a 16% reduction in the amount of Lac mRNA in cells transformed with pβ17KW compared with cells transformed with p492KW. This reduction was similar for two independent transformants. The data obtained is consistent with a very high degree of translational coupling between the *atpB* and *atpE* genes when expressed in *Synechocystis* 6803. The magnitude of this effect is virtually identical when coupling in *E. coli* and *Synechocystis* 6803 are compared (Tables 1 and 2).

DISCUSSION

We show that translation of chloroplast ATP synthase ε subunits is coupled to translation of the β subunits. Premature termination of translation of β subunits almost completely blocks translation of the ε subunits, indicating that coupling of the two genes is stringent. The tight translational coupling of the β and ε genes could be a mechanism to ensure production of the two subunits in a highly coordinated and regulated manner during biosynthesis of the ATP synthase complex.

Examination of the published sequences of β and ε genes from higher plant chloroplast DNA reveals two common arrangements in which termination and initiation codons either overlap (7–11) or are spaced apart by 22 base pairs (bp) (34, 35). Our data show that the overlapping sequences for the maize β and ε genes result in translational coupling, and this coupling probably occurs for the other known examples of this arrangement of genes. In species where the 22-bp-spacer region is present, there is remarkable conservation in the nontranslated nucleotides. Between pea (34) and sweet potato (35) there is 86% identity in this region. This conservation in the nontranslated region might indicate that it has an important regulatory function—perhaps a sequence to ensure a structure that governs translational coupling—even though termination and initiation codons are spaced apart. The nonflowering plant *Marchantia polymorpha* also has a close positioning of the termination and initiation codons for the β and ε genes, and although they are 5 bp apart, the Shine–

Dalgarno sequence of the ε subunit gene is located within the structural gene for the β subunit (36). There are, however, examples where the β and ε subunit genes are not closely linked. The β and ε genes of *Chlamydomonas reinhardtii* are 92 kilobase pairs apart (37), which suggests that a different regulatory mechanism for coordination may be used in this species.

The mechanism of translational coupling is not clearly understood. Two possibilities have been considered for a tightly coupled system (12, 38). (*i*) Translational initiation of the distal gene is defective because of a weak ribosome-binding site, and only a translating ribosome approaching from the upstream gene can initiate translation. (*ii*) Translation could initiate successfully by independent ribosome entry at the distal gene but is prevented from doing so by RNA structures that require perturbation by an upstream translating ribosome to unmask the downstream ribosome initiation site. For several ribosomal protein operons in *E. coli*, the synthesis rates of each of the translationally coupled proteins are equimolar, and this could be more easily explained by the first mechanism "sequential translation" (see ref. 17). In the other system—e.g., the L10 operon in *E. coli*—the protein from the second gene is produced at higher levels than the protein from the first gene, indicating that the second possibility is the likely mechanism (16). For the chloroplast, *atpBE* gene system, we cannot distinguish between the two mechanisms. The ATP synthase holoenzyme is known to probably contain three β and one ε subunits (39). If translational coupling of the maize β and ε genes resulted in equal numbers of subunits being synthesized, then proteolytic degradation of excess ε subunits would presumably occur. Alternatively, the efficiency of translational initiation, or re-initiation, of the ε gene may be lower than that of the β gene—that is, not all ribosomes that terminate translation of β would be involved in initiation of ε translation. Should this be the case, one would then have to ask whether and how the 3-fold reduction of translation is ensured at the junction of these two genes.

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