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Short Communication

Nucleotide Sequence of ATPase Subunit 6 Gene of Maize Mitochondria[1]

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R. E. DEWEY, CHARLES S. LEVINGS III*, AND D. H. TIMOTHY
Departments of Crop Science (R.E.D., D.H.T.) *and Genetics* (G.S.L.), *North Carolina State University, Raleigh, North Carolina 27695*

ABSTRACT

The ATPase subunit 6, located in the inner mitochondrial membrane, is encoded by mitochondrial genomes in animals and fungi. We have isolated and characterized a mitochondrial gene, designated *atp* 6, that encodes the subunit 6 polypeptide of *Zea mays*. Nucleotide and predicted amino acid sequence comparisons have revealed a homology of 44.6 and 33.2% with the yeast ATPase subunit 6 gene and polypeptide, respectively. The predicted protein in maize contains 291 amino acids with a molecular weight of 31,721. Hydropathy profiles generated for the maize and yeast polypeptides are very similar and contain large hydrophobic domains, characteristic of membrane bound proteins. RNA transfer blot analysis indicates that *atp* 6 is actively transcribed. Interestingly, 122 base pairs of nucleotide sequence interior to *atp* 6 have extensive homology with the 5′ end of the cytochrome oxidase subunit II gene of maize mitochondria, suggesting recombination between the two genes.

The mt[2] ATPase complex, located in the inner mt membrane, consists of three components designated F_0, F_1, and the oligomycin-sensitivity-conferring protein (OSCP) (27). The various subunits making up the complex are encoded either by the nuclear or mt genomes. In yeast, subunits 6, 8, and 9 of the F_0 component are mt gene products while the other subunits are of nuclear origin (16, 27, 28). Animal systems and certain fungi differ in that subunit 9 is encoded within the nucleus (25). Higher plant mt genomes contain a gene coding for ATPase subunit 9 (8), yet differ from both animals and fungi in that they also code for the alpha subunit of the F_1 component (4, 11).

Two different methods have been used to identify protein encoding genes of the maize mt genome. The Cyt oxidase subunit II and apocytochrome *b* genes were located with heterologous probes of the corresponding genes from *Saccharomyces cerevisiae* and *Kluyveromyces lactis*, respectively (7, 9). The other approach involved the isolation and sequencing of an actively transcribed clone selected from a mtDNA library, followed by computer searches of gene banks to identify the gene encoded by the clone. The ATPase subunit 9 gene of maize mitochondria was identified in this manner (8). Using the latter method, we have isolated and identified the maize mt F_0-ATPase subunit 6 gene. We present the nucleotide sequence of the subunit 6 gene and evidence that it is actively transcribed.

MATERIALS AND METHODS

Isolation of Nucleic Acids. Mitochondrial DNA and RNA were isolated from 6 to 7 d old dark-grown seedlings of *Zea mays* L, W182BN *cms-SC* or B73 *cms-T* as previously described (21, 24). The *cms-SC* cytoplasm is a member of the T (Texas) group of male-sterile cytoplasms (10).

Construction of Mitochondrial DNA Library. *Bam*HI digests of total maize mtDNA were ligated into the plasmid vector pUC 8 (29), and transformed into *Escherichia coli* strain JM 83. Ampicillin-resistant, lac- colonies were selected, replicated and fixed onto nitrocellulose filters (17).

Radioactive Labeling of DNA and RNA. Double-stranded DNA was labeled with [α-^{32}P]dATP (NEN, 3200 Ci/mmol) by nick translation (22). Single-stranded DNA clones in bacteriophage M13 were labeled using the back priming technique of Hu and Messing (13). Total mtRNA was 5′ end-labeled with [γ-^{32}P] ATP (ICN, 7000 Ci/mmol) using T_4 polynucleotide kinase (18).

Gel Electrophoresis and Nucleic Acid Hybridizations. DNA fragments were separated by electrophoresis on 0.8% agarose gels in TPE buffer (80 mM Tris-phosphate, 8 mM EDTA (pH 7.8]) and transferred to nitrocellulose according to Wahl *et al.* (30). MtRNA was heat denatured and fractionated by electrophoresis in 1.2% agarose gels containing 6% formaldehyde and blotted to nitrocellulose as described by Thomas (26). The 18S (1986 nt) and 26S (3546 nt) ribosomal RNAs of maize mitochondria were used as markers for estimating RNA sizes.

All nucleic acid hybridizations were performed under conditions previously described (8).

DNA Sequence Analysis. Cloning for sequence analysis was carried out using M13 bacteriophage vectors mp10 and mp11 (18). Ligation and transformation procedures were as outlined by New England Biolabs. DNA sequences were determined by the chain-termination method of Sanger *et al.* (23) with a universal primer (PL Biochemicals). Sequencing gels were either 6 or 8% polyacrylamide and 0.4 mm thick. The sequencing strategy is shown in Figure 1.

Sequence analyses were performed with computer programs furnished by Bionet or with a dot matrix computer program provided by M. Edgell (University of North Carolina, Chapel Hill).

RESULTS

Identification and Analysis of the Maize ATPase Subunit 6 Gene. To locate mtDNA clones actively involved in transcription, end-labeled mtRNA was hybridized to a *Bam*Hi mtDNA

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[2] Abbreviations: mt, mitochondrial; kb, kilobase(s); nt, nucleotides; bp, base pairs

library from SC cytoplasm, a maize T-type male-sterile cytoplasm (10). Among the clones exhibiting positive hybridization was a 6.5 kb *Bam*HI clone designated T25B. Hybridization of end-labeled mtRNA to southern blots of restriction digests of T25B revealed that significant hybridization was confined to a 2.7 kb *Hin*dIII fragment interior to the 6.5 kb *Bam*HI clone. This fragment was inserted into plasmid vector pUC 13 and designated T25H. T25H was also cloned into the viral vector M13 and the complete nucleotide sequence of 2583 bp was determined. A restriction map and sequencing strategy of T25H are given in Figure 1.

Using a dot matrix computer program (M. Edgell, University of North Carolina, Chapel Hill) the nucleotide sequence of T25H was compared with the mtDNA sequences of yeast. Sequence homology was found between a segment of T25H and the yeast mitochondrial gene coding for ATPase subunit 6; no other yeast gene contained significant sequence homology with T25H. The nucleotide sequence of the maize gene is shown in Figure 2. DNA sequence homology between the maize and yeast ATPase subunit 6 genes is 44.6%. Based on this homology we have concluded that this sequence codes for the ATPase subunit 6 gene and have selected the symbol *atp* 6 to designate the gene in maize. Unlike the cytochrome oxidase subunit II gene in maize mitochondria (9), *atp* 6 does not appear to contain intervening sequences. Due to low homologies at the terminal regions of the gene, however, we cannot exlude the possibility that introns exist near the 5′ or 3′ ends of the gene.

Amino Acid Sequence. As a translational initiation site for the *atp* 6 gene, we have selected the ATG codon closest to the initiator methionine of the homologous gene in yeast and *Aspergillus*. This ATG site (beginning at position 1 in Fig. 2) is distantly located from the next adjacent in frame ATG codons in both the 3′ and 5′ directions. In the 5′ direction, the next ATG codon begins at position −294 (Fig. 2) and would increase the size of the polypeptide by 98 amino acids. These additional amino acids are not homologous with ATPase subunit 6 protein sequences from other organisms and would generate a polypeptide much larger than observed in other organisms. In the 3′ direction, the next ATG codon starts at position 162 (Fig. 2) and would decrease the polypeptide by 53 amino acids, portions of which contain significant homology with the yeast protein. It has not been unequivocally demonstrated, however, that translation always begins with AUG in maize mitochondria. In mammalian mitochondria the entire AUN family is capable of translational initiation (1, 2).

Assuming translation initiates as proposed in Figure 2, the protein sequence of *atp* 6 contains 291 amino acids. The predicted protein sequence is the same regardless of whether the universal code or the higher plant mitochondrial code is used (9). The predicted maize protein is 32 amino acids longer than

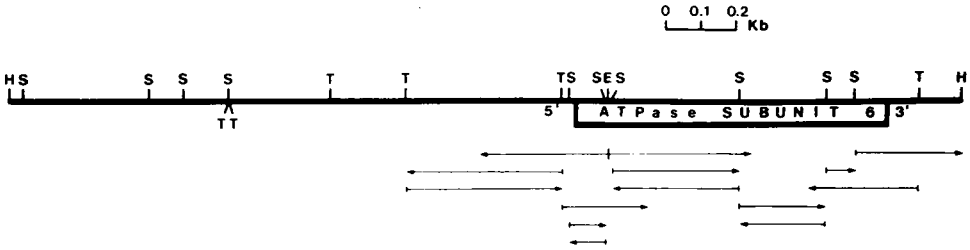

FIG. 1. Restriction map of the maize mitochondrial ATPase subunit 6 gene and flanking sequences. Arrows below the map show the direction and extent of sequence analysis from each restriction site. Restriction sites are indicated by vertical lines: E, *Eco*RI; H, *Hin*dIII; S, *Sau* 3A; T, *Taq* I.

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                                asn gln val ser phe gly ser arg leu tyr leu ile leu tyr ser tyr ser met met met met thr arg trp ser ser thr asp met lys
-423  5'-GATTTCGTTGGGTAG AAC CAA GTC TCT TTT GGG AGC AGA TTG TAT TTG ATT TTA TAT AGT TAC TCC ATG ATG ATG ATG ACT AGA TGG AGT TCC ACT GAT ATG AAG

      arg arg asn arg ile leu ala asn met val pro ile arg asn leu ser leu pro asp tyr tyr glu tyr glu glu glu tyr his pro val ser arg glu ala
-318  AGA AGA AAT AGA ATA TTG GCT AAT ATG GTG CCA ATT CGT AAT TTA AGT TTA CCT GAT TAT TAT GAA TAT GAA GAA GAA TAC CAT CCA GTT TCA AGA GAG GCA

      thr arg gly val cys ile leu leu arg ile asp arg tyr leu ser ser ile gly arg ser ile gln asp arg glu val leu arg asp phe arg gln trp leu
-216  ACC AGA GGG GTC TGT ATA CTC CTA CGA ATA GAC AGA TAT TTA TCT TCA ATT GGA AGG AGC ATT CAA GAC CGT GAG GTT CTA CGC GAT TTC CGC CAA CGG TTA

      leu phe pro gln arg glu ala gly tyr ser phe ser glu ile tyr asp asp ile arg ala his gly val glu ala ser arg leu gly gln pro leu arg asp
-114  CTC TTT CCC CAA CGC GAG GCT GGG TAC AGC TTT TCC GAA ATA TAT GAT GAT ATA CGA GCG CAT GGG GTA GAA GCA AGT CGA TTG GGT CAG CCT CTA AGA GAT

      leu tyr asp glu Met Glu Arg Asn Gly Glu Ile Val Asn Asn Gly Ser Ile Ile Ile Pro Gly Gly Gly Gly Pro Val Thr Glu Ser Pro Leu Asp Gln Phe
 -12  CTG TAC GAT GAG ATG GAA AGG AAC GGC GAG ATA GTA AAT AAC GGC TCA ATC ATT ATC CCT GGA GGC GGC GGA CCA GTA ACA GAA AGC CCA TTG GAT CAA TTT

      Gly Ile His Pro Ile Leu Asp Leu Asn Ile Gly Lys Tyr Tyr Val Ser Phe Thr Asn Leu Ser Leu Ser Met Leu Leu Thr Leu Gly Leu Val Leu Leu Leu
  91  GGA ATT CAC CCA ATT CTG GAT CTG AAT ATT GGC AAG TAC TAT GTC TCA TTC ACA AAT CTA TCC TTG TCT ATG CTA CTC ACT CTC GGT TTG GTC CTA CTT CTG

      Val Phe Val Val Thr Lys Lys Gly Gly Gly Lys Ser Val Pro Asn Ala Phe Gln Ser Leu Val Glu Leu Ile Tyr Asp Phe Val Pro Asn Leu Val Asn Glu
 193  GTT TTT GTT GTT ACG AAA AAA GGA GGG GGA AAG TCA GTG CCA AAT GCA TTT CAA TCC TTG GTG GAG CTT ATT TAT GAT TTC GTG CCG AAC CTG GTA AAC GAA

      Gln Ile Gly Gly Leu Ser Gly Asn Val Lys His Lys Phe Phe Pro Cys Ile Ser Val Thr Phe Thr Phe Ser Leu Phe Arg Asn Pro Gln Gly Met Ile Pro
 295  CAA ATA GGT GGT CTT TCC GGA AAT GTG AAA CAC AAG TTT TTC CCT TGC ATC TCG GTC ACT TTT ACT TTT TCG TTA TTT CGT AAT CCC CAG GGT ATG ATA CCC

      Phe Ser Phe Thr Val Thr Ser His Phe Leu Ile Thr Leu Ala Leu Ser Phe Ser Ile Phe Ile Gly Ile Thr Ile Val Gly Phe Gln Arg His Gly Leu His
 397  TTT AGC TTC ACA GTG ACA AGT CAT TTT CTC ATT ACT TTG GCT CTT TCA TTT TCT ATT TTT ATA GGC ATT ACG ATC GTT GGA TTT CAA AGA CAT GGG CTT CAT

      Phe Phe Ser Phe Leu Leu Pro Ala Gly Val Pro Leu Pro Leu Ala Pro Phe Leu Val Leu Leu Glu Leu Ile Ser His Cys Phe Arg Ala Leu Ser Ser Gly
 499  TTT TTT AGC TTC TTA TTA CCA GCG GGA GTC CCA CTG CCA TTA GCA CCT TTT TTA GTA CTC CTT GAG CTA ATC TCT CAT TGT TTT CGT GCA TTA AGC TCA GGA

      Ile Arg Leu Phe Ala Asn Met Met Ala Gly His Ser Ser Val Lys Ile Leu Ser Gly Phe Ala Trp Thr Met Leu Phe Leu Asn Asn Ile Phe Tyr Phe Leu
 601  ATA CGT TTA TTT GCT AAT ATG ATG GCC GGT CAT AGT TCA GTA AAG ATT TTA AGT GGG TTT GCT TGG ACT ATG CTA TTT CTG AAT AAT ATT TTC TAT TTC TTA

      Gly Asp Leu Gly Pro Leu Phe Ile Val Leu Ala Leu Thr Gly Leu Glu Leu Gly Val Ala Ile Ser Gln Ala His Val Ser Thr Ile Ser Ile Cys Ile Tyr
 703  GGA GAT CTT GGT CCC TTA TTT ATA GTT CTA GCA TTA ACC GGT CTG GAA TTA GGT GTA GCT ATA TCA CAA GCT CAT GTT TCT ACG ATC TCA ATT TGT ATT TAC

      Leu Asn Asp Ala Thr Asn Leu His Gln Asn Glu Ser Phe His Asn Cys Ile Lys Thr Arg Ser Gln Ser
 805  TTG AAT GAT GCT ACA AAT CTC CAT CAA AAT GAG TCA TTT CAT AAT TGC ATA AAA ACG AGG AGC CAA TCA TAG AACTACATATGGTCTGATACTACTAAC-3'
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FIG. 2. Nucleotide sequence of the maize ATPase subunit 6 gene. The predicted amino acid sequence is translated according to the higher plant mitochondrial code (9) and is indicated in Roman type. The amino acid sequence of the open reading frame extending beyond the putative ATG initiation codon is in italics.

the corresponding yeast protein with most of the additional amino acids located at both the amino and carboxyl termini (Fig. 3). The 5′ end of the *atp* 6 open reading frame extends 408 nucleotides upstream of the putative ATG start site shown in Figure 2. However, analysis of the DNA sequence and predicted protein sequence of this region reveals no significant homology with other DNA or protein sequences in the sequence libraries of NIH GenBank or National Biomedical Research Foundation. The carboxyl terminus is predicted by a TAG stop codon at position 873, 45 nucleotides beyond the stop site of the yeast gene. A mol wt of 31,721 is calculated from the predicted protein sequence.

The maize and yeast proteins share an amino acid sequence homology of 33.2% (Fig. 3). When conservative replacements are included (Asn-Gln), (Lys-Arg), (Ser-Thr), (Phe-Tyr-Trp), (Ile-Leu-Val-Met), the homology increases to 48.6%. Comparisons of the maize protein to the predicted mitochondrial proteins from *Aspergillus nidulans*, *Drosophila yakuba*, and mouse (2, 6, 19) show amino acid homologies of 35.6, 20.5, and 20.2%, respectively (data not shown). A homology of 16.7% exists between the maize ATPase subunit 6 protein and the analogous bacterial protein from *Escherichia coli* (20).

As expected for membrane associated proteins, the predicted amino acid sequence of maize ATPase subunit 6 contains a majority of hydrophobic residues and relatively few charged amino acids. To analyze the distribution of these residues, a hydropathy profile was constructed according to the values of Kyte and Doolittle (Fig. 4) (15). Hydrophobic domains located throughout the protein indicate the portions of the molecule most likely to lie within the membrane. The maize *atp* 6 profile is similar to the plot of the yeast ATPase subunit 6 protein with

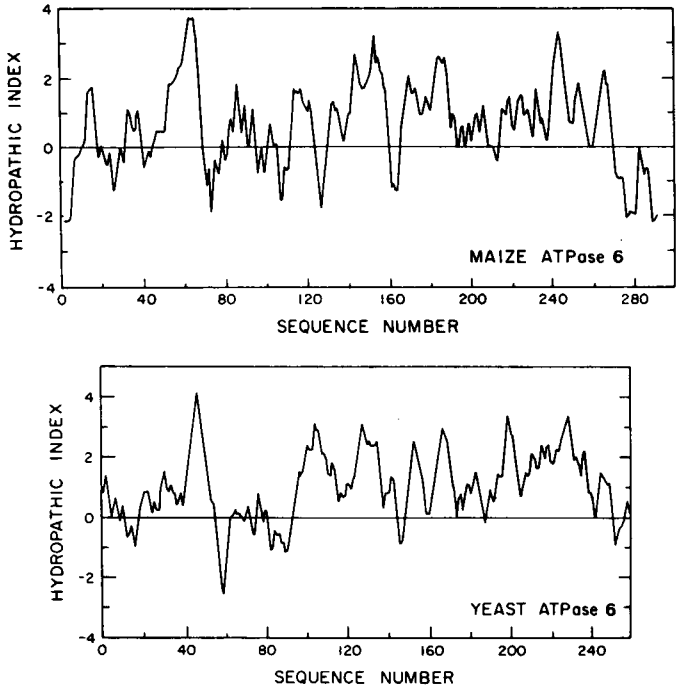

FIG. 4. Hydropathy profiles of the predicted maize and yeast ATPase subunit 6 proteins. The *y* axis represents arbitrary hydrophobic values (15). The *x* axis indicates the positions of the individual amino acids. Area above the line shows domains with increased probability of being located in the lipid bilayer.

Maize	Met	Glu	Arg	Asn	Gly	Glu	Ile	Val	Asn	Asn	Gly	Ser	Ile	Ile	Ile	Pro	Gly	Gly	Gly	Gly	20
Yeast	Met	-	Phe	Asn	-	-	Leu	Leu	Asn	Thr	-	-	-	-	-	-	-	-	-	-	
	Pro	Val	Thr	Glu	Ser	Pro	Leu	Asp	Gln	Phe	Gly	Ile	His	Pro	Ile	Leu	Asp	Leu	Asn	Ile	40
	Tyr	Ile	Thr	-	Ser	Pro	Leu	Asp	Gln	Phe	Glu	Ile	Arg	Thr	Leu	Phe	Gly	Leu	Gln	Ser	
	Gly	Lys	Tyr	Tyr	Val	Ser	Phe	Thr	Asn	Leu	Ser	Leu	Ser	Met	Leu	Leu	Thr	Leu	Gly	Leu	60
	Ser	Phe	Ile	Asp	Leu	Ser	Cys	Leu	Asn	Leu	Thr	Thr	Phe	Ser	Leu	Tyr	Thr	Ile	Ile	Val	
	Val	Leu	Leu	Leu	Val	Phe	Val	Val	Thr	-	Lys	Lys	Gly	Gly	Gly	Lys	Ser	Val	Pro	Asn	80
	Leu	Leu	Val	Ile	Thr	Ser	Leu	Tyr	Thr	Leu	Thr	Asn	Asn	Asn	Asn	Lys	Ile	Ile	Gly	Ser	
	Ala	Phe	Gln	Ser	Leu	Val	Glu	Leu	Ile	Tyr	Asp	Phe	Val	Pro	Asn	Leu	Val	Asn	Glu	Gln	100
	Arg	Trp	Leu	Ile	Ser	Gln	Glu	Ala	Ile	Tyr	Asp	Thr	Ile	Met	Asn	Met	Thr	Lys	Gly	Gln	
	Ile	Gly	Gly	Leu	Ser	Gly	Asn	Val	Lys	His	Lys	Phe	Phe	Pro	Cys	Ile	Ser	Val	Thr	Phe	120
	Ile	Gly	Gly	Lys	Asn	Trp	Gly	Leu	-	-	-	Tyr	Phe	Pro	Met	Ile	Phe	Thr	Leu	Phe	
	Thr	Phe	Ser	Leu	Phe	Arg	Asn	Pro	Gln	Gly	Met	Ile	Pro	Phe	Ser	Phe	Thr	Val	Thr	Ser	140
	Met	Phe	Ile	Phe	Ile	Ala	Asn	Leu	Ile	Ser	Met	Ile	Pro	Tyr	Ser	Phe	Ala	Leu	Ser	Ala	
	His	Phe	Leu	Ile	Thr	Leu	Ala	Leu	Ser	Phe	Ser	Ile	Phe	Ile	Gly	Ile	Thr	Ile	Val	Gly	160
	His	Leu	Val	Phe	Ile	Ile	Ser	Leu	Ser	Ile	Val	Ile	Trp	Leu	Gly	Asn	Thr	Ile	Leu	Gly	
	Phe	Gln	Arg	His	Gly	Leu	His	Phe	Phe	Ser	Phe	Leu	Leu	Pro	Ala	Gly	Val	Pro	Leu	Pro	180
	Leu	Tyr	Lys	His	Gly	Trp	Val	Phe	Phe	Ser	Leu	Phe	Val	Pro	Ala	Gly	Thr	Pro	Leu	Pro	
	Leu	Ala	Pro	Phe	Leu	Val	Leu	Leu	Glu	Leu	Ile	Ser	His	Cys	Phe	Arg	Ala	Leu	Ser	Ser	200
	Leu	Val	Pro	Leu	Leu	Val	Ile	Met	Glu	Thr	Leu	Ser	Tyr	Ile	Ala	Arg	Ala	Ile	Ser	Leu	
	Gly	Ile	Arg	Leu	Phe	Ala	Asn	Met	Met	Ala	Gly	His	Ser	Ser	Val	Lys	Ile	Leu	Ser	Gly	220
	Gly	Leu	Arg	Leu	Gly	Ser	Asn	Ile	Leu	Ala	Gly	His	Leu	Leu	Met	Val	Ile	Leu	Ala	Gly	
	Phe	Ala	Trp	Thr	Met	Leu	Phe	Leu	Asn	Asn	Ile	Phe	Tyr	Phe	Leu	Gly	Asp	Leu	Gly	Pro	240
	Leu	Thr	Phe	Asn	Phe	Met	-	Leu	Ile	Asn	Leu	Phe	Thr	Leu	Val	Phe	Gly	Phe	Val	Pro	
	Leu	Phe	Ile	Val	Leu	Ala	Leu	Thr	Gly	Leu	Glu	Leu	Gly	Val	Ala	Ile	Ser	Gln	Ala	His	260
	Leu	Ala	Met	Ile	Leu	Ala	Ile	Met	Ile	Leu	Glu	Phe	Ala	Ile	Gly	Ile	Ile	Gln	Ser	Tyr	
	Val	Ser	Thr	Ile	Ser	Ile	Cys	Ile	Tyr	Leu	Asn	Asp	Ala	Thr	Asn	Leu	His	Gln	Asn	Glu	280
	Val	Trp	Thr	Ile	Leu	Thr	Ala	Ser	Tyr	Leu	Lys	Asp	Thr	Leu	Tyr	Leu	His				
	Ser	Phe	His	Asn	Cys	Ile	Lys	Thr	Arg	Ser	Gln	Ser									

FIG. 3. A comparison of the predicted amino acid sequence of maize ATPase subunit 6 with the corresponding protein from yeast. Boxed regions indicate amino acids that are conserved. A dash indicates an amino acid that is absent.

the exception of the terminal regions. In maize both termini are hydrophilic whereas in yeast the terminal domains are mostly hydrophobid (Fig. 4). The additional amino acids present at the termini of the maize protein and absent in yeast contain several hydrophilic residues (Fig. 3) and account for the differences in the profiles in these regions. The hydropathy plot for the amino acids that extend beyond the putative initiator methionine (indicated in italics in Fig. 2) is very hydrophilic; this is uncharacteristic of a membrane bound polypeptide and supports our proposal that translation initiates as indicated in Figure 2.

The secondary structure of the maize ATPase subunit 6 protein was deduced from the amino acid sequence by the method of Chou and Fasman (5). A large amount of β-sheet conformation (61%) is predicted by this procedure. Analysis of yeast and *Aspergillus* ATPase subunit 6 proteins also predicts the β-sheet conformation to be the most prevalent secondary structure, with estimates of 73 and 66%, respectively. In contrast, the predicted secondary structure of the amino acid sequence extending beyond the putative start methionine contains relatively low β-sheet conformation (30%).

A summary of codon usage for *atp* 6 is given in Figure 5.

Homology Between atp 6 and the Cytochrome Oxidase Subunit II Gene of Maize Mitochondria. Nucleotide sequence comparison of the *atp* 6 gene to the NIH GenBank sequence library revealed extensive homology to a region of the Cyt oxidase subunit II (COII) gene in male-fertile maize mitochondria (9). The homology extends from the *Eco*RI site, where the published COII sequence begins, to a position 8 bp 5′ of an ATG codon designated by Fox and Leaver (9) as the "second possible initiation codon" (positions −104 to −8, Fig. 6). This corresponds to positions 97 to 194 of *atp* 6 (Figs. 2, 6). To examine the extent of the homology 5′ of the *Eco*RI site, we cloned and sequenced this portion of the COII gene from *cms*-T maize cytoplasm. Homology was found to continue 25 bp 5′ of the *Eco*RI site, indicating a continuous homologous region of 122 bp. The *atp* 6 and COII sequences in this segment differ only by 3 bp substitutions and a single insertion/deletion of the sequence TATCAA at position 88 of *atp* 6 (Fig. 6). Comparison of the predicted amino acid sequences in this region shows 40 of 43 amino acids (93%) are conserved. The portion of *atp* 6 that is homologous with the maize COII gene is located in the coding region (positions 73–194, Fig. 2). The predicted amino acid sequence and hydropathy profiles of this region are homologous to the yeast and *Aspergillus* ATPase subunit 6 proteins, whereas no homology is seen with the yeast Cyt oxidase subunit II protein (data not shown). Thus, it is probable that the maize COII gene derived the homologous sequences from recombination with *atp* 6 and thereby may be considered a chimeric gene.

Southern Blot Analysis. Hybridization of clones containing *atp* 6 to Southern blots of *Bam*HI and *Hind*III mtDNA digests revealed intense hybridization to a 6.5 kb *Bam*HI fragment and a 2.7 kb *Hind*III fragment, respectively (data not shown). In addition, several fragments showed weak hybridization after long exposure in both digests. The weakly hybridizing bands are probably due to poorly matched or short homologous sequences. In fact, we have previously described a short sequence (122 bp)

	U		C		A		G	
U	Phe	UUU: 21	Ser	UCU: 4	Tyr	UAU: 3	Cys	UGU: 2
		UUC: 7		UCC: 3		UAC: 2		UGC: 2
	Leu	UUA: 12		UCA: 10		UAA: -		UGA: -
		UUG: 6		UCG: 2		UAG: 1	Trp	UGG: 1
C	Leu	CUU: 7	Pro	CCU: 3	His	CAU: 8	Arg	CGU: 3
		CUC: 5		CCC: 3		CAC: 2		CGC: -
		CUA: 6		CCA: 7	Gln	CAA: 7		CGA: -
		CUG: 7		CCG: 1		CAG: 1	Trp	CGG: -
A	Ile	AUU: 12	Thr	ACU: 5	Asn	AAU: 13	Ser	AGU: 3
		AUC: 6		ACC: 1		AAC: 4		AGC: 5
		AUA: 8		ACA: 5	Lys	AAA: 4	Arg	AGA: 1
	Met	AUG: 6		ACG: 4		AAG: 4		AGG: 2
G	Val	GUU: 6	Ala	GCU: 6	Asp	GAU: 5	Gly	GGU: 8
		GUC: 4		GCC: 1		GAC: -		GGC: 6
		GUA: 6		GCA: 4	Glu	GAA: 4		GGA: 10
		GUG: 5		GCG: 1		GAG: 4		GGG: 3

FIG. 5. Codon usage in the *atp* 6 gene.

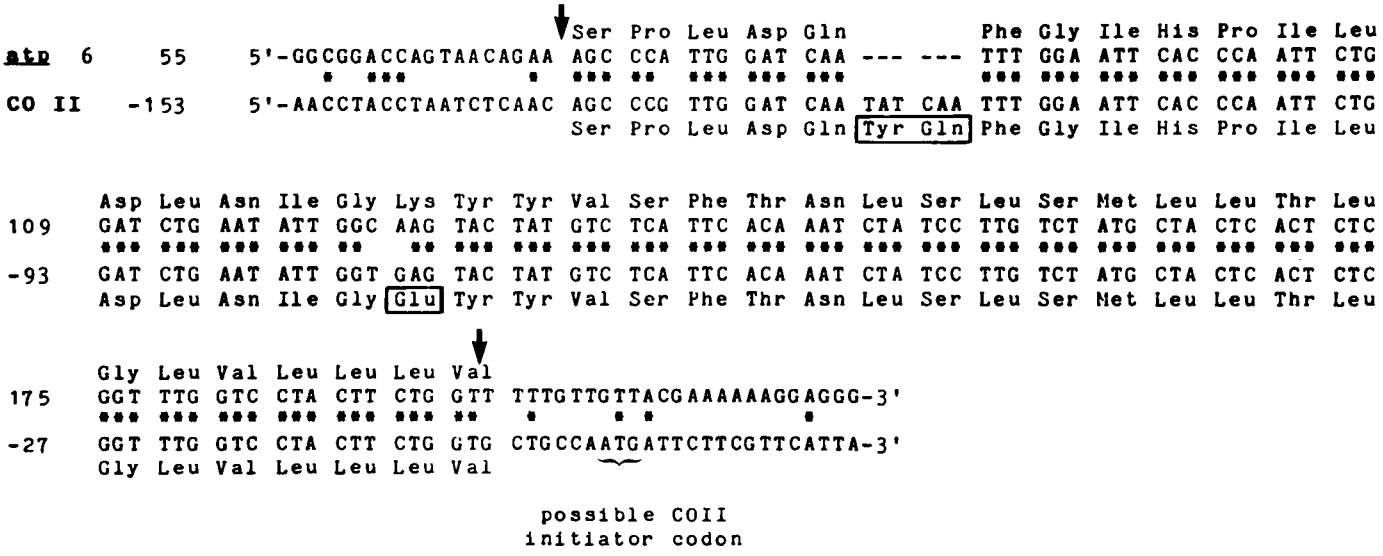

FIG. 6. Nucleotide and amino acid sequence homology between *atp* 6 and COII genes of maize mitochondria. Nucleotide positions of *atp* 6 are as described in Figure 2. Positions of COII nucleotides are in relation to the possible ATG initiator codon indicated with a bracket. Homologous nucleotides are indicated with an asterisk. Amino acids of COII nonhomologous with *atp* 6 are boxed. Proposed points of recombination are designated with arrows.

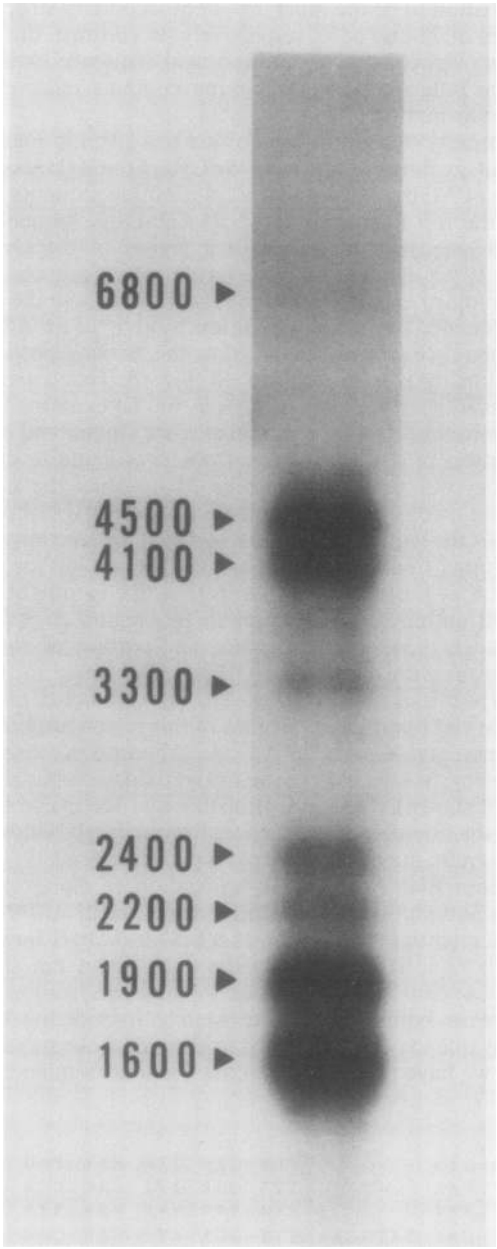

FIG. 7. Hybridization of 2.7 kb T25H clone to an RNA blot of maize mtRNA. Approximate transcript sizes are indicated in nucleotides.

in the COII gene with substantial homology to the *atp* 6 gene. These results, together with the transcriptional studies given below, suggest that the complete *atp* 6 gene is present as a single copy in this genome.

Transcriptional Processing of the atp 6 Message. When the *atp* 6 sequence was hybridized to a Northern blot of total maize mtRNA, a strong and complex hybridization pattern was revealed (Fig. 7). The largest detectable transcript is approximately 6800 nt in length and may be the primary transcript. The most predominant forms of the transcript are approximately 4500, 1900, and 1600 nt long. Further studies are needed to determine unequivocally the primary and mature forms of the message. As expected, single-stranded M13 probes of the noncomplementary strand showed no detectable hybridization to RNA blots (data not shown). These results indicate that the *atp* 6 sequence is an actively transcribed gene.

DISCUSSION

Subunit 6 of the mitochondrial ATPase is an inner membrane polypeptide of the F_0 component, encoded within the mitochondrial genomes of all eukaryotic organisms examined to date. The nucleotides and amino acid sequence homologies of *atp* 6 with the ATPase subunit 6 genes from yeast and other organisms, along with evidence of active transcription, indicates that the ATPase subunit 6 is also encoded by a mitochondrial gene in maize. Our characterization of the maize *atp* 6 nucleotide sequence is the first evidence that ATPase subunit 6 is mitochondrially encoded in higher plants.

It has been proposed that the code in higher plant mitochondria differs from that found in yeast; the triplet CGG is translated as tryptophan rather than arginine, and TGA codons are nontranslatable rather than specifying tryptophan residues (9). The *atp* 6 sequence contains no TGA codons, thus supporting the view that it is a nonsense codon in higher plant mitochondria. The triplet CGG is also absent, making it impossible to confirm its usage as either a tryptophan or arginine residue in the mitochondrial genome of maize.

The amino acid homology between the maize and yeast ATPase subunit 6 proteins (32.2%) is less than that found between the other maize genes and their yeast counterparts. This is not surprising considering the general lack of conservation among ATPase subunit 6 proteins of distantly related species (6). For example, the amino acid homology between *Drosophila* and yeast ATPase subunit 6 proteins is 23.0%. The homology between the *Drosophila* and mouse polypeptides is 35.7% (6). Of particular interest is the overall size differences observed among the species. The maize protein is 32 amino acids longer than the yeast protein and 55 amino acids longer than the corresponding protein from mouse. Almost all of these additional amino acids are located at the terminal regions and not within the interior of the protein. Interestingly, the open reading frame containing the maize ATPase subunit 6 protein extends 408 base pairs upstream beyond the putative ATG initiation codon. Thus the maize protein could be even larger than the 291 amino acids proposed here. It is unlikely, however, that these additional amino acids could be part of the mature ATPase subunit 6 polypeptide since these amino acids are very hydrophilic. It is possible that ATPase subunit 6 in maize mitochondria is translated as a precursor, with the hydrophilic amino acids at the amino terminus undergoing cleavage to produce the mature form of the protein.

Extensive nucleotide and amino acid homology is observed bewteen a portion of *atp* 6 and the 5′ end of the COII gene in maize mitochondria (Fig. 6). This homology is presumably due to recombination between the two genes. Homology among mitochondrial COII proteins of *Oenothera*, rice, and wheat, (3, 12, 14) with the predicted maize COII sequence begins at the 'possible ATG initiator codon' indicated in Figure 6 and does not include any of the amino acids homologous with *atp* 6. Although *Oenothera*, rice, and wheat share nucleotide homology in the 5′ flanking region of the Cyt oxidase subunit II gene, no homology is observed with the corresponding maize sequence where the recombination with *atp* 6 has occurred. This recombination, therefore, does not appear to be a common characteristic of higher plants. Nucleotide sequence analysis of the COII gene of *Zea diploperennis*, a wild relative of maize, indicates that the recombination with *atp* 6 is also found in this species (R. E. Dewey, C. S. Levings III, D. H. Timothy, unpublished results). It is therefore likely that this phenomenon is common to the genus *Zea*.

A complex hybridization pattern is observed when the *atp* 6 gene is hybridized to Northern blots of total mtRNA. Complex RNA hybridization patterns are also observed for the COII, apocytochrome b, and ATPase subunit 9 genes of maize mitochondria (7–9). Part of the *atp* 6 hybridization complexity may

be due to cross-hybridization of the *atp* 6 sequence with the transcript produced by the COII gene, since *atp* 6 and the COII gene contain nucleotide homology (Fig. 6). Likewise, some of the complexity observed when the COII gene is hybridized to RNA blots may be caused by cross-hybridization to *atp* 6 transcripts. Because intramolecular recombination is relatively common in the maize mitochondrial genome, rearrangements may be partially responsible for the complex hybridization patterns detected by Northern blot analysis.

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