Plant Physiol. (1985) 79, 571–577
0032-0889/85/79/0571/07/$01.00/0

Short Communication

Nucleotide Sequence of the F_1-ATPase α Subunit Gene from Maize Mitochondria[1]

Received for publication May 30, 1985

CARL J. BRAUN AND CHARLES S. LEVINGS III*
Department of Genetics, Box 7614, North Carolina State University, Raleigh, North Carolina 27695-7614

ABSTRACT

The α subunit of the F_1-ATPase complex of maize is a mitochondrial translational product, presumably encoded by the mitochondrial genome. Based on nucleotide and amino acid homology, we have identified a mitochondrial gene, designated *atpα*, that appears to code for the F_1-ATPase α subunit of *Zea mays*. The *atpα* gene is present as a single copy in the maize Texas cytoplasm and is actively transcribed. The maize α polypeptide has a predicted length of 508 amino acids and a molecular mass of 55,187 daltons. Amino acid homologies between the maize mitochondrial α subunit and the tobacco chloroplast CF_1 and *Escherichia coli* α subunits are 54 and 51%, respectively. The origin of the *atpα* gene is discussed.

A vital component of all mitochondria is the F_1-F_0 ATPase complex that is involved in the generation of energy in the terminal event of oxidative phosphorylation. This complex reversibly catalyzes either the hydrolysis or synthesis of ATP, depending upon the direction of an electrochemical gradient that is formed by the passage of protons through the complex (24).

The complex consists of three parts: F_0, F_1, and the oligomycin-sensitivity-conferring protein (24). The F_0 is located within the inner mitochondrial membrane and consists of an undetermined number of hydrophobic subunits. The F_1 is the best characterized segment of the ATPase complex, consisting of five subunits located in the matrix of mitochondria. These F_1 subunits exist in a 3 α:3 β:1 γ:2 δ:1 ϵ stoichiometric ratio (26). Between the membrane-bound F_0 and the matrix F_1 is the oligomycin-sensitivity-conferring protein.

The biogenesis of the F_1-F_0 ATPase complex requires both nuclearly and mitochondrially encoded genes (6). In most organisms, the hydrophobic proteins of the F_0 are encoded by the mitochondria. The F_0-subunit 9 is an exception, being encoded in the nucleus of animals, *Aspergillus* and *Neurospora* (31). In most organisms, the genes for the five F_1-subunits are encoded by the nuclear genome (16). These F_1-subunits are translated on cytosolic ribosomes as precursors with amino-terminal signals that facilitate transport into the mitochondria (16). In maize mitochondria, however, a protein with a molecular mass of 58 kD has been identified that is translated on mitochondrial ribosomes and immunoprecipitated by antisera to the F_1-α subunit of yeast (10). The F_1-α mitochondrial gene product has also been demonstrated in *Vicia faba* (1).

We have isolated and characterized an actively transcribed gene from the Texas male-sterile cytoplasm of maize (*cms-T*). Based on DNA sequence and amino acid homologies, we have identified this gene as coding for the α subunit of F_1-ATPase.

MATERIALS AND METHODS

Isolation of Nucleic Acids. Mitochondrial DNA and RNA (mtRNA) were isolated from 5- to 7-d-old, etiolated coleoptiles from *Zea mays* L., *cms*[2]-*T* cytoplasm. Detailed protocols for the isolation of mtDNA (19) and mtRNA (22) have been previously described.

Cloning, Hybridization, and Electrophoresis Technique. The construction of maize mitochondrial DNA libraries with pUC plasmid vectors, and hybridization conditions for detecting transcribed clones have been previously described (5).

DNA was digested, fractionated by electrophoresis, and transferred to nitrocellulose as described by Wahl *et al.* (30). RNA electrophoresis and transfer to nitrocellulose were as described by Thomas (29). The 18S (1986 nt) and 26S (3546 nt) ribosomal RNAs of maize mitochondria and S/RU-RNA-b (750 nt) of *cms*-S maize mitochondria (22) were used as markers for estimating RNA sizes.

Preparation of Probes. DNA fragments were isolated from low melting point agarose gels and purified by NACS 52 resin according to conditions described by the supplier (Bethesda Research Laboratories). Purified fragments were labeled by nick-translation (20), and M13 clones by backpriming (11), each with [α-^{32}P]dATP (New England Nuclear, 3200 Ci/mmol, 1Ci = 37 Gbq). RNA was 5′ end-labeled (14) with T4 polynucleotide kinase and [γ-^{32}P]ATP (ICN, >7000 Ci/mmol).

DNA Sequence Analysis. Clones T22 and TA22 (defined in "Results") were subcloned for sequencing into the M13 bacteriophage vectors mp18 and mp19 (17). A shotgun cloning strategy was used for subcloning. To complete the sequence, it was necessary to supplement this strategy by force cloning several specific fragments. Transformation into the *Escherichia coli* host JM103 followed protocols supplied by New England Biolabs. Conditions for sequencing by the dideoxy chain-terminator method have been previously described (21). Sequence analysis and predictions of polypeptide mol wt were performed with computer programs provided by Bionet.

RESULTS

Identification of the F_1-α Gene. A *Bam*HI library of maize *cms-T* mtDNA was constructed and screened for actively tran-

[1] Supported in part by the National Science Foundation and Agrigenetics, Inc. Paper No. 10045 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7601.

[2] Abbreviations: *cms*, cytoplasmic male sterile; mtRNA, mitochondrial RNA; ORF, open reading frame; kb, kilobase(s); bp, base pair; nt, nucleotides.

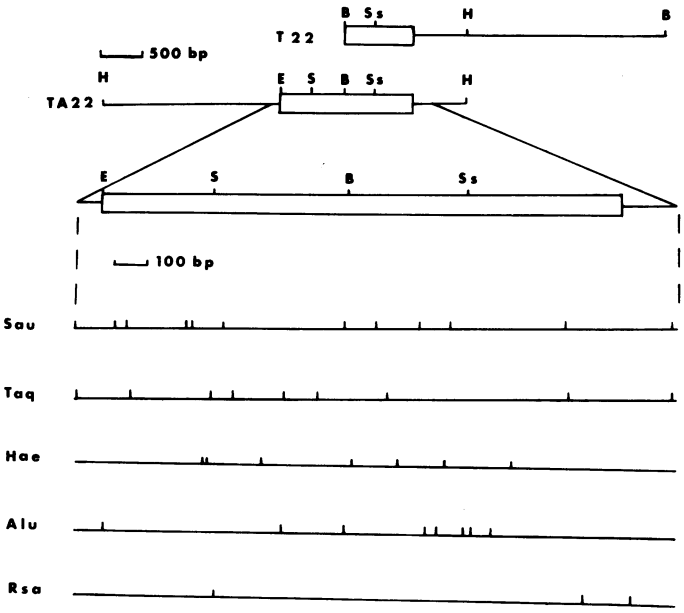

FIG. 1. Restriction map of *cms-T* mtDNA including the ATPase F_1-ATPase α gene. The coding region of the gene is enclosed in the box. The restriction map was constructed from two mtDNA clones: a 3.7-kb *Bam*HI fragment (T22), and a 4.2-kb *Hin*dIII clone (TA22). Restriction sites are indicated by vertical hash marks: B, *Bam*HI; H, *Hin*dIII; S, *Sal*I; Ss, *Sst*I; E, *Eco*RI; Sau, *Sau*3A; Taq, *Taq*I; Hae, *Hae*III; Alu, *Alu*I; Rsa, *Rsa*I.

scribed genes with end-labeled mtRNA from *cms-T* maize. Mitochondrial clones from the 26S, 18S, and 5S rRNA genes (2) were used as labeled probes to identify and eliminate clones containing rRNA genes. An actively transcribed 3.7 kb clone, designated T22, was selected from the library for study (Fig. 1). Hybridization studies with end-labeled mtRNA indicated that transcription was limited to a 1.4-kb *Bam*HI to *Hin*dIII fragment of the T22 clone. Subsequently, hybridization to strand-specific M13 subclones showed that only one strand was transcribed. The sequence of the 1.4-kb fragment was determined and a large ORF was found. Comparison of the ORF translation to the National Biomedical Research Foundation protein library identified significant homology between the ORF and the carboxy end of the CF_1 and F_1 α genes from tobacco chloroplast (4) and *E. coli* (8), respectively. Sequence comparisons indicated that the *Bam*HI site of the T22 clone was located near the middle of the α gene coding region, and that approximately 700 base pairs (bp) of the 5′ end of the gene were missing from T22. To isolate the fragment containing the 5′ end of the putative α gene, a second *cms-T* mtDNA library was constructed using *Hin*dIII restriction fragments. The 1.4 kb *Bam*HI to *Hin*dIII fragment of the T22 clone was nick-translated and used to probe the *Hin*dIII library for the 5′ end of the gene by colony hybridization. A 4.2-kb *Hin*dIII clone, designated TA22, was selected by its intense hybridization for further analysis. Restriction endonuclease analysis of the TA22 clone identified the 1.4-kb *Bam*HI to *Hin*dIII fragment initially found in clone T22. Adjacent to the 1.4-kb *Bam*HI to *Hin*dIII fragment was a 716-bp *Eco*RI to *Bam*HI fragment that contained the 5′ end of the α gene (Fig. 1). The 716-bp fragment was nick-translated and used to probe for M13 subclones of the TA22 fragment for sequencing.

Hybridization studies were used to determine the number of α genes present in the mitochondrial genome, and to verify that the genes were derived from the mitochondrial and not the chloroplast genome. A 716-bp *Eco*RI to *Bam*HI fragment from the coding region of the TA22 clone was labeled by nick-translation and used to probe Southern blots of either *Xho*I (Fig. 2), *Bam*HI, or *Eco*RI (data not shown) digested maize mtDNA. In each digest, a single hybridization signal was observed in the *cms-T* and *cms-C* cytoplasms. This indicates that the maize mitochondrial α gene is a single copy gene in these two cytoplasms. In the *Xho*I digest, two hybridization signals were observed in the *cms-S* and normal cytoplasm lanes (Fig. 2), suggesting the possibility that two copies of the α gene are present in these cytoplasms. Longer exposures of this hybridization showed weak signals in the chloroplast lane. These weak signals are the same size as the intense mitochondrial signal in normal cytoplasm and are probably due to mtDNA contamination in the chloroplast preparation, rather than cross-hybridization to the CF_1-α gene of the chloroplast genome. The chloroplast DNA was prepared from tissue containing normal mtDNA.

Analysis of the F_1-α Gene. The coding region of the F_1-ATPase α gene has been determined by aligning its predicted amino acid sequence with corresponding genes from tobacco chloroplast and *E. coli* (Fig. 4). Two possible methionine initiation codons are found at positions 1 and 52 in Figure 3. An UAA stop codon at position-6 eliminates the possibility of any other 5′ initiation sites. The first methionine codon (position 1, Figs. 3 and 4) has been tentatively designated as the initiation codon based on the homology among α genes that is observed upstream of the second possible initiation codon. Substantial homology is found between the amino termini of the *E. coli* F_1-α and tobacco chloroplast CF_1-α, and the area between the two possible initiation codons of the maize mitochondrial α gene (Fig. 4). A glutamic acid residue, position 9, and an arginine residue, position 17, are common to the three genes (Fig. 4). A leucine residue, position 13, is found in the *E. coli* and mitochondrial α genes, and the tobacco chloroplast and mitochondrial α genes share arginine and alanine residues (positions 6 and 7, respectively, Fig. 4). If we allow for the conservative replacement of amino acids in this region (Ile = Leu; Ser = Thr), the *E. coli* and mitochondrial α genes contain 6 out of 16 matches, and the tobacco chloroplast and maize mitochondrial α genes share 9 out of 16 equivalent residues (positions 1–17, Fig. 4). Moreover, if the first methionine codon is the initiation codon, the predicted molecular mass of the maize F_1-α polypeptide is within 43 D of the predicted *E. coli* F_1-α subunit, and within 258 D of the CF_1-α subunit of tobacco chloroplast. Alternatively, if the second methionine codon is the initiation codon, the difference between the maize α and the *E. coli* and tobacco chloroplast α polypeptides would increase by 2096 D.

The carboxy end of the F_1-α gene is predicted by a UGA stop codon (position 1524, Fig. 3). Recently, it has been reported that UGA is a stop codon in the apocytochrome *b* gene of *Oenothera* mitochondria (23). This is in contrast to mammal and fungal mitochondrial codes where UGA codes for tryptophan. The corresponding α genes from *E. coli* and tobacco chloroplast each terminate within nine amino acid residues of the maize gene (Fig. 4).

Based on the proposed initiation and termination sites, the maize mitochondrial F_1-α gene contains 508 codons, and encodes for a protein with a predicted molecular mass of 55,187 D. This is in reasonable agreement with the 58-kD previously estimated by SDS-gel electrophoresis (10). The DNA homologies between the maize mitochondrial α gene and the tobacco chloroplast CF_1 and *E. coli* F_1 α genes are 55 and 51%, respectively. Homologies at the amino acid level are 54% between the maize mitochondrial and tobacco chloroplast α genes and 51% for the maize and *E. coli* α genes. Large, highly conserved regions are observed in the middle of the different α genes, while the terminal regions are much less conserved. The maize F_1-α gene does not appear to contain an intron, although the possibility of an intron in the poorly conserved 5′ and 3′ termini cannot be excluded.

Although the maize F_1-α gene encodes for a large protein, the

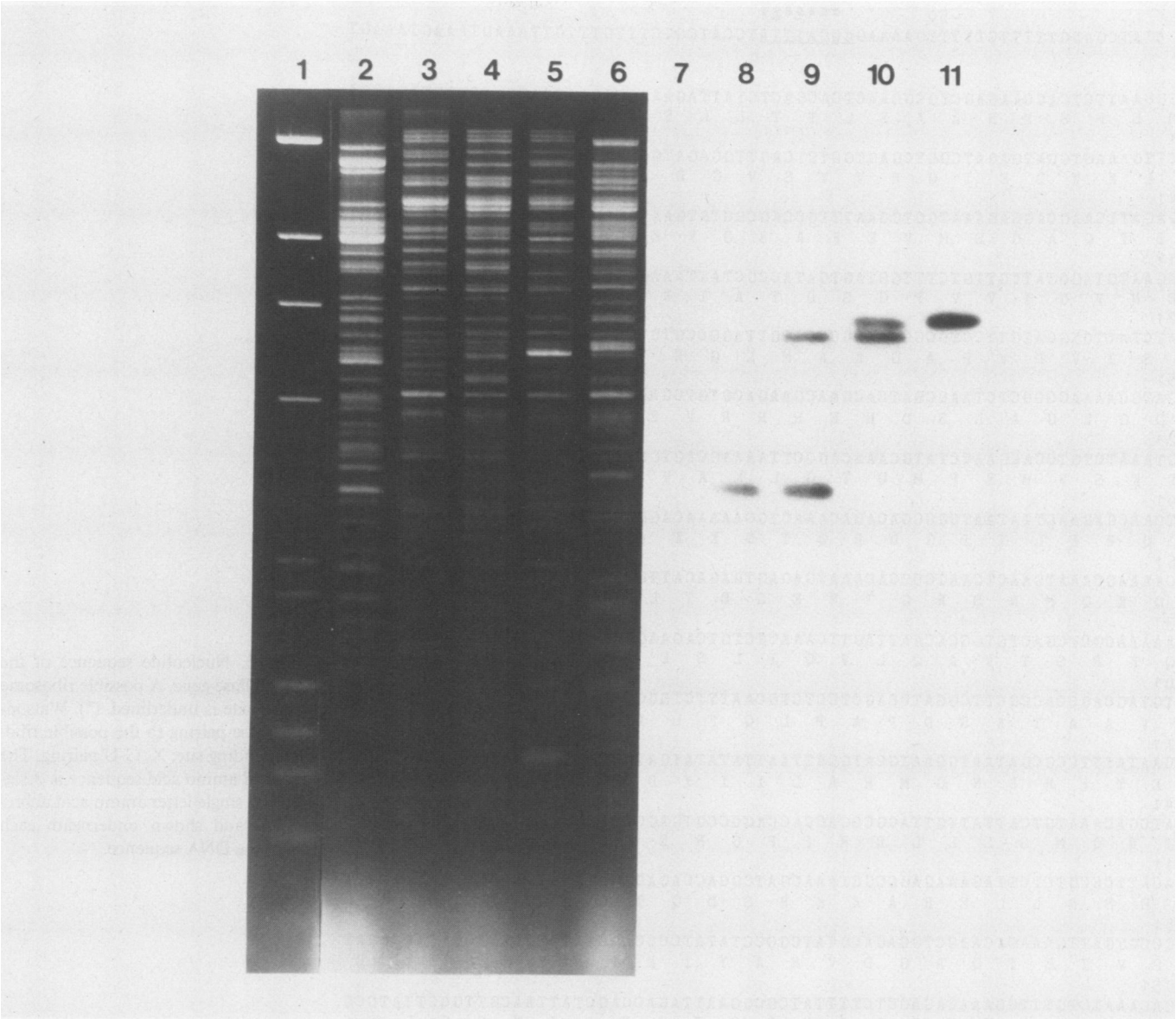

FIG. 2. *Xho*I restriction digests of maize chloroplast DNA (lane 2), and the different maize mitochondrial DNAs: lane 3, *cms-C*; lane 4, normal; lane 5, *cms-S*; and lane 6, *cms-T*. *HindIII digested* λ, *and Hae*III digested φX 174 were used for DNA size markers (lane 1). The autoradiogram of the transferred digest (lanes 7–11), was probed with the nick-translated 716-bp *Eco*RI to *Bam*HI fragment from the α gene. Lane 7, chloroplast DNA; lane 8, *cms-C* mtDNA; lane 9, normal mtDNA; lane 10, *cms-S* mtDNA; and lane 11, *cms-T* mtDNA.

codon usage figure (Fig. 5) shows that three codons are not used. Of the 22 proline residues present in the α gene, none are coded by the CCG codon. In other maize mitochondrial genes, ATPase subunit 9 (5), Cyt oxidase subunit II (7), ATPase subunit 6 (R. Dewey, personal communication), and apocytochrome *b* (3), the CCG proline codon is used approximately 20% of the time. The maize mitochondrial F_1-α gene does not encode a tryptophan codon (UGG and CGG codons). The CF_1-α subunit of tobacco chloroplast also does not contain a tryptophan codon, while *E. coli* F_1-α has only one tryptophan codon, located at the carboxy terminus (Fig. 4).

Among maize mitochondrial genes, including the α subunit, there is a significant bias favoring thymine and adenine residues in the third codon position (3, 5, 7). These two residues together account for approximately 66% of the third position residues. In the α gene, adenine residues predominated (34%) over thymine residues (31%) in the third position. This is in contrast to other maize mitochondrial genes that have a preference for thymine residues (38%) over adenine residues (27%) in the third position.

Analysis of the 5′ and 3′ flanking regions and the area surrounding the proposed initiator codon reveals two interesting sequences. First, it has been proposed that an octanucleotide segment from the 3′ end of the mitochondrial 18S rRNA may act as a mRNA binding site in an analogous fashion to the prokaryotic Shine and Dalgarno sequence (3, 25). In several maize mitochondrial genes, up to 5 out of the 8 residues are capable of base pairing. This octanucleotide segment is normally encountered 11 to 23 bp from the initiator site. Similar homology is not found in the corresponding 5′ flanking region of the F_1-α gene; however, an excellent match (6 of 8, or 7 of 8 with G-U pairing) is detected further upstream to the α initiation site (positions −42 to −35, Fig. 3). Second, the sequence surrounding the initiator codon agrees with the eucaryotic cytosolic message consensus Pu (A > G)NN*AUG*Pu (G > A) (Pu = Purine, N = any residue, initiation codon underlined) (12). Although other maize mitochondrial genes contain purine residues in the −3

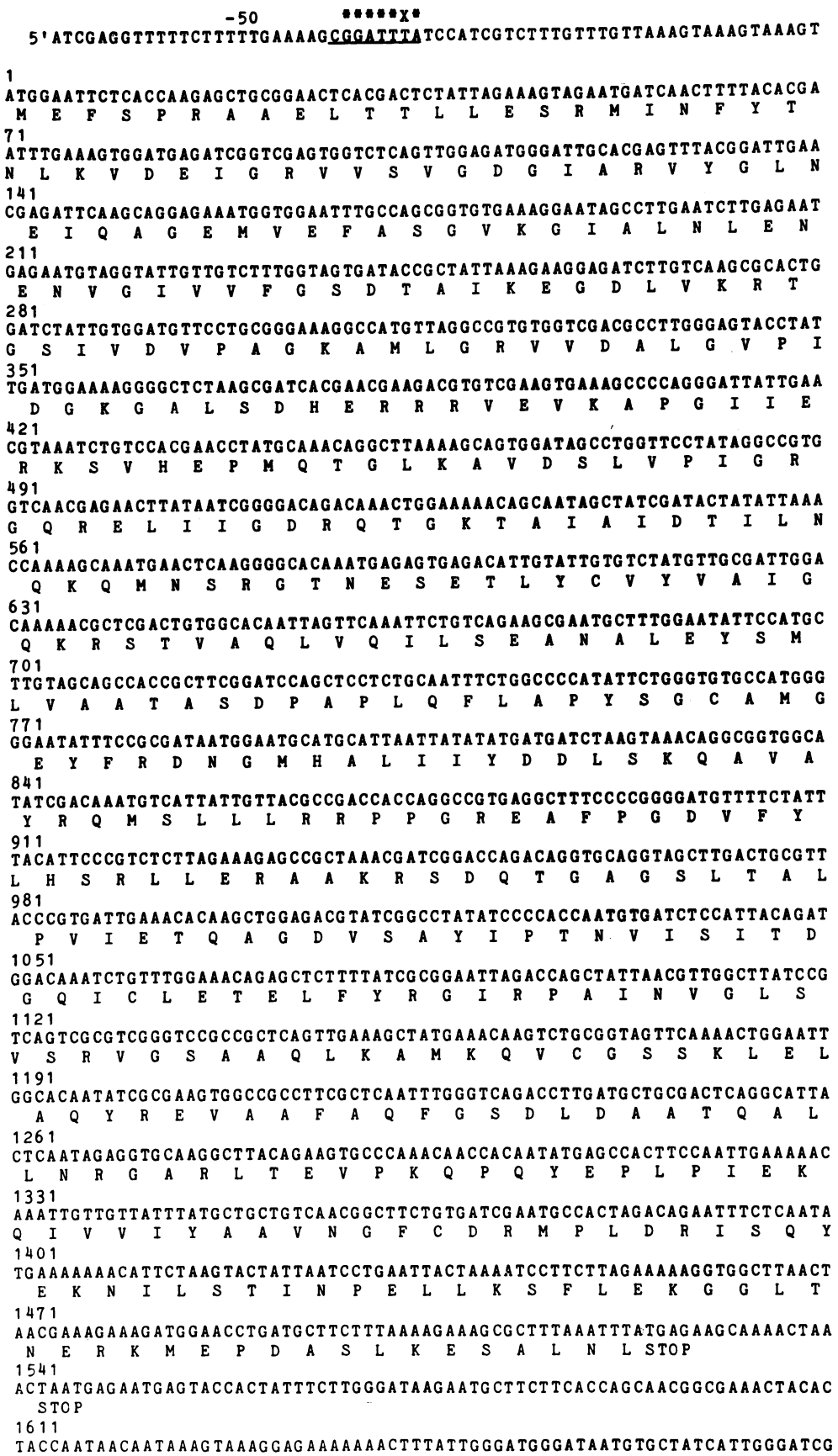

FIG. 3. Nucleotide sequence of the F_1-α ATPase gene. A possible ribosome binding site is underlined. (*), Watson-Crick base pairing to the possible ribosome binding site; X, G-U pairing. The predicted amino acid sequence is designated by single letter amino acid abbreviations and shown underneath each line of the DNA sequence.

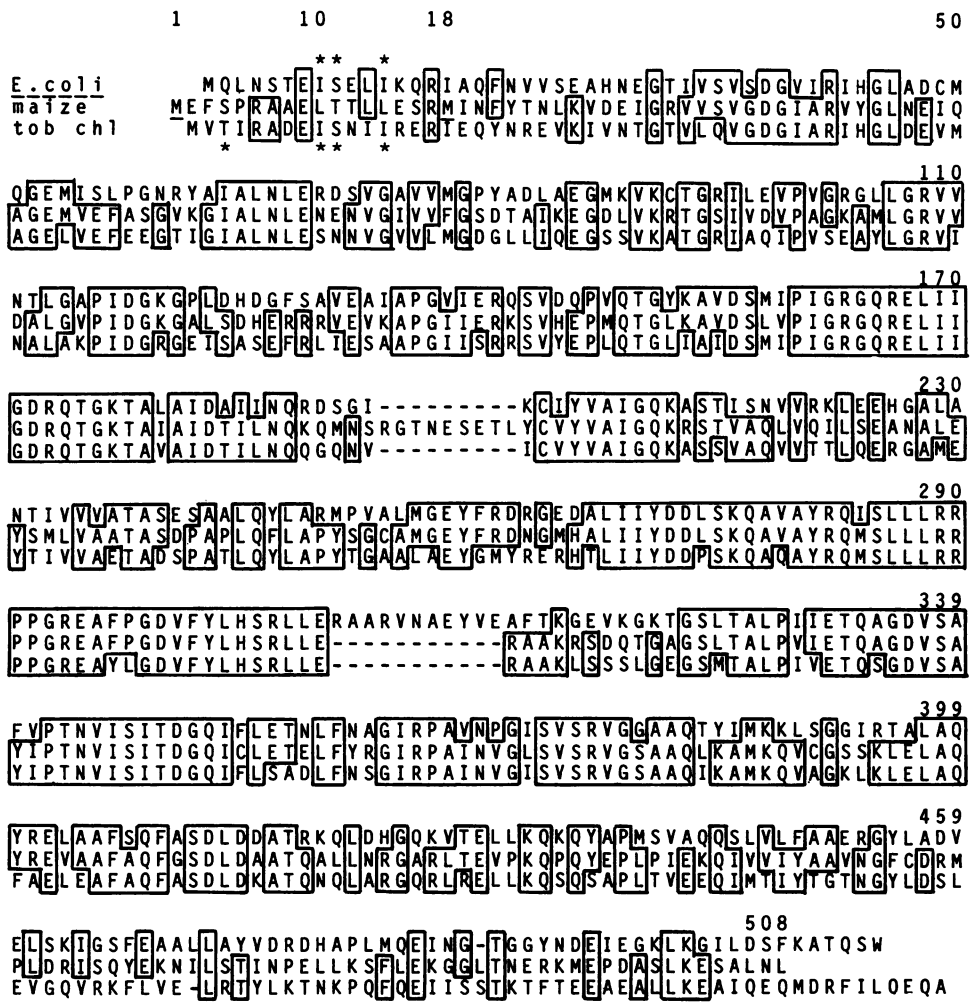

FIG. 4. Comparison of the predicted amino acid sequence of ATPase α genes from *E. coli* (top line), maize mitochondria (second line), and tobacco chloroplast (third line). Conserved regions in reference to maize are boxed. (*), Matches between amino acids allowing for conservative replacement (Ser = Thr; Ile = Leu). Conservative replacement is only displayed between the first two underlined methionine residues.

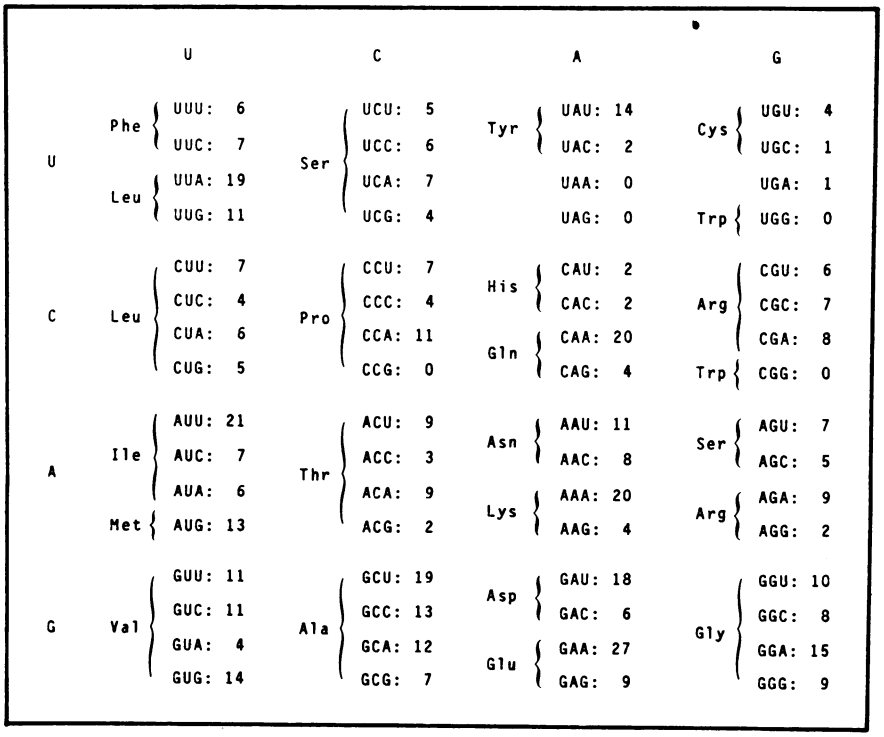

FIG. 5. Codon usage for the ATPase F_1-α gene.

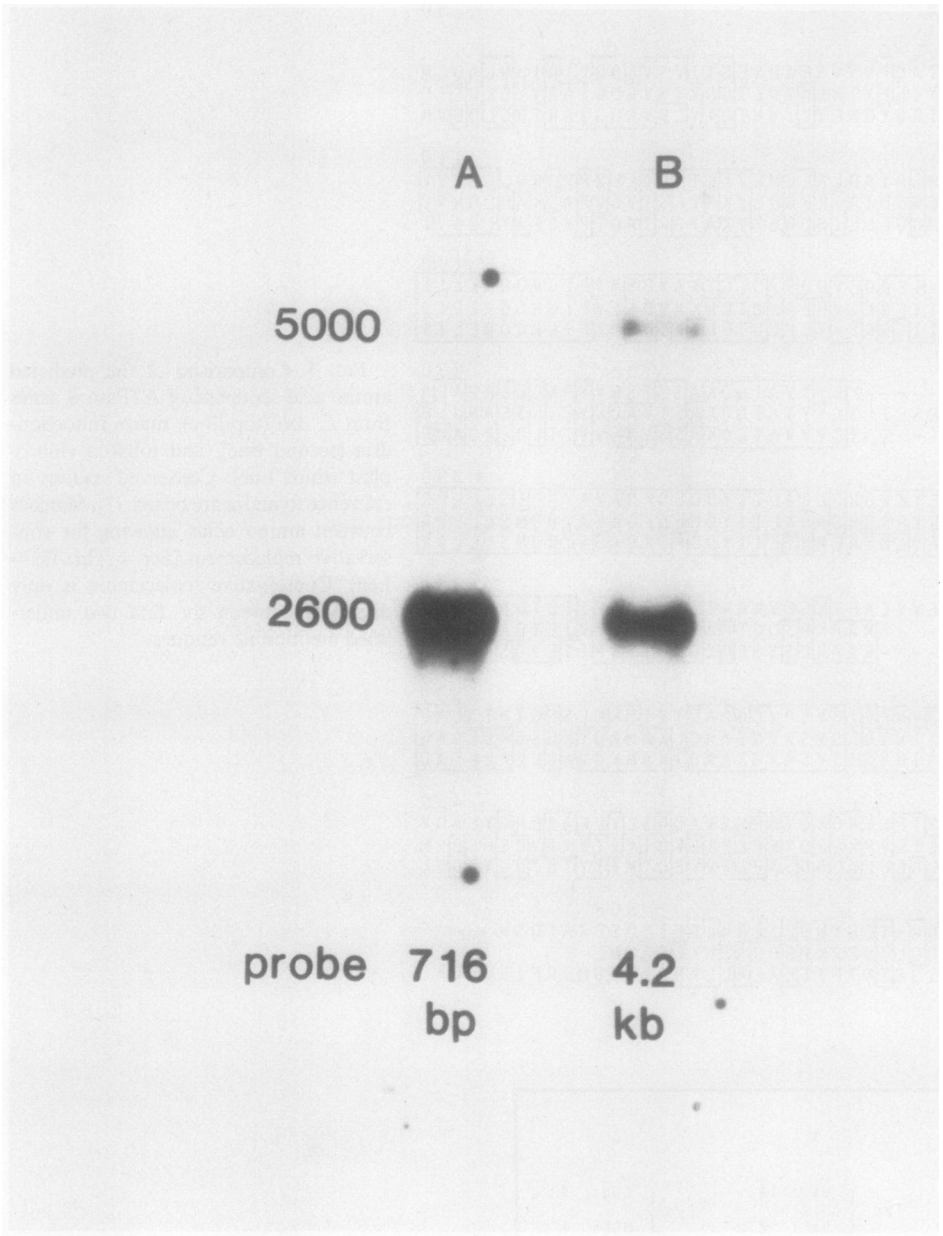

FIG. 6. RNA blot analysis of the ATPase F_1-α gene. Duplicate mtRNA filters were probed with: A, an internal *Bam*HI to *Eco*RI, 716-base pair fragment; and B, the 4.2-kb TA22 clone. Approximate RNA sizes are expressed in nucleotides.

and +1 positions, only the α gene has both of the preferred purine residues.

Transcriptional Analysis of the α Gene. To determine the transcriptional pattern of the F_1-α gene, mtRNA blots were probed with two nick-translated DNA fragments. The first probe contains a 716-bp *Eco*RI to *Bam*HI fragment located entirely within the coding region (Fig. 6, lane 1). The second probe is the 4.2-kb *Hin*dIII clone that contains approximately 2 kb of 5′ flank, 600 base pairs of 3′ flank, and the entire coding region (Fig. 6, lane 2). Both fragments hybridize to two major RNA bands of approximately 5000 and 2600 nucleotides (Fig. 6). Each probe hybridizes intensely to the smaller 2600 band, which may be the mature transcript. The larger 5000 nucleotide band may be the primary transcript. Hybridization to the larger 5000 nucleotide band is stronger with the 4.2-kb *Hin*dIII fragment than with the 716-bp fragment. The α transcriptional pattern appears simpler than other mitochondrial messages. For example, analysis of the ATPase 9 (5) and ATPase 6 (R. Dewey, personal communication) genes reveals complex transcriptional patterns; up to eight bands are observed in the ATPase 6 transcription pattern, and 5 bands are seen in the ATPase 9 pattern.

DISCUSSION

The α subunit of the F_1-ATPase complex of maize (10) and broad bean (1) is a mitochondrial translational product and thus is presumably encoded by the mitochondrial genome. We have identified by nucleotide and amino acid homology a mitochondrial gene, designated *atp*α, that appears to code for the F_1-ATPase α subunit of maize. The gene is actively transcribed and occurs as a single copy in the mitochondrial genome of *cms-T* maize. The maize subunit contains substantial amino acid homology with the α subunits of *E. coli* and tobacco chloroplast. This high level of conservation among corresponding subunits of the two organelles and the prokaryote suggests that functional constraints have strongly influenced the evolution of the α genes.

The vast majority of mitochondrial polypeptides are encoded by nuclear genes, synthesized on cytosolic ribosomes, and imported into the mitochondria (16). This includes polypeptides of the matrix, inner mitochondrial membrane, inner mitochondrial space, and the outer mitochondrial membrane (16). A small number of mitochondrial polypeptides, chiefly those of the inner mitochondrial membrane, are mitochondrial gene products. Sev-

eral polypeptides seem to be exclusively encoded by mitochondrial genes; for examle, the Cyt oxidase I, II, and III, the apocytochrome *b*, and the F_0-ATPase subunit 6. In contrast, a few mitochondrial proteins are encoded by nuclear genes in some organisms and by mitochondrial genes in others. The F_1-ATPase α subunit is such a case; it is a mitochondrial gene product in higher plants but a nuclear product in fungi and animals (1, 10). Interestingly, a second case occurs with another subunit of the ATPase complex. The F_0-ATPase subunit 9 is encoded by nuclear genes in animals and certain fungi, *Aspergillus* and *Neurospora*, but by mitochondrial genes in yeast and maize (16).

Comparisons of the apocytochrome b and Cyt oxidase subunit I and II genes from maize and *Oenothera* have shown that the residue following the AUG initiation codon is most often an adenine residue (3). It has been suggested that this fourth residue may be involved in additional base pairing with the formylmethionine tRNA anticodon loop allowing for greater specificity in translational initiation (3). Sequence data from the wheat (9) and maize (18) mitochondrial formylmethionine tRNA anticodon loops show identical 5′UCAU patterns, which are capable of a four-base pair interaction with a 5′AUGA mRNA. The F_1-ATPase α subunit as well as ATPase subunits 6 (R. Dewey, personal communication) and 9 (5) genes from maize mitochondria contain either guanine (subunit 6 and α) or thymine (subunit 9) residues in the fourth position. This suggests that additional fourth base pairing between formylmethionine tRNA and the initiator codon is not essential for translational initiation.

The discovery that the F_1-ATPase α subunit is encoded by a mitochondrial gene in maize and possibly all higher plants raises a question about the origin of the gene. The endosymbiotic theory proposes that after the colonization of the protoeukaryotes had occurred, there was a selective transfer of genes from the invading genome to the host genome (15). Presumably this transfer continued even after the nucleus and mitochondria were established as unique organelles. Variation in selective conditions and the possibility of multiple colonization events could lead to differential gene transfer and to the establishment of distinct mitochondrial genomes. Differential gene transfer could account for the fact that the α gene remained a part of the mitochondrial genome in higher plants. Alternatively, the mitochondrial α gene could have originated from the chloroplast or nuclear genome. In higher plants the transfer of DNA from chloroplast to mitochondrial genomes is well documented (13, 27, 28).

Acknowledgments—We gratefully acknowledge the expert technical assistance of Jane Suddith.

LITERATURE CITED

1. Boutry M, M Briquet, A Goggeau 1983 The α subunit of a plant mitochondrial F_1-ATPase is translated in mitochondria. J Biol Chem 258: 8524–8526
2. Chou S, RR Sederoff, CS Levings III 1984 Nucleotide sequence and evolution of the 18S ribosomal RNA gene in maize mitochondria. Nucleic Acids Res 12: 6629–6644
3. Dawson AJ, VP Jones, CJ Leaver 1984 The apocytochrome b gene in maize mitochondria does not contain introns and is preceded by a potential ribosome binding site. EMBO J 3: 2107–2113
4. Deno H, K Shinozaki, M Sugiura 1983 Nucleotide sequence of tobacco chloroplast gene for the α subunit of proton-translocating ATPase. Nucleic Acids Res 11: 2185–2191
5. Dewey RE, AM Schuster, CS Levings III, DH Timothy 1985 Nucleotide sequence of F_0-ATPase proteolipid (subunit 9) gene of maize mitochondria. Proc Natl Acad Sci USA 82: 1015–1019
6. Dujon B 1983 Mitochondrial genes, mutants, and maps, a review. *In* RJ Schweyen, K Wolf, F Kaudewitz, eds, Mitochondria 1983. DeGruyter, Berlin-New York, pp 1–24
7. Fox TD, CJ Leaver 1981 The *Zea mays* mitochondrial gene coding cytochrome oxidase subunit II has an intervening sequence and does not contain TGA codons. Cell 26: 315–323
8. Gay NJ, JE Walker 1981 The atp operon: nucleotide sequences of the region encoding the α-subunit of *Escherichia coli* ATP-synthase. Nucleic Acids Res 9: 2187–2194
9. Gray MW, DF Spencer 1983 Wheat mitochondrial DNA encodes a eubacterial-like initiator methionine transfer RNA. FEBS Lett 161: 323–327
10. Hack E, CJ Leaver 1983 The α-subunit of the maize F_1-ATPase is synthesised in the mitochondrion. EMBO J 2: 1783–1789
11. Hu N, J Messing 1982 The making of strand-specific M13 probes. Gene 17: 271–277
12. Kozak M 1983 Comparison of initiation of protein synthesis in procaryotes, eucaryotes, and organelles. Microbiol Rev 47: 1–45
13. Lonsdale DM, TP Hodge, CJ Howe, DB Stern 1983 Maize mitochondrial DNA contains a sequence homologous to the ribulose-1,5-bisphosphate carboxylase large subunit gene of chloroplast DNA. Cell 34: 1007–1014
14. Maniatis T, EF Fritsch, J Sambrook 1982 Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
15. Margulis L 1970 Origin of Eukaryotic Cells. Yale University Press, New Haven, pp 45–68
16. Neupert W, G Schatz 1981 How proteins are transported into mitochondria. Trends Biochem Sci 6: 1–4
17. Norrander J, T Kempe, J Messing 1983 Construction of improved M13 vectors using oligodeoxynucleotide-directed mutagenesis. Gene 26: 101–106
18. Parks TD, WG Dougherty, CS Levings III, DH Timothy 1984 Identification of two methionine transfer RNA genes in the maize mitochondrial genome. Plant Physiol 76: 1079–1082
19. Pring DR, CS Levings III 1978 Heterogeneity of maize cytoplasmic genomes among male-sterile cytoplasms. Genetics 89: 121–136
20. Rigby PWJ, M Dieckmann, C Rhodes, P Berg 1977 Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with polymerase I. J Mol Biol 113: 237–251
21. Sanger F, S Nicklen, AR Coulson 1977 DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74: 5463–5467
22. Schuster AM, PH Sisco, CS Levings III 1983 Two unique RNAS in *cms*-S and RU maize mitochondria. Plant Mol Biol 12: 437–444
23. Schuster W, A Brennicke 1985 TGA-termination codon in the apocytochrome b gene from *Oenothera* mitochondria. Curr Genet 9: 157–164
24. Senior A 1979 The mitochondrial ATPase. *In* R Capaldi, ed, Membrane Proteins in Energy Transduction. Dekker, New York, pp 233–278
25. Shine J, L Dalgarno 1974 The 3′-terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. Proc Natl Acad Sci USA 71: 1342–1346
26. Spitsberg VL, NE Pfeiffer, B Partridge, DE Wylie, SM Schuster 1985 Isolation and antigenic characterization of corn mitochondrial F_1-ATPase. Plant Physiol 77: 339–345
27. Stern DB, JD Palmer 1984 Extensive and widespread homologies between mitochondrial DNA and chloroplast DNA in plants. Proc Natl Acad Sci USA 81: 1946–1950
28. Stern DB, DM Lonsdale 1982 Mitochondrial and chloroplast genomes of maize have a 12-kilobase DNA sequence in common. Nature 299: 698–702
29. Thomas PS 1980 Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc Natl Acad Sci USA 77: 5201–5205
30. Wahl GM, M Stern, GR Stark 1979 Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. Proc Natl Acad Sci USA 76: 3683–3687
31. Yaffe M, G Schatz 1984 The future of mitochondrial research. Trends Biochem Sci 9: 179–181