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Plastome mutation affecting the chloroplast ATP synthase involves a post-transcriptional defect

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Summary. In a plastid genome (plastome) mutation of *Oenothera hookeri,* at least two of the plastome-coded polypeptides (the β and ϵ subunits) of the chloroplast ATP synthase are directly affected. As in other plastid chromosomes, the genes for the β and ϵ subunits are located next to each other on the *Oenothera* ptDNA molecule and are cotranscribed. Immunoanalysis and peptide mapping of in vivo products suggests that a fusion of the two genes may have occurred in the plastome mutant. In contrast to the in vivo data, in vitro translation of the RNA using a heterologous system results in polypeptides which cannot be distinguished from those of wild-type. In addition, neither the mRNA sizes nor plastid DNA restriction fragment patterns differ from wild-type. To reconcile the paradox of these results, it is suggested that either a defect in a translational signal or some other post-transcriptional event is responsible for the mutant phenotype.

Key words: Chloroplast – Organelle genetics – photosynthesis – Plastome mutant – ATP synthase

Introduction

The chloroplast ATP synthase, CF_1-CF_0, is composed of at least eight different polypeptide species. Five of the subunits (α, β, ϵ, CF_0-I, and the proteolipid CF_0-III) are synthesized on plastid ribosomes (Mendiola-Morgenthaler et al. 1976; Ellis 1977; Doherty and Gray 1980; Nelson et al. 1980), and their genes have been physically mapped on the plastid DNA (ptDNA) molecule (Westhoff et al. 1981; Krebbers et al. 1982; Howe et al. 1982; Alt et al. 1983; Herrmann et al. 1983). The β and ϵ subunit genes lie next to each other and are cotranscribed (Zurawski et al. 1982; Whitfeld et al. 1983). Since the ratio of β to ϵ is 3:1 in the holoenzyme (Maroney et al. 1983; Merchant et al. 1983) some type of post-transcriptional control must exist to accomplish the correct stoichiometry. In spinach, maize and wheat, the 5′ end of the ϵ gene overlaps the 3′ end of the β gene by one to four bases (Zurawski et al. 1982; Krebbers et al. 1982; Howe et al. 1982) and a prokaryotic ribosomal binding site for the ϵ message is found within the β gene. In pea, a 20 base pair sequence separates the two genes and also contains a ribosomal binding sequence for the translation of ϵ (Whitfeld et al. 1983).

In this paper we report our investigations of the plastome I mutant *iota* (I-iota) of *Oenothera hookeri* which was initially recognized as having a mottled phenotype, suggesting variable expressivity of chlorophyll and/or certain genes. Gel electrophoresis of plastid proteins showed none or only reduced amounts of the plastome coded α and β subunits of the ATP synthase (Herrmann et al. 1980; Sears and Herrmann 1983). We undertook this analysis to determine the effect of this lesion on the other ATP synthase subunits, particularly the β and ϵ genes which are cotranscribed. Our analysis indicated that the mutational defect was different from any plastome mutation reported previously. In this paper we describe an initial molecular characterization of this mutant. The ultrastructural analysis will be the subject of another report.

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Materials and methods

Plant material. Mutant I-iota of *Oenothera hookeri* was initially isolated by Dr. H. Kutzelnigg under the direction of Prof. Wilfried Stubbe who kindly made the material available to us. Mutant and wild-type plants were grown as shoot cultures on NT medium (Nagata and Takebe 1971) supplemented with 4 μM benzylamino purine and 16 μM napthyl acetic acid according to the procedure established by Mehra-Palta and described by Stubbe and Herrmann (1982). To radioactively label proteins in vivo, shoot cultures were grown for 2 weeks on this medium containing ^{35}S in the form of $MgSO_4$ as the sole sulfur source. Wild-type *Oenothera hookeri* plants were also grown in the greenhouse.

Protein analysis. Plastids were isolated as previously described (Gordon et al. 1980). To separate thylakoid membranes from soluble proteins, the plastids were osmotically shocked by centrifuging them out of the wash medium which contained 0.3 M sorbitol and suspending them in 50 mM Tris-HCl, pH 8.0. The membranes were then washed three times with 10 mM NaCl, 50 mM Tris-HCl, pH 8.0 to remove the soluble proteins. The soluble proteins were precipitated by the addition of TCA to 15%; they were then washed twice with acetone, and suspended in sample buffer (10% glycerol, 5% SDS, 5% mercapto-ethanol, 62 mM Tris-HCl, pH 6.8) for polyacrylamide gel electrophoresis. The membrane proteins were also dissolved and stored in sample buffer.

For comparison and use as size standards on our polyacylamide gels, purified CF_1 complex from spinach and *Oenothera* was kindly provided by Dr. Peter Westfhoff (Westhoff et al. 1981). The Laemmli buffer system was used, and the acrylamide/bisacrylamide ratio was 60:0.8%. 5 M urea was added to the resolving gel, and not to the stacking gel. Other details have been described by Westhoff et al. (1981).

Immunological techniques for the protein analysis are detailed by Westhoff and Zetsche (1981) and Towbin et al. (1979). The polyclonal antibodies used in these experiments were monospecific and were kindly provided by Drs. N.-H. Chua and N. Nelson.

For one-dimensional peptide mapping, protein bands representing the α and β polypeptides of CF_1 and the large subunit of ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) were cut out of lightly stained polyacrylamide gels, soaked in buffer (0.1% SDS, 1 mM EDTA, 125 mM Tris-HCl, pH 6.8), and then dried on aluminum foil at 40 °C. The pieces were then inserted into slots of a second gel and V-8 protease was layered over them, with subsequent digestion and electrophoresis as described by Cleveland et al. (1977). Two techniques were used to amplify the peptide signal: 1) The peptides were transferred electrophoretically from a Cleveland gel to nitrocellulose filters, following the technique of Towbin et al. (1979). The filters were incubated with specific antisera, washed and then incubated with ^{125}I-Protein A. The peptides that had been recognized as antigens by the polyclonal antisera could be visualized by autoradiography. 2) Proteins were isolated from plants which were grown on medium containing ^{35}S. Following protease digestion and electrophoresis, the labelled peptide bands were visualized by fluorography.

RNA analysis. The basic techniques for preparation of ptRNA and poly A^- RNA, in vitro protein synthesis using rabbit reticulocyte lysate, and hybrid-selection of specific RNA transcripts using cloned DNA bound to a matrix have been described elsewhere (Westhoff et al. 1981; Bünemann et al. 1982; Bünemann 1982). To insure that the quality of *Oenothera* RNA would be adequate for in vitro translation, the RNA was dissolved in Tris/borate buffer (1 mM EDTA, 0.08 M Tris, 0.12 M boric acid), and precipitated by addition of 4 M LiCl to an end concentration of 2 M (Baltimore 1966). This precipitation step was performed 2–3 times before the mRNA was used for gel electrophoresis or in vitro translation.

RNAs were elctrophoretically separated on agarose gels using glyoxal (McMaster and Carmichael 1977). For Northern analysis, the RNAs were transferred to nitrocellulose (Thomas 1980) and were hybridized overnight with nick-translated DNA fragments (Rigby et al. 1977) from the coding region of the β or ϵ genes. These gene specific probes were obtained by SacI (β) or XbaI (ϵ) digestion of cloned EcoRI fragments of spinach ptDNA (c.f. Zurawski et al. 1982) which had been generously provided by Dr. Warwick Bottomley. The plasmid DNA was prepared essentially as described by Maniatis et al. (1982). SacI cuts the 1960 bp β-specific EcoR1 fragment of pSocE40 into 725 and 1,135 bp segments, while XbaI dissects the 1,670 bp ϵ-specific EcoR1 fragment of pSocE55 into 420 bp and 1,250 bp pieces. In each case, the smaller fragment was used for gene-specific hybridizations. These DNA fragments were electro-eluted from a 1.5% agarose gel, ethanol-precipitated, and dissolved in 1 mM EDTA, 10 mM Tris-HCl, pH 8.0.

DNA analysis. PtDNA was isolated as described previously (Gordon et al. 1980). Reaction conditions for the restriction enzymes were as specified by the manufacturer (Boehringer and Biolabs Inc.). DNA fragments were electrophoretically separated on agarose slab gels with a running buffer composed of 0.2 M sodium acetate, 10 mM EDTA, and 0.4 M Tris-HCl (pH 7.5) and 0.5 mg/ml ethidium bromide.

Results

Comparison of spinach and Oenothera CF_1 Polypeptides. The two ATP synthase proteins of highest molecular weight have practically the same mobility on denaturing polyacrylamide gels in *Oenothera* (data not shown). The addition of 5 M urea allowed the electrophoretic separation of these polypeptides (Fig. 1). In both *Oenothera* and spinach, the CF_1 complex is composed of five subunits. The identities of the CF_1 polypeptides were determined by immunoblotting using antisera prepared against spinach CF_1 subunits (lanes 3–12). The α subunit of *Oenothera* (which cross-reacts less strongly with the spinach α antisera) has a slightly faster mobility than does the β subunit in this gel system, in contrast to the ATP synthase polypeptides of spinach. While the γ subunit is conserved in size, the ϵ subunit of *Oenothera* has a faster mobility than the spinach subunit, and the *Oenothera* δ subunit does not migrate as rapidly as the spinach δ subunit (lanes 9 and 10).

Immunoanalysis of α, β, and ϵ polypeptides from mutant I-iota. The Western technique of immunoblotting was used to assay for the presence of the plastome-coded proteins of CF_1 (Fig. 2). Since the lanes of the gel in Panel A of Fig. 2 were equally loaded with respect to the other major thylakoid proteins, the membranes of the mutant I-iota contain reduced amounts of both

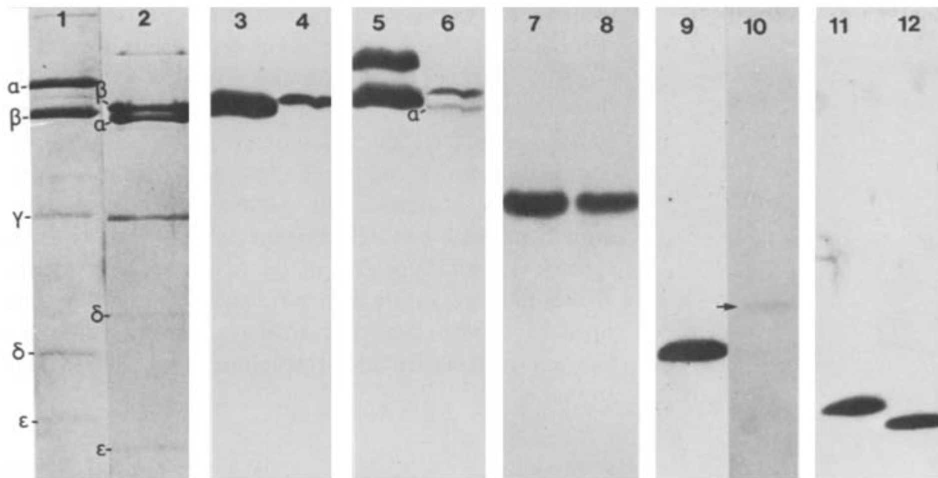

Fig. 1. Comparison of the CF_1 polypeptides of spinach and *Oenothera*. Each pair of lanes shows a gel or autoradiograph of spinach proteins on the left and *Oenothera* proteins on the right. The first pair of lanes contains isolated CF_1 complex from spinach (lane 1) and *Oenothera hookeri* (lane 2) electrophoretically separated on a 12–17% polyacrylamide gel containing 5 M urea and stained with Coomassie Blue. The ATP synthase subunits of spinach CF_1 are identified on the left. A higher molecular weight aggregate is also visible in these lanes. In the other lanes, chloroplast membrane polypeptides from spinach (lanes 3, 5, 7, 9, 11) and *Oenothera* (lanes 4, 6, 8, 10, 12) were electrophoretically separated as above, transferred electrophoretically to a nitrocellulose filter, and then incubated with antisera against the subunits of spinach ATP synthase. Following ^{125}I-Protein A incubation, bands were visualized by autoradiography. Lane 3 and 4 show a filter which was incubated with β-specific antisera. The same filter is shown in lanes 5 and 6 following subsequent incubation with α-specific antisera: the relatively faint α band of *Oenothera* is marked. Single incubations were performed with antisera specific for γ (lanes 7 and 8), δ (lanes 9 and 10), or ϵ (lanes 11 and 12). The faint δ-band from *Oenothera* is indicated with an arrow

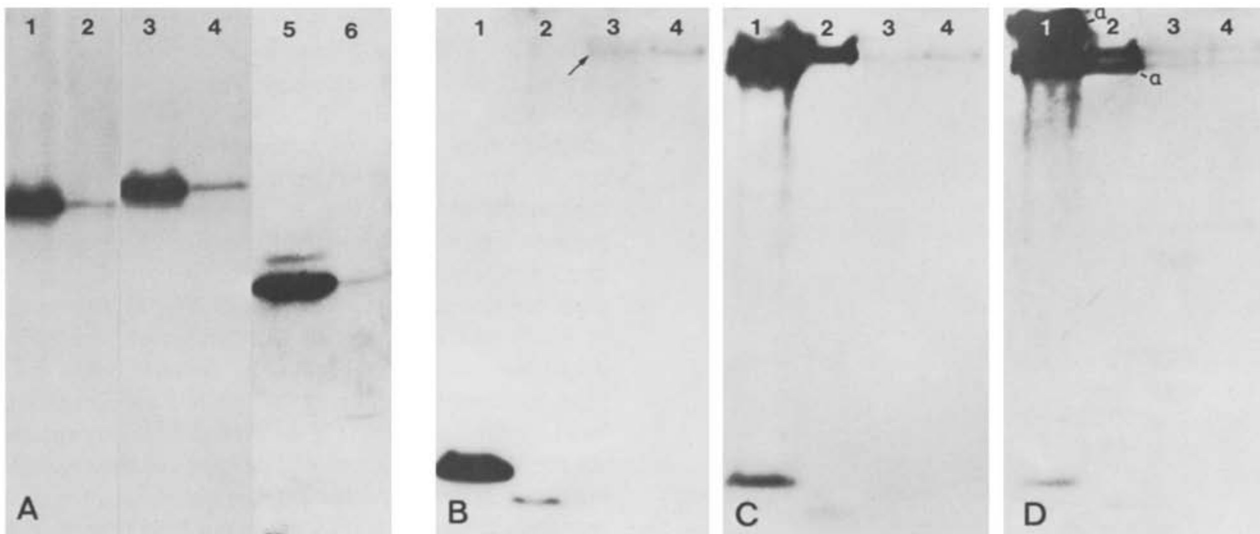

Fig. 2. Identification of the polypeptides in Mutant I-iota. Thylakoid membrane polypeptides were electrophoretically separated on a 5 M urea, 10–15% polyacrylamide gel and transferred to nitrocellulose. Panel A. Identical filters from the same gel were incubated with α-specific antisera (lanes 1, 2) or β-specific antisera (lanes 3, 4) prior to handling with radioactive protein A and autoradiography. Another gel provided the Western blot which was incubated with γ-specific antisera (lanes 5 and 6). Lanes 1, 3, and 5 contain polypeptides from wild-type *Oenothera hookeri* while lanes 2, 4, and 6 carry mutant I-iota proteins. The other panels show autoradiographs from a single filter which was sequentially handled with antisera prepared against the ϵ subunit (panel B), the β-subunit (panel C) and the α-subunit (panel D). In each panel, chloroplast membrane proteins were analyzed from spinach (lane 1), wild-type *Oenothera hookeri* (lane 2), and *Oenothera* mutant I-iota (lane 3); soluble proteins from the mutant were also checked (lane 4). In panel B, the arrow indicates the faint band in the mutant material following the incubation with the ϵ-specific antisera. In panel D, new bands resulting from the binding of α-specific antibodies to spinach and wild-type *Oenothera* proteins are indicated. Since the β and α antisera give stronger signals, the autoradiograph in this experiment was exposed for progressively less time, which is why the ϵ signal seems to fade

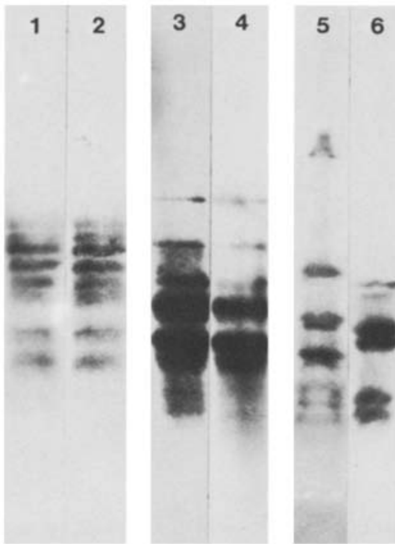

Fig. 3. One dimensional peptide mapping of three proteins from mutant and wild-type *Oenothera* plants grown on ^{35}S. Chloroplast membranes were harvested from about 10 g of plant material which had been growing for 2 weeks on agar medium containing ^{35}S-$MgSO_4$. The proteins were electrophoretically separated on a small preparative gel. Bands representing the large subunit of Rubisco (lanes 1, 2) as a control and the α (lanes 3, 4) and β (lanes 5, 6) subunits of CF_1 were cut out and used for the proteolytic analysis. Peptides from wild-type *Oenothera hookeri* are in lanes 1, 3, and 5; peptides from the mutant I-iota are in lanes 2, 4, and 6

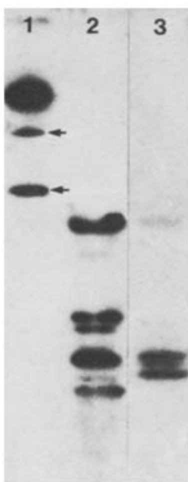

Fig. 4. V8-protease digestion of β-subunit followed by immunoblotting with β-antisera. The proteins at the β-position were cut from preparative gels of thylakoid membranes from both mutant and wild-type plant material. Following V8-protease digestion and separation of protein fragments on a second gel, the peptides were transferred electrophoretically to a nitrocellulose filter. The filter was incubated with β-specific antisera, followed by radioactive Protein A and autoradiography. Undigested β protein from the wild-type (lane 1) was loaded on the gel next to the proteins from wild-type (lane 2) and mutant (lane 3) which received V8-protease treatment. Minor bands representing partial digestion products in lane 1 are marked with arrows and indicate that a slight contamination of this lane with the protease occurred

the α and β polypeptides. When antisera against the ϵ subunit were used, no protein was ever recognized at the ϵ position (15.5 kd) in the mutant plant material. However, a faint band could be detected with this serum at the position of the β-subunit in the mutant material (Panel B, lanes 3 and 4). Exposure of the same blot to β-antisera increased the relative intensity of the aforementioned band (compared to the ϵ-bands from spinach or wild-type *Oenothera* which are less intense at this reduced autoradiographic exposure). Subsequent incubation with α-specific antisera confirmed that the band in question had an electrophoretic mobility similar to that of β.

Comparison of wild-type and mutant polypeptides by one-dimensional peptide mapping. For a more precise comparison of the "β"-sized polypeptides from mutant and wild-type plants, one-dimensional peptide mapping was performed, using protein bands cut out of polyacrylamide gels. To visualize the ATP synthase subunits which were low in abundance in the mutant, radioactive label was employed in two different ways. Using plants grown on agar medium containing ^{35}S, we compared the ATP synthase α and β subunits, and the large subunit of Rubisco from wild-type and mutant plants (Fig. 3). In the comparisons of wild-type and mutant peptide fragment patterns, the Rubisco subunits are identical, as are the ATP synthase α-subunits, but the β-subunits differ. To verify the β-subunit differences, we used a second amplification technique. Peptides were electrophoretically transferred from the Cleveland gel to a nitroccelluslose filter for immunoblotting analysis. β-specific antisera revealed significant differences between the mutant and wild-type "β" proteins. The undigested proteins are the same apparent size (Fig. 2). Upon digestion, the β-polypeptide from the wild-type control gives rise to eight peptide fragments (Fig. 3), five or six of which give strong signals when immunoblotted with polyclonal β-antisera (Fig. 4). In contrast, only four peptides from the mutant's "β" protein are recognized at all by the β-antisera (Fig. 4). Not all of these peptides are the same size as those of wild-type and their relative intensities differ. ϵ-specific antisera did not react against any peptide fragments, but this could have been due to destruction of an antigenic site by the protease.

Comparison of plastid DNA restriction fragment patterns. PtDNAs isolated from mutant and wild-type plants were digested with BamH1 or with both SalI and PstI and were subjected to electrophoresis on a 1.0% agarose gel. No differences in fragment sizes could be detected between the wild-type and mutant (Fig. 5), although quite a few differences are apparent when ptDNA from the more distant relative *Oenothera argillicola* is compared.

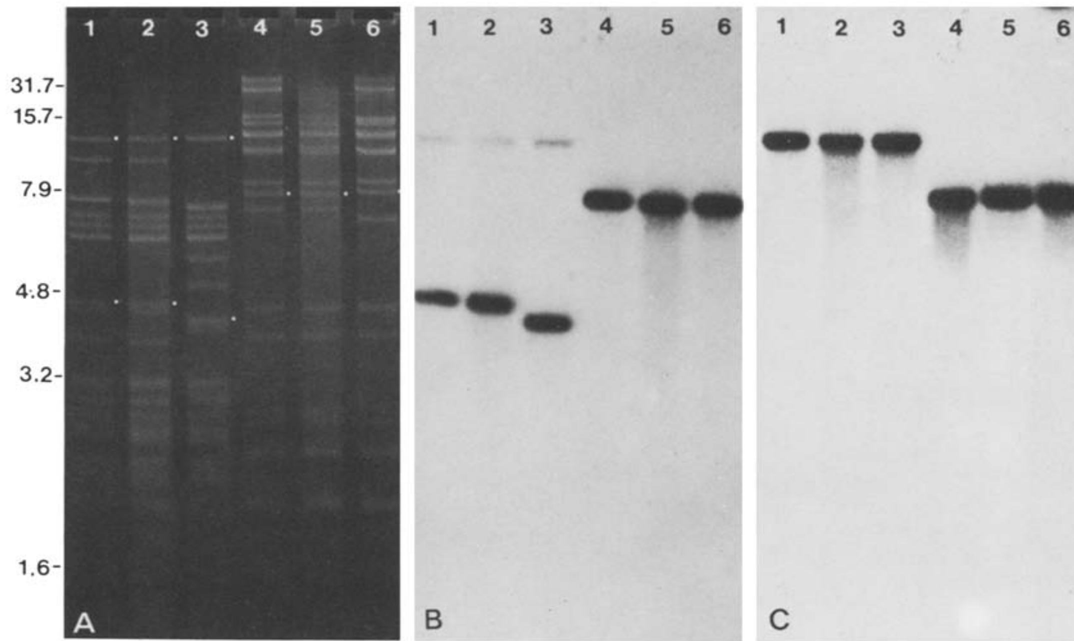

Fig. 5. Endonuclease digests and Southern analysis of mutant and wild-type ptDNA. Panel A shows restriction digests of ptDNA from wild-type *Oenothera hookeri* (lanes 1 and 4) and mutant I-iota (lanes 2 and 5), and *O. argillicola* (lanes 3 and 6). The DNA in lanes 1–3 was digested with BamHI; in lanes 4–6, it was digested with both SalI and PstI. The small white points mark the restriction fragments which contain coding sequence for the β and ϵ genes, as shown in panels B and C. The DNA was transferred to a nitrocellulose filter and was hybridized with an internal probe specific for either the β (Panel B) or ϵ (Panel C) gene. Molecular weights are indicated in kbp on the left of the figure

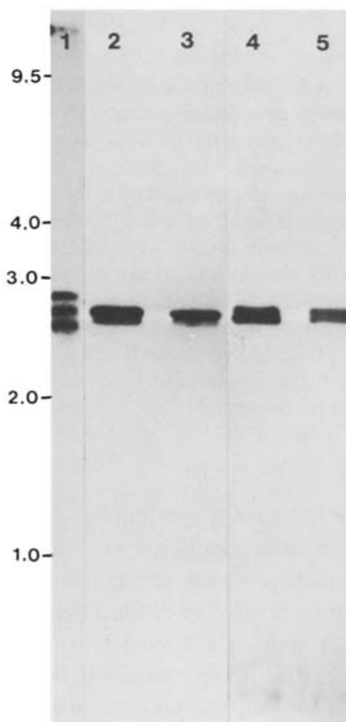

Fig. 6. Northern analysis of mutant and wild-type RNAs. LiCl-precipitated RNAs were separated on 1.2% agarose gels using glyoxal. RNAs from spinach (lane 1), wild-type *Oenothera hookeri* (lanes 2 and 4) and mutant I-iota (lanes 3 and 5) were transferred to nitrocellulose filters which were probed using nick-translated DNA fragments from the coding region of the β gene (lanes 1–3) or the ϵ gene (lanes 4 and 5)

Most importantly, the fragments which contain the genes for β and ϵ are unchanged in the mutant, as determined by Southern blot analysis using probes specific for β (Panel B) or ϵ (Panel C).

Comparison of β- and ϵ-specific RNAs and their in vitro translation products. The protein analysis revealed alterations in the ATP synthase of mutant I-iota, yet the DNA comparisons did not show any major differences. Therefore, mutant and wild-type RNAs and their in vitro translation products were examined to compare sizes of transcripts and to determine if the messages were properly decoded. RNA was prepared from chloroplasts which had been isolated from spinach and wild-type *Oenothera hookeri.* For mutant I-iota, poly A^- RNA was used as a source of ptRNA for the in vitro translations in order to obtain a higher yield from the mutant tissue. According to Westhoff et al. (1981), when the rRNAs are removed, poly A^- RNA is practically identical to ptRNA.

To determine the transcript sizes, RNAs were denatured with glyoxal, electrophoretically separated on agarose gels, and transferred to nitrocellulose. Hybridization was conducted using nick-translated subfragments from spinach which contained only coding sequences for the β or ϵ genes (Zurawski et al. 1982) (Fig. 6). In both cases, the RNAs from mutant and wild-type were

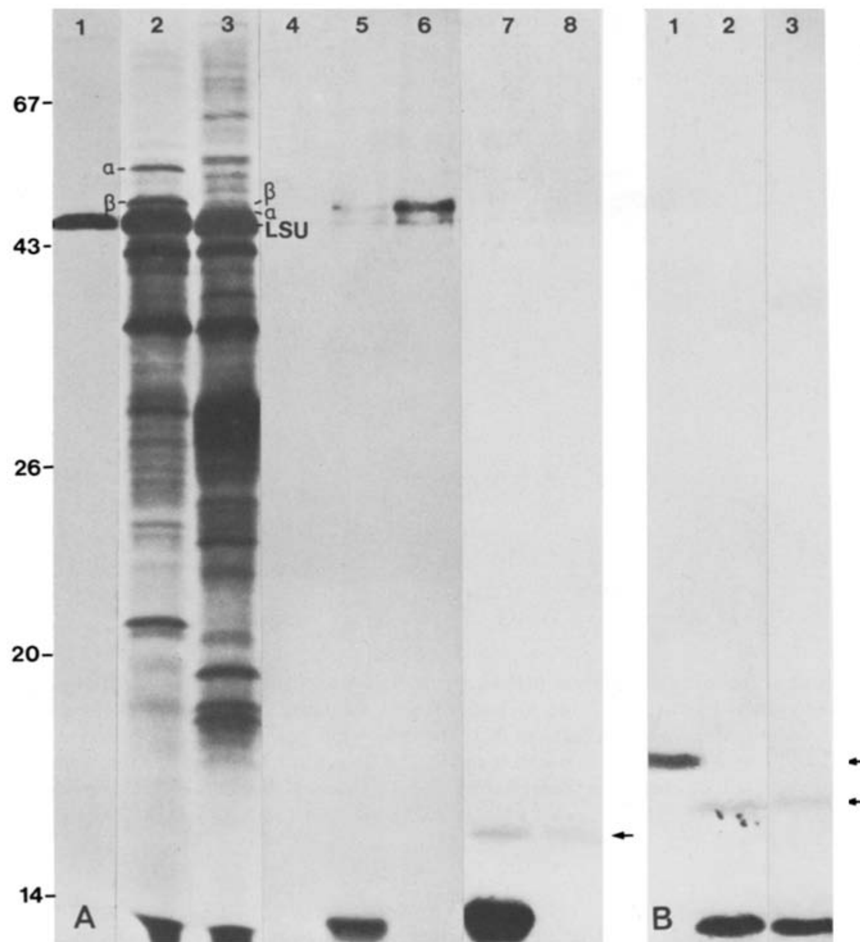

Fig. 7. In vitro translation of RNAs and immunoprecipitation of β and ε products. Panel A: RNAs from spinach (lane 2), wild-type *Oenothera* (lanes 3, 5, and 7) and mutant I-iota (lanes 4, 6, and 8) were translated using a rabbit reticulocyte lysate system. Lane 1 shows the "no message" control in which a protein endogenous to the rabbit reticulocyte translation system is evident, while lanes 2 and 3 show the results of the in vitro translation when the rabbit reticulocyte system was given mRNA. Antisera against β (lanes 5 and 6) or ε (lanes 7 and 8) were added to immunoprecipitate these products specifically. Non-immune serum was used as a control in lane 4. The position of the faint bands in lanes 7 and 8 which were immunoprecipitated by the ε antisera is indicated with an arrow. The dark band of very low molecular weight in lanes 5 and 7 is a product which was non-specifically precipitated from the wild-type reaction mix regardless of the antibody used in this experiment. (In addition to the β- and ε-antisera, two other antisera were tested and also precipitated this product.) Panel B: Immunoprecipitation of ε-specific antigens made in vitro from hybrid-selected RNAs. An ε-specific EcoR1 fragment isolated from cloned spinach ptDNA was covalently bound to sephacryl S-500. This DNA was used to specifically select RNAs from spinach (lane 1), wild-type *Oenothera* (lane 2) and *Oenothera* mutant I-iota (lane 3). The RNAs thus selected were translated in a rabbit reticulocyte system, and the products were immunoprecipitated using ε-specific antisera. The upper arrow indicates the size of the authentic ε subunit from spinach, while the lower arrow indicates the size of ε isolated from *Oenothera*

identical in size. Confirming the report of Zurawski et al. (1982) with spinach, our data indicate that the β and ε genes are cotranscribed in *Oenothera.* The doublet band in the Northern gel reveals two size classes of the mRNA, each of which contains both the β and ε messages.

PtRNAs from the wild-type and mutant *Oenothera* were translated in vitro using a rabbit reticulocyte system. β and ε-specific antisera were added to the translation products to immunoprecipitate the polypeptides by the technique of Westhoff and Zetsche (1981). The translation products of the mutant are identical to those of the wild-type for both the β and ε polypeptides (Fig. 7). Since the ε-bands from the in vitro translation products were weak, the mRNA from this region was enriched by hybrid selection (Westhoff et al. 1981; Bünemann 1982; Bünemann et al. 1982). Using the cloned spinach ε-specific EcoR1 fragment, the RNAs were hybrid-selected and translated in vitro, and the products were immunoprecipitated with ε-specific antisera. As shown in Panel B of Fig. 7, the ε-antisera precipitated proteins which were clearly identical in mutant I-iota (lane 3) and wild-type *Oenothera.* The immunoprecipitated

spinach product has the correct molecular weight of 16 kg, while the α-antigens from in vitro translation of both wild-type and mutant *Oenothera* consist of a faint band at the correct molecular weight and a prominant band of lower molecular weight. Such partial products are routinely produced when ptRNA is translated in a rabbit reticulocyte system, especially after hybrid selection. These partials are repeatable and characteristic for each particular protein; possibly they represent premature termination of translation (Westhoff et al. 1981).

Discussion

The results of the mutant analysis described here initially presented a paradox: the protein defects observed in vivo could not be substantiated by in vitro translation of mRNAs and immunoprecipitation of their products. Specifically, analysis of the thylakoid proteins from the mutant plant showed that it lacked an ATP synthase ϵ subunit of the correct molecular weight. Instead, a faint band at the position of the β subunit was visible after immunoblotting with ϵ-specific antisera (Fig. 3). Since the genes for β and ϵ lie next to each other on the ptDNA molecule and are cotranscribed, the results could be explained if a deletion of about 400 nucleotides occurred in the mutant, resulting in the fusion of the β and ϵ genes. Our analysis of ptDNA and ptRNA would have detected such an alteration, yet no major differences were observed between mutant and wild-type (Figs. 5 and 6). On the other hand, one-dimensional peptide mapping of the β-sized polypeptides from mutant I-iota and wild-type *Oenothera hookeri* following V-8 protease digestion clearly indicated that the number and position of peptides differed between the mutant and wild-type (Figs. 3 and 4). Although we had expected to see the "fusion product" in our analysis of in vitro translation, we found that the β and ϵ proteins made from mutant I-iota mRNA were indistinguishable from those synthesized from wild-type mRNA (Fig. 7). These findings ruled out the existence of a large deletion, and suggest that an error in translation in vivo could be responsible for the apparent β-ϵ fusion. Either the translational signals are altered or the translational system itself is defective. In both cases, the apparent specificity of the mutational lesion for the β and ϵ subunits of the ATP synthase can be explained by the physical proximity of the two genes and their transcription onto the same message (Fig. 6).

It should be kept in mind that although β and ϵ are decoded from the same transcript, their stoichiometries in the ATP synthase appear to be 3:1 (Maroney et al. 1983; Merchant et al. 1983). Therefore a separate ribosomal binding site most certainly exists for the ϵ gene. Because of the proximity of the genes, the ϵ ribosomal binding site must lie within or closely adjacent to the β-gene sequence (Zurawski et al. 1982; Whitfeld et al. 1983). If the I-iota mutation created a new ribosomal binding site within the β-gene sequence with translation initiation beginning at an earlier *met* codon, a large protein with ϵ-antigenic sites could result if translation continued into the correct reading frame of ϵ. The hypothetical new site would be recognized by the chloroplast ribosomes, but not by the heterologous ribosomes of the rabbit reticulocyte system, which may well have a different specificity than do the chloroplast ribosomes. By coincidence, the protein produced in vivo might be approximately the same size as β, which is why the β- and ϵ-antisera appear to bind to a single protein band. Since the mutation in the ribosomal binding site for the ϵ gene is postulated to occur in the β-sequence, it might alter that gene as well, causing a missense or frameshift mutation which would be reflected by the altered peptide fragments (Figs. 3 and 4).

The second explanation evokes a lesion in the chloroplast translation system. Thus the structural genes and the messages for β and ϵ would not themselves contain the primary defect. For that reason, the ptDNA (Fig. 5), RNA (Fig. 6), and in vitro translation comparisons (Fig. 7) would indicate no alteration in the structural genes. However if a translational defect exists, other proteins which are translated on the chloroplast ribosomes should be altered too. We have assayed for other plastome-coded proteins, including the large subunit of Rubisco (Fig. 3), the $CF_1\alpha$ subunit (Fig. 3), the apoprotein of P700, and cytochrome f (data not shown), and they appear to have the correct molecular weight in the mutant. Of course, we cannot rule out the possibility that other proteins are altered or that size differences of a few amino acids may exist.

Cloning and sequencing the β-ϵ region in mutant I-iota should reveal which explanation is more likely. If the defect lies in the translational system of the mutant, construction of a homologous in vitro translation system from mutant and wild-type *Oenothera* plants could allow one to pinpoint the lesion. In either case, our data strongly indicate that posttranscriptional controls are involved in the expression of plastid genes.

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