Cell, Vol. 44, 439–449, February 14, 1986, Copyright © 1986 by Cell Press

Novel Recombinations in the Maize Mitochondrial Genome Produce a Unique Transcriptional Unit in the Texas Male-Sterile Cytoplasm

R. E. Dewey,* C. S. Levings, III,† and D. H. Timothy*
* Department of Crop Science
† Department of Genetics
North Carolina State University
Raleigh, North Carolina 27695

Summary

We have characterized a 3547 bp DNA fragment from male-sterile (*cms-T*) maize mitochondria, designated TURF 2H3, selected because of its unique and abundant transcripts. Sequence analysis indicated that TURF 2H3 originated by recombinations among portions of the flanking and/or coding regions of the maize mitochondrial 26S ribosomal gene, the ATPase subunit 6 gene, and the chloroplast tRNA-Arg gene. TURF 2H3 contains two long open reading frames that could encode polypeptides of 12,961 Mr and 24,675 Mr. The larger open reading frame hybridizes to transcripts in all maize cytoplasms, the smaller to transcripts only in T cytoplasm. TURF 2H3 transcripts appear to be uniquely altered in *cms-T* plants restored to fertility by the nuclear restorer genes *Rf1* and *Rf2*. A possible relationship between TURF 2H3, nuclear restorer genes, and the male sterility trait in T cytoplasm is suggested.

Introduction

Mitochondrial genomes of higher plants are larger and more complex than those of other eukaryotic organisms (Levings, 1983a). The complexity is due in part to intramolecular recombinational events in which populations of subgenomic circles are generated from a master genomic circle (Palmer and Shields, 1984; Lonsdale et al., 1984). Large repeated sequences often appear to be the sites of intramolecular recombination (Lonsdale et al., 1984). Intergenomic rearrangements involving the exchange of DNA between the chloroplast, nuclear, and mitochondrial genomes of higher plants have also been reported (Stern and Lonsdale, 1982; Kemble et al., 1983; Stern and Palmer, 1984).

The mitochondrial genome of Zea mays from normal (male-fertile) cytoplasm is estimated to be 570 kilobases (kb) in length by cosmid mapping (Lonsdale et al., 1984). Restriction endonuclease digestion analysis of the mitochondrial DNA (mtDNA) from normal and mutant cytoplasms of maize, however, has revealed considerable heterogeneity among the different cytoplasms (Pring and Levings, 1978). Restriction analysis of various members of the genus Zea shows that recombination is the major force accounting for mtDNA heterogeneity among the species (Sederoff et al., 1981).

Abundant evidence indicates that the mitochondrial genome contains the genetic information responsible for the cytoplasmic male-sterile (cms) trait (see review, Levings, 1983b). Soon after tassel emergence, male-fertile (normal) maize plants exert anthers and shed viable pollen. In contrast, plants containing male-sterile cytoplasms do not exert anthers and viable pollen is not produced. The cms trait has been used extensively in hybrid seed production to avoid the costly procedure of hand emasculation. The three major types of maize male-sterile cytoplasms, *cms-T*, *cms-S*, and *cms-C*, are distinguished on the basis of the specific nuclear genes that restore pollen fertility. The Texas male-sterile cytoplasm (*cms-T*) is unique in that an inseparable association appears to exist between disease susceptibility to the fungal pathogen Bipolaris maydis (Helminthosporium maydis), race T, and male sterility in this cytoplasm (Hooker et al., 1970; Gengenbach et al., 1977; Brettell et al., 1979). *Cms-T* was widely used in commercial hybrid maize production before severe disease outbreaks in 1970 forced the industry to curtail its use.

We have isolated and characterized a fragment of *cms-T* mtDNA from maize, designated TURF 2H3, that presumably arose through a series of novel recombinations within and between the mitochondrial and chloroplast genomes. The DNA organization of TURF 2H3 is unique to T cytoplasm and part of the fragment is uniquely transcribed in T. The sequence contains two open reading frames that could encode proteins of 12,961 Mr and 24,675 Mr. Our evidence indicates that the TURF 2H3 sequence is a part of a 6.6 kb Xho I fragment whose alteration is associated with reversion to male fertility (Umbeck and Gengenbach, 1983). Furthermore, the processing of the TURF 2H3 message appears to differ in the presence of nuclear genes that restore *cms-T* to male fertility. We suggest that the TURF 2H3 sequence may be associated with the T type of male sterility because of the chimeric and unstable nature of the sequence, the novel and abundant transcripts, the open reading frames, and the apparent differential processing of its transcript by nuclear restorer genes.

Results

Identification of a Highly Transcribed mtDNA Clone in *cms-T*

End-labeled mtRNA (*cms-T*) was hybridized to a Bam HI maize mtDNA library from T cytoplasm to identify mtDNA clones containing sequences actively participating in mitochondrial transcription. Also, duplicate filters of the same library were hybridized to end-labeled mtRNA from normal cytoplasm. A 9.0 kb Bam HI fragment, designated TURF 2B, was identified that hybridized more intensely to mtRNA from T cytoplasm than to mtRNA from normal. Hybridization of end-labeled mtRNA (*cms-T*) to restriction digests of TURF 2B revealed that significant hybridization was confined to three Hind III fragments within the 9.0 kb TURF 2B clone. The Hind III fragments were found to be in consecutive alignment (Figure 1) and the complete

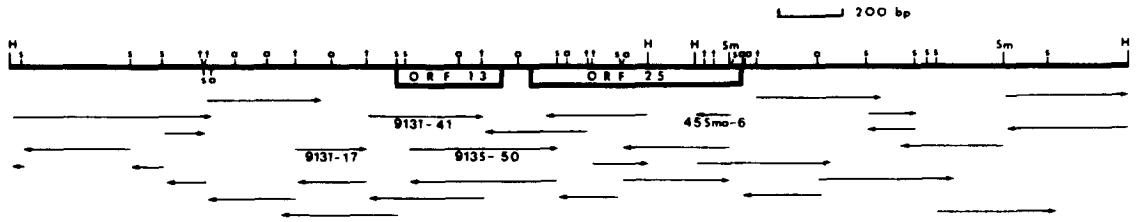

Figure 1. Restriction Map of TURF 2H3

Open reading frames are indicated by boxed regions. Arrows below the map show the direction and extent of sequence analysis from each restriction site. Clones cited in the text are designated with numbers. Restriction sites are indicated by vertical lines: a, Alu I; H, Hind III; s, Sau 3A; Sm, Sma I; t, Taq I.

```
     TURF  2H3       1   5'-AAGCTTTAGG TAGTTCGCCG TGCGATGGGC CTGAGGATCT ATTCCAAAGG GTGATGGGTC GTGGGTACGA
                             ********** ********** ********** ********** ********** ********** **********
     atp 6       -1589   5'-AAGCTTTAGG TAGTTCGCCG TGCGATGGGC CTGAGGATCT ATTCCAAAGG GTGATGGGTC GTGGGTACGA

   71 CTCTTCAGAA GGCGCTTTTG CCAACCACCG AGCACCAACA AATGAAGAGA GTTACCACAA GTATGAGACC ACTGAAACTA CTTCGCCTGC
      ********** ********** ********** ********** ********** ********** ********** ********** **********
-1519 CTCTTCAGAA GGCGCTTTTG CCAACCACCG AGCACCAACA AATGAAGAGA GTTACCACAA GTATGAGACC ACTGAAACTA CTTCGCCTGC

  161 TTTGAATCAG AGCAAACTGC TTTTCAGTAT AAGACCACCA ATCTAGTACG CAACCATTGC CTATTATGCC ATCCATATTG TTGACTTTCA
      ********** ********** ********** ********** ********** ********** ********** ********** **********
-1429 TTTGAATCAG AGCAAACTGC TTTTCAGTAT AAGACCACCA ATCTAGTACG CAACCATTGC CTATTATGCC ATCCATATTG TTGACTTTCA

  251 CAGACCCACT ATGACTGCCT ACGCCCATAT AGATAGAGAA AAGTTCACCT AAGGATAATA AAACGTTTGA GGAAGGTTAC CGCTTACGAT
      ********** ********** ********** ********** ********** ********** ********** ********** **********
-1339 CAGACCCACT ATGACTGCCT ACGCCCATAT AGATAGAGAA AAGTTCACCT AAGGATAATA AAACGTTTGA GGAAGGTTAC CGCTTACGAT

  341 ATACCTATAT ATAGATAGTG TAGTGTTGTA GTGTGTTAAG TAGATCGGTC CCATATAAAG TTGCAATGTA TCTGCAATGA GTTCATCTAC
      ********** ********** ********** ********** ********** ********** ********** ********** **********
-1249 ATACCTATAT ATAGATAGTG TAGTGTTGTA GTGTGTTAAG TAGATCGGTC CCATATAAAG TTGCAATGTA TCTGCAATGA GTTCATCTAC

  431 TCCGGGGTGT TCTCCGCACT TCTCAAGAAA GAAATAGTTG GAAATTCCCC GGATCGTCTG CGCTTGGCTT GGTATTCTTA TACTGAATCG
      ********** ********** ********** ********** ********** ********** ********** ********** **********
-1159 TCCGGGGTGT TCTCCGCACT TCTCAAGAAA GAAATAGTTG GAAATTCCCC GGATCGTCTG CGCTTGGCTT GGTATTCTTA TACTGAATCG

  521 CTGTTGGATA AAAGAAAGCA TAAAGTTTTT AAGCCTGCGT TCTCGTTGGC GAAACCACTA GGAGGCAACC ATGAAAGCGG TTTCTATGTT
      ********** ********** ********** ********** ********** ********** ********** ********** **********
-1069 CTGTTGGATA AAAGAAAGCA TAAAGTTTTT AAGCCTGCGT TCTCGTTGGC GAAACCACTA GGAGGCAACC ATGAAAGCGG TTTCTATGTT

  611 TCGATCGAAC CAACACAGCT TTACGCTTCT TTCCAACTCT ACCCACGCTT CGGGTTCGCA CTTACTTTAC GCCTTACCGA ACTTGGGCTT
      ********** ********** ********** ********** ********** ********** ********** ********** **********
 -979 TCGATCGAAC CAACACAGCT TTACGCTTCT TTCCAACTCT ACCCACGCTT CGGGTTCGCA CTTACTTTAC GCCTTACCGA ACTTGGGCTT

  701 AGTTCAGCCA GCTTCGTAAC ATGCCTGCTC AAAGTGAGTA GTTGGAAGTT CCTGTAAGCA TCTTCCCATG AATTGACGTA TATAAGCGTA
      ********** ********** ********** ********** ********** ********** ********** ********** **********
 -889 AGTTCAGCCA GCTTCGTAAC ATGCCTGCTC AAAGTGAGTA GTTGGAAGTT CCTGTAAGCA TCTTCCCATG AATTGACGTA TATAAGCGTA

  791 GTTATCAGGT ACGCCGTGAG CTGGATTCTA TCCCCGAGGA TAGAGATTGA TGGGTAATAG TTTTGATAGA AAACTCGTAT GTTAAGTTCT
      ********** ********** ********** ********** ********** ********** ********** ********** **********
 -799 GTTATCAGGT ACGCCGTGAG CTGGATTCTA TCCCCGAGGA TAGAGATTGA TGGGTAATAG TTTTGATAGA AAACTCGTAT GTTAAGTTCT

  881 CTAGTTGATG TGATGCGGCT CGATTCATCA TTGCTCTCAC ATCAATGTTT TCGGTTCCCT TTTTACTCTC CCATTTTCAT AGAGAAAGAT
      ********** ********** ********** ********** ********** ********** ********** ********** **********
 -709 CTAGTTGATG TGATGCGGCT CGATTCATCA TTGCTCTCAC ATCAATGTTT TCGGTTCCCT TTTTACTCTC CCATTTTCAT AGAGAAAGAT

  971 GTTCTGATTC AGTTCTCTCT GAAAAGGAAG ACGGGGCCCT TAGCTTAGGG ACACAGTAGT ACCATTTCCA TTGTGCGAAA GGTCGTGTCC
      ********** ********** ********** ********** ********** ********** ********** ********** **********
 -619 GTTCTGATTC AGTTCTCTCT GAAAAGGAAG ACGGGGCCCT TAGCTTAGGG ACACAGTAGT ACCATTTCCA TTGTGCGAAA GGTCGTGTCC

 1061 TGGTAGCCTC TCTGCTGTAG ACGGTCTCAT TGGTAAATTC TTCCATTTCT GAAATTGTCA AATCGAGATT GTGTGGGTGT TCAGTTCATG
      ********** ********** ********** ********** ********** ********** ********** ********** *****
 -529 TGGTAGCCTC TCTGCTGTAG ACGGTCTCAT TGGTAAATTC TTCCATTTCT GAAATTGTCA AATCGAGATT GTGTGGGTGT TCAGT

                                                                                    ORF 13  met ile thr thr phe leu asn
 1151 AAATGGGTGA AGTCTCTTTC AAGAAAAGAG CAGAG-GGAG CGGAAAGGAA AACACTTTTT CGTCA ATG ATC ACT ACT TTC TTA AAC
      ********** ** ******* * *** ** ******* ** ******* ***** *** *** ** *** *** *** ***
      26S   4170 AGTCTCTTTC AACAAAAGAG CGGAGCGG-- ---AAAGGAA AAAACTTTTT CGTCA ATG ATC ACG ACT TTC TTA AAC

      leu pro pro phe asp gln gly leu val phe phe gly ser ile phe ile phe phe leu cys ile leu leu ile lys
 1236 CTT CCT CCC TTT GAT CAA GGT TTG GTA TTT TTC GGT TCT ATT TTT ATT TTT TTT TTG TGC ATA TTA TTG ATA AAG
      *** *** *** *** ***  ** *** *** *** *** *** *** *** *** *** *  **  ** **  * *  **        *  **  **
 4241 CTT CCT CCC TTT GAT AAA GGT TTG GTA TTT TTC GGT TCT ATT TTT GTG TTC ATT TTT TTC ATT AGG CAG ATT AAA

      gly tyr leu arg lys met asp asp ser tyr leu ala gln leu ser glu leu ala asn his asn arg val glu ala
 1311 GGA TAT CTC CGT AAA ATG GAT GAT TCC TAT TTG GCT CAA CTC TCC GAG TTA GCC AAC CAC AAT AGA GTG GAA GCG
      *** *** **   **  **  **  **  **    * *** *** **  *** **  **  *** **  *** *** *** *** *** *** *** ***
 4316 GGA TAT CTA AGT AAG ATA GAC GAG AGC TAT TTG ACT CAA CTA TCT GAG TTT GCC AAC CAC AAT AGA GTG GAA GCG

      ala lys ala gly his val ala leu his glu leu ser phe ser       trp leu arg gly val gln ile arg val
 1386 GCA AAA GCG GGC CAC GTG GCC CTG CAT GAG CTA TCC TTC TCG ----- TGG TTG AGG GGG GTT CAA ATT AGG GTG
      *** *** *** *** *** *** *** *** *** *** *** *** ** **       *** *** *** *** ***  ** **  *** ***
 4391 GCA AAA GCG GGC CAC GTG GCC CTG CAT GAG CTA TCC TTC TCA TCTCA TGG TTG AGG GGG GTT AAA ATG AGG GTG
```

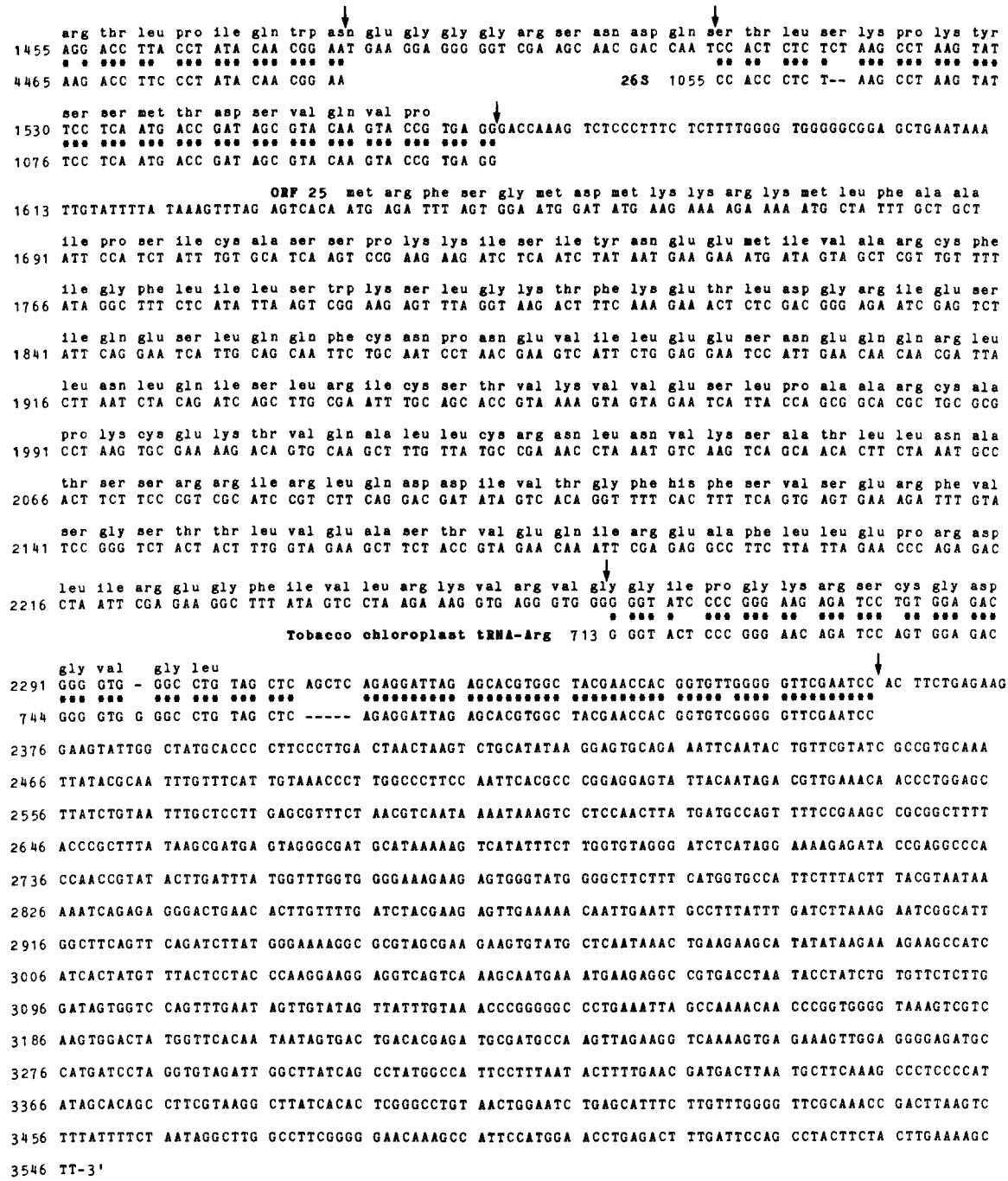

Figure 2. Nucleotide Sequence of TURF 2H3

Sequences homologous with portions of *atp* 6, 26S ribosomal gene, and tobacco chloroplast tRNA-Arg are indicated by an asterisk. Sequence numbers of *atp* 6 are in relation to the *atp* 6 putative start codon (Dewey et al., 1985b). Sequence numbers of the 26S ribosomal and tobacco tRNA-Arg genes are as published (Dale et al., 1984; Kato et al., 1985). Points of recombination are designated with arrows. The predicted amino acid sequences of reading frames ORF 13 and ORF 25 are translated according to the mitochondrial genetic code of higher plants (Fox and Leaver, 1981), beginning at the first ATG codon.

nucleotide sequence of 3547 base pairs (bp) was determined. The 3547 bp region of TURF 2B was designated TURF 2H3 (Figure 2).

Analysis of TURF 2H3 Nucleotide Sequence

Significant sequence homology was observed between the 5′ end of TURF 2H3 and the 5′ flanking region of the maize ATPase subunit 6 gene, *atp* 6 (Figure 2). The first 1145 bp of TURF 2H3 are identical with the 5′ flanking DNA sequence of *atp* 6 extending from positions −1589 to −445 with respect to ATPase subunit 6 initiator ATG codon (Dewey et al., 1985b). Hybridization to Southern blots of maize mtDNA with clones specific to this region confirms that these sequences are repeated in the

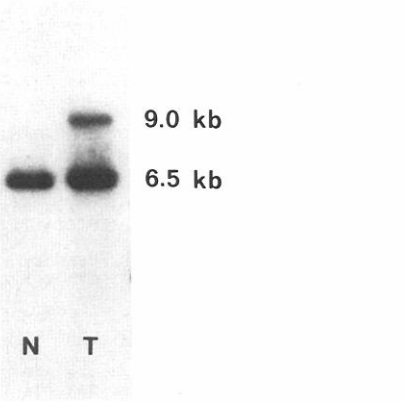

Figure 3. Hybridization of Clone 913T-17 to Bam HI Restriction Digests of Normal and *cms-T* Maize mtDNA.

Clone 913T-17 (Figure 1) is interior to the repeat common to TURF 2H3 and the 5′ flank of *atp* 6. N, normal; T, *cms-T*.

mitochondrial genome in *cms-T*. In Bam HI digests of T mtDNA, both the 9.0 kb TURF 2B fragment and the 6.5 kb fragment that contains *atp* 6 (Dewey et al., 1985b) hybridize strongly to probes interior to the repeat, whereas in digests of normal only the *atp* 6 fragment is detected (Figure 3). Similarly, *cms-S* and *cms-C* genomes contain only one copy of this DNA segment (data not shown). The extent of the repeat is unknown beyond the sequenced region in *cms-T*.

The nucleotide sequence of TURF 2H3 was compared with the National Institutes of Health GenBank and European Molecular Biology Laboratories DNA libraries using the computer programs provided by Bionet. Three segments of TURF 2H3 contain significant homology with sequences of the maize mitochondrial 26S ribosomal gene or its flanking regions (Dale et al., 1984). The first homologous segment occurs at the end of the long repeat common with *atp* 6. Twenty-nine of 30 bp are identical between positions 1116 to 1145 of TURF 2H3 and a DNA segment in the 5′ flanking region of the 26S ribosomal gene (positions 450–479) (Figure 4). The homology extends past the repeat when the comparison is made only between the 5′ flanking sequences of *atp* 6 and the 26S gene. An additional 11 bp identity is observed between the *atp* 6 and 26S gene 5′ flanking regions, interrupted only by a 5 bp duplication of the sequence TCTAC in *atp* 6 (Figure 4).

The second region of homology between TURF 2H3 and the 26S gene occurs from positions 1161 to 1477 of TURF 2H3 and a segment of the 3′ flanking region of the 26S gene extending from positions 4170 to 4487 (Figure 2). These segments share a sequence homology of 85%. Five mismatches in this region result from a 5 bp duplication of the sequence TCTCA in the 26S 3′ flanking sequence (position 4433).

The third region of homology with the 26S gene is found between positions 1507 and 1564 of TURF 2H3 and positions 1055 and 1110 of the 26S gene (Figure 2). In contrast with the prior homologous sequences, this homologous region is located in the coding sequence of the 26S ribosomal gene. Ninety-five percent homology is observed between these segments, including a continuous stretch of 47 bp with perfect identity. This portion of the 26S gene is also highly conserved among diverse species (Dale et al., 1984). TURF 2H3 matches 44 of 53 bp (83%) with the corresponding E. coli sequence in this region (data not shown).

Computer searches of the N.I.H. GenBank and E.M.B.L. gene libraries also revealed significant homologies between a fragment of TURF 2H3 and the chloroplast tRNA-Arg genes of tobacco, Spirodela oligorhiza, and Euglena gracilis (Kato et al., 1985; Keus et al., 1984; Orozco and Hallick, 1982). The homology extends from positions 713 to 812 of tobacco, corresponding with positions 2260 to 2363 of the TURF 2H3 sequence (Figure 2). This region of TURF 2H3 is homologous with 37 bp of the tobacco tRNA-Arg 5′ flank, along with all of the coding region except the last 10 bp. A homology of 90% is detected between these segments of DNA. Five mismatches in this region result from a short duplication of the sequence AGCTC in TURF 2H3 at position 2309. The homologies between the S. oligorhiza and E. gracilis tRNA-Arg genes and TURF 2H3 are 89% and 83%, respectively. However, no homology is found in the 5′ flanking sequence of the E. gracilis gene as in the tobacco and S. oligorhiza genes. The homology at the 3′ end with the tobacco, S. oligorhiza and E. gracilis genes ends in the stem of the T loop. The maize sequence is therefore unable to complete pairing in the T stem and the aminoacyl stem (Figure 5). The significant homology with chloroplast tRNAs from various species suggests that this portion of TURF 2H3 originated from the maize chloroplast genome. TURF 2H3 subclones containing the tRNA-like region hybridize to the same 12.7 kb Eco RI maize chloroplast fragment on which Selden et al. (1983) have mapped a tRNA-Arg (data not shown). Since the nucleotide sequence of this maize chloroplast tRNA-Arg has not been determined, it is not possible for us to compare the maize chloroplast tRNA-Arg sequence directly with the homologous sequences of TURF 2H3. Nevertheless, due to its probable chloroplast origin and inability to complete pairing, the tRNA-Arg found in TURF 2H3 must be regarded as a pseudogene.

The extensive sequence homologies between TURF 2H3 and the 5′ flank of *atp* 6, various portions of the flanking and coding regions of the 26S ribosomal gene, and a chloroplast tRNA gene indicate that several recombinational events have occurred to form this fragment. Assuming the TURF 2H3 sequence arose via recombination, a minimum of seven recombination points (positions 1146, 1160, 1478, 1506, 1565, 2259, and 2364 of Figure 2) are identified.

Transcript Analysis of TURF 2H3

When the 9.0 kb TURF 2B clone was hybridized to Northern blots of maize mtRNA from *cms-C*, normal, *cms-S*, and *cms-T*, a complex pattern was observed (Figure 6A). Hybridization to *cms-T* mtRNA, however, was much greater than to the other three cytoplasms. Hybridization of subclones of TURF 2B to Northern blots revealed that significant transcription was limited to the area of TURF 2B contained in TURF 2H3 (data not shown). Single-stranded

```
TURF 2H3   1116  TGTCAAATCGAGATTGTGTGGGTGTTCAGT  1145
                 ****************************
atp 6      -474  TGTCAAATCGAGATTGTGTGGGTGTTCAGTCTACTCTACCGCTCAT  -428
                 ******** ************************  *******
26S         450  TGTCAAATTGAGATTGTGTGGGTGTTCAGTCTAC-----CGCTCAT   490
```

Figure 4. Sequence Homologies between TURF 2H3 and 5′ Flanking Sequences of *atp* 6 and the 26S Ribosomal Gene

Sequences are numbered as described in Figure 2. A 5 bp repeat in *atp* 6 is boxed.

M13 subclones of TURF 2H3 complementary to the sense strand from the Taq I site at position 1123 to the Sma I site at position 3137 (Figure 2) demonstrated positive hybridization to the same set of transcripts in *cms-T* as the 9.0 kb TURF 2B fragment. Probes outside of this region did not significantly hybridize to mtRNA blots. M13 subclones of the noncomplementary strand throughout the entire TURF 2H3 fragment showed no detectable hybridization to Northern blots (data not shown).

To study the transcripts of TURF 2H3, individual subclones of the transcribed region were hybridized to Northern blots. When clone 913T-41 (Figure 1) was hybridized to maize mtRNA blots of all four cytoplasms, transcripts were seen only in *cms-T* mtRNA preparations (Figure 6B). Clone 913T-41 contains the DNA sequences homologous to the 3′ flanking region of the 26S ribosomal gene. When a clone that also contains the homologous sequences interior to the 26S gene (clone 913S-50, Figure 1) was used as a hybridization probe, the 26S ribosomal RNA transcript was visualized in all cytoplasms along with other minor transcripts (Figure 6C). Different transcripts appeared in mtRNA preparations of *cms-C*, normal, and *cms-S* when a more 3′ distal clone (clone 45Sma-6, Figure 1) was used as a probe (Figure 6D). In *cms-T*, the largest detectable transcript is approximately 3900 nucleotides long. Transcripts of approximately 2000 nucleotides, 1800 nucleotides, 1500 nucleotides, and 1100 nucleotides are also seen (Figure 6). The 3900 nucleotide transcript is considerably larger than the length of DNA in TURF 2H3 that hybridizes positively to Northern blots (approximately 2 kb). No other fragment within TURF 2B, however, hybridizes to the 3900 nucleotide transcript. The explanation of this discrepancy is unknown.

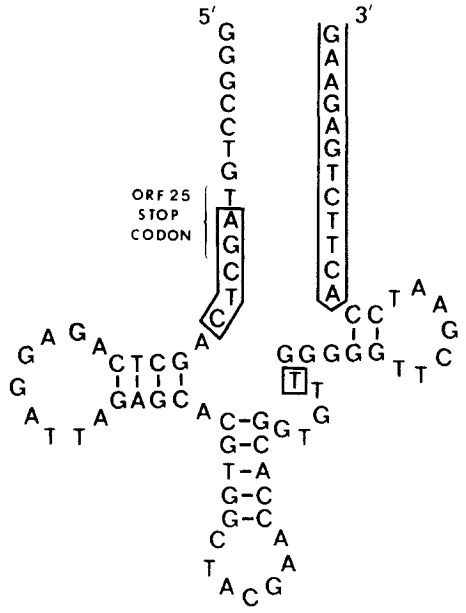

Figure 5. PseudotRNA-Arg Gene in TURF 2H3

Positions 2296 to 2363. Sequences nonhomologous with tobacco chloroplast tRNA-Arg gene are boxed, including a 5 bp repeat in TURF 2H3. Stop codon of reading frame ORF 25 is indicated by bracket.

Open Reading Frames of TURF 2H3

Two large open reading frames are situated in the transcribed region of TURF 2H3 (Figure 2). The first frame, designated ORF 13, is located from positions 1161 to 1559, where a TGA stop codon occurs. According to recent studies, TGA is a termination codon in higher plant mitochondria (Schuster and Brennicke, 1985; Braun and Levings, 1985). Assuming translation initiates with an AUG codon, ORF 13 could start at position 1215 (Figure 2), and encode a polypeptide 115 amino acids long with a predicted molecular weight of 12,961. A smaller polypeptide is predicted if initiation occurs at the next ATG codon at position 1326. Because the complex rearrangements of DNA from the 26S ribosomal gene and its flank occur in this region, the complete open reading frame is unique to *cms-T*. ORF 13 is also in the segment of TURF 2H3 that is uniquely transcribed in T cytoplasm (Figure 6B).

The second open reading frame, designated ORF 25, extends from positions 1547 to 2302, where a TAG stop codon is encountered. ORF 25 occurs in a different reading frame than ORF 13 and overlaps with ORF 13 from positions 1547 to 1559. The first ATG codon, however, is located at position 1640. Assuming translation begins with the ATG codon at position 1640 (Figure 2), ORF 25 could code for a protein 221 amino acids long with a predicted molecular weight of 24,675. The DNA sequences encoding ORF 25 hybridize to transcripts in all four cytoplasms (Figure 6D). Hybridization of ORF 25-specific subclones to Southern blots of mtDNA from bean, wheat, pea, and rice shows that homologous sequences are present in the mitochondrial genomes of these higher plants (data not shown). Interestingly, the TAG termination codon of ORF 25 is situated at the beginning of the chloroplast tRNA-Arg pseudogene (Figure 5). Computer searches of the predicted translation products of ORF 13 and ORF 25 to amino acid sequences in the NBRF protein library detected no significant homologies.

Effect of Restorer Genes on TURF 2H3 Transcript

Nuclear genes that countermand the cms trait in maize by restoring it to full pollen fertility are called restorers (*Rf*). Two dominant restorer genes, designated *Rf1* and *Rf2*, are required to re-establish fertility in the *cms-T* phenotype (Duvick, 1965). That is, *cms-T* is restored to pollen fertility by the nuclear genotype *Rf1* ___ *Rf2*

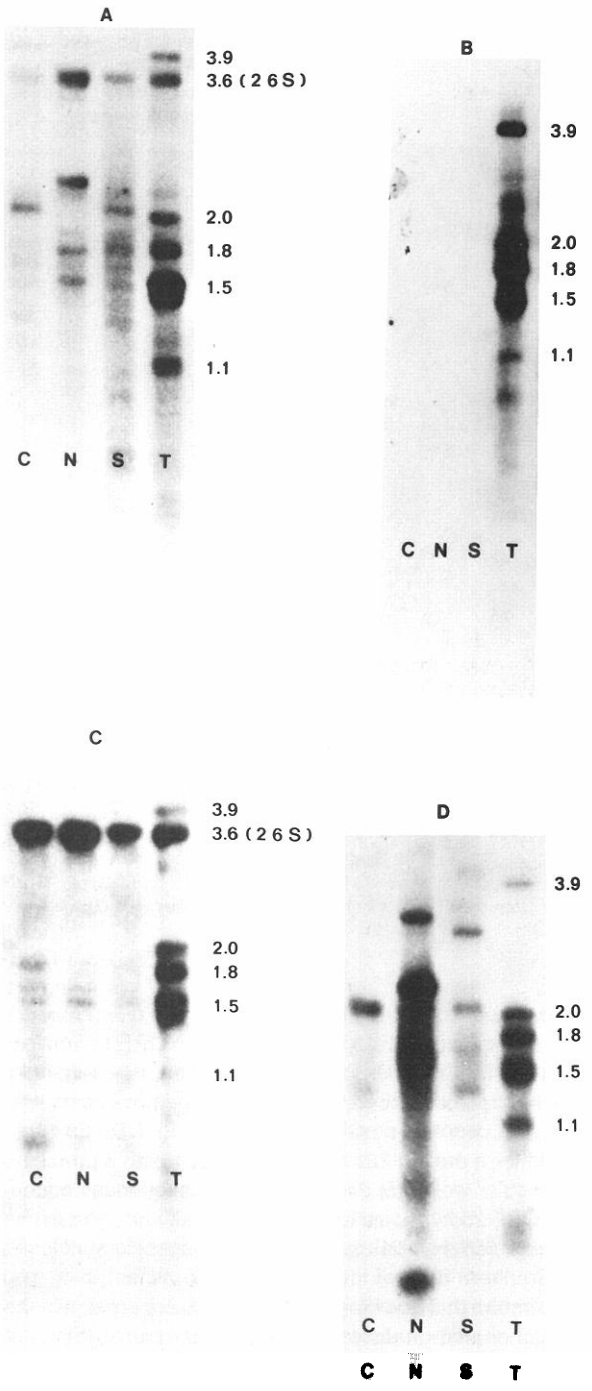

Figure 6. Northern Blot Analysis of TURF 2B

Approximate sizes are indicated in kilonucleotides. Each lane contains equal amounts of RNA from *cms-C*, normal (N), *cms-Vg* (S), or *cms-T* mt preparations. *Cms-Vg* cytoplasm is a member of the S group of male-sterile cytoplasms. The following clones were used as hybridization probes: A, 9.0 kb TURF 2B clone; B, 913T-41 (Figure 1); C, 913S-50 (Figure 1); D, 45Sma-6 (Figure 1).

___, but is not restored by *Rf1* ___ *rf2 rf2*, *rf1 rf1 Rf2* ___, or *rf1 rf1 rf2 rf2*. To determine the effects of restorer genes on TURF 2H3 transcripts, we have examined TURF 2H3 transcripts in restored (fertile) and nonrestored (sterile) lines of *cms-T*. Portions of TURF 2H3 were hybridized to Northern blots of total mtRNA from the male-sterile line, B37 *cms-T* (sterile) and the fertility-restored line, B37 *cms-T* (*Rf1 Rf1 Rf2 Rf2*) (Figure 7). The lines were grown simultaneously and mtRNAs were isolated under identical conditions. Two major differences were detected in the RNA banding patterns between the two lines. Clone 913T-41, containing most of ORF 13 (Figure 1), hybridized to a 1600 nucleotide mtRNA species in the *cms-T* (restored) but not in the *cms-T* (sterile) mitochondria. In contrast, 913T-41 hybridized to a 1500 nucleotide RNA species of *cms-T* (sterile) but not in *cms-T* (restored). In addition, a unique RNA species approximately 600 nucleotides long was detected in *cms-T* (restored) mtRNA preparations that was absent in *cms-T* (sterile). Hybridization of ORF 25-specific probes also distinguished the 1500 nucleotide RNA in *cms-T* (sterile) and the 1600 nucleotide species in *cms-T* (restored), yet did not appear to hybridize to the unique 600 nucleotide RNA species found in the *cms-T* (restored) (Figure 7B). Similarly, clones containing maize mitochondrial genes *atp* 9 (Dewey et al., 1985a) and *atp* 6 (Dewey et al., 1985b) were hybridized to mtRNA blots of restored and sterile *cms-T*. Unlike TURF 2H3, no alterations were detected in the transcripts of these mitochondrial genes when in a nuclear genotype containing *Rf1* and *Rf2* (data not shown).

To further characterize the differences in the transcripts of TURF 2H3 between restored and sterile *cms-T* mitochondria, two distinct oligonucleotides complementary to the message were prepared and hybridized to Northern blots of total mtRNA. A 17 base long oligonucleotide, complementary to positions 1400 to 1416 of TURF 2H3 and located in the middle of ORF 13, gave the same hybridization pattern as clone 913T-41 (data not shown). A 21 base long oligonucleotide, complementary to positions 1235 to 1255, located near the 5′ end of ORF 13 and 181 bp 5′ of the 17 base long oligonucleotide, was also hybridized to mtRNA blots. This 21 base long oligonucleotide hybridized to the same set of transcripts in *cms-T* (restored) as the 17 base long oligonucleotide and clone 913T-41, yet failed to hybridize to the 1500 nucleotide RNA species in *cms-T* (sterile) preparations (Figure 7C). Similar results were observed using the maize cross B73 × Ky21 (*cms-T*) *Rf1* ___ *Rf2* ___ as a source of restored cytoplasm (data not shown). These data indicate that TURF 2H3 transcripts are altered by the same restorer genes (*Rf1* and *Rf2*), which are able to suppress the male-sterility trait of *cms-T*.

Southern Blot Analysis of TURF 2B

When the 9.0 kb TURF 2B fragment is hybridized to Southern blots of mtDNA from normal cytoplasm digested with Bam HI, four fragments hybridize intensely and a fifth fragment hybridizes weakly (Figure 8A). The 6.5 kb *atp* 6-containing Bam HI fragment of normal cytoplasm hybridizes to the segment of TURF 2B containing the 5′ flanking repeat common with *atp* 6 (Figure 3). Subclones of TURF 2H3 containing sequences homologous to the 26S ribosomal gene hybridize uniquely with a 13.7 kb Bam HI fragment of normal cytoplasms (Figure 8B). This is consistent with the finding of Iams and Sinclair (1982), show-

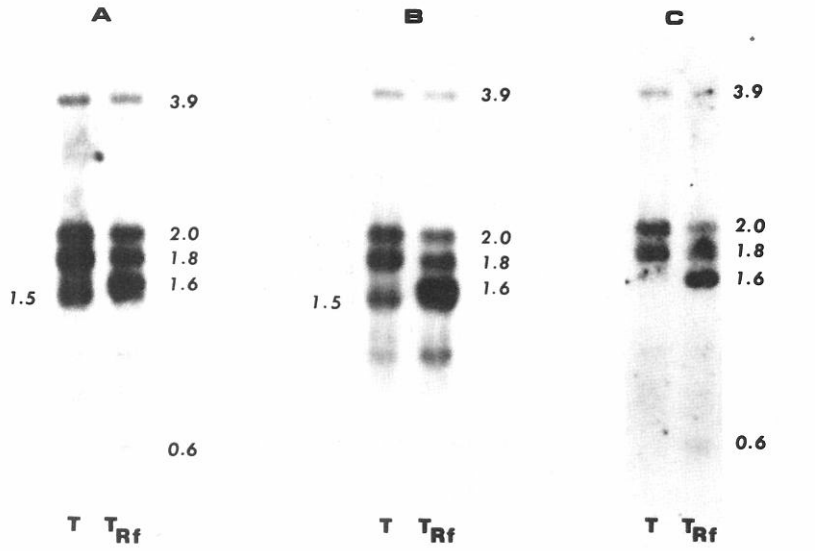

Figure 7. Northern Blot Analysis of TURF 2H3 Message in Male-Sterile *cms-T* and Fertility Restored *cms-T* Lines of the Maize Inbred B37

Male-sterile *cms-T*, T; fertility restored *cms-T*, T_{Rf}. The following hybridization probes were used: A, clone 913T-41 (Figure 1); B, Bgl II–Sma I fragment of TURF 2H3, specific to ORF 25 (positions 1722 to 2266, Figure 2); C, 21 base long oligonucleotide complementary to positions 1235 to 1255 of TURF 2H3 (Figure 2). Approximate sizes are indicated in kilonucleotides.

ing the 26S ribosomal gene from normal cytoplasm to be located in a 13.7 kb Bam HI segment of maize mtDNA. Subclones of TURF 2H3 specific to ORF 25 hybridize to the 12.0 kb Bam HI fragment (Figure 8C). We have not characterized the TURF 2B sequences that hybridize to the 3.7 kb and 1.0 kb fragments.

The 9.0 kb TURF 2B clone contains an internal 6.6 kb Xho I fragment. When the first Hind III fragment of TURF 2H3 (positions 1 to 2013, Figure 1) is hybridized to Xho I digests of normal and T mtDNA, major fragments of 6.6 kb and 4.5 kb are detected in *cms-T* and a major band of 4.5 kb is seen in normal (Figure 9). Minor bands are also observed in both lanes due to cross hybridization to the fragments encoding the 26S ribosomal gene and ORF 25. The entire 3.5 kb region of TURF 2H3 hybridizes to the 6.6 kb fragment in *cms-T* while only subclones containing the repeat segment homologous to the 5′ flank of *atp* 6 hybridize to the 4.5 kb fragment in normal and T cytoplasm (data not shown). Therefore, we assume the 4.5 kb Xho I fragment contains the *atp* 6 gene in normal and T, while the 6.6 kb Xho I fragment in T contains the entire 3.5 kb TURF 2H3 sequence.

Discussion

The complexity of plant mitochondrial genomes has been demonstrated in numerous studies. These studies have described rearrangements involving intramolecular recombination within the mitochondrial genome and intermolecular recombination between the mitochondrial genome and plasmid-like DNAs, and between the chloroplast and mitochondrial genomes (Lonsdale et al., 1984; Schardl et al., 1984; Stern and Palmer, 1984). For the most part, hybridization techniques have been used to delineate these rearrangements. In this study we characterized, by nucleotide sequence analysis, a fragment of maize mtDNA from T cytoplasm that originated from intramolecular recombinations as well as intermolecular recombination between the chloroplast and mitochondrial genomes.

Substantial sequence homologies between various regions of TURF 2H3 and portions of flanking and/or coding sequences of the 26S ribosomal gene, *atp* 6, and a chloroplast tRNA gene indicate that multiple recombinational events have occurred to form this fragment. In fact, at least seven points of recombination are indicated in the fragment. The first site of recombination (position 1146, Figure 2) may be due to the duplication of a large segment of DNA 5′ of the *atp* 6 gene, creating the repeat unique to *cms-T* (Figure 3). Since this region is repeated only in *cms-T* and not in normal, *cms-C*, or *cms-S*, we favor a duplication rather than a deletion to explain this rearrangement. Rearrangements involving the 26S ribosomal gene and the chloroplast tRNA gene have most likely arisen either by nonhomologous recombination or by homologous recombination involving small areas of nucleotide homology. We cannot discriminate between these alternatives with our data. Because of the large amount of chloroplast DNA found in the mitochondria of maize, it is possible that the chloroplast tRNA sequence in TURF 2H3 came about indirectly from the chloroplast genome via intramolecular recombination between chloroplast-like sequences already present in the mitochondrial genome, rather than through a direct intermolecular recombinational event. Although several instances of intermolecular recombination between chloroplast and mitochondrial genomes are described in maize, the molecular mechanism is not yet determined.

The maize mitochondrial genome of normal cytoplasm contains six major sequence reiterations, approximately 1 kb, 2 kb, 3 kb, 10 kb, 12 kb, and 14 kb in length (Lonsdale et al., 1984). Each repeat is found twice within the master genomic circle and all except the 10 kb repeat are implicated in the formation of smaller subgenomic circles. We have identified and partially sequenced a large repeat unique to the T cytoplasm (Figure 3). The presence of a unique repeat suggests that a novel set of subgenomic cir-

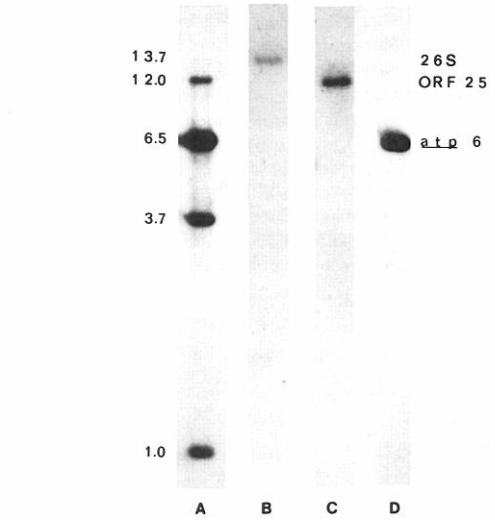

Figure 8. Hybridization of Clones

(A) Hybridization of clone TURF 2B to a Bam HI mtDNA blot of normal maize cytoplasm. Approximate sizes are indicated in kb. (B–D) Hybridization of clones 913T-41, 45Sma-6, and 913T-17 (Figure 1), respectively, to Bam HI mtDNA digests of normal maize.

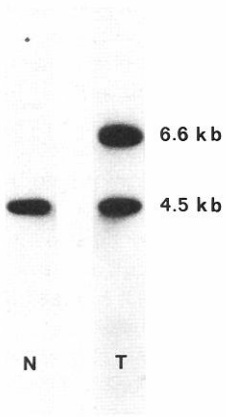

Figure 9. Hybridization of 2013 bp Hind III Fragment of TURF 2H3 (Figure 1) to Xho I Restriction Digests of Normal (N) and *cms-T* (T) Maize mtDNA.

cles could arise from recombination with this repeat in T cytoplasm. Indeed, electron microscopy studies have shown differences in the sizes and distributions of subgenomic circles between T and normal cytoplasms (Levings et al., 1979).

Sequence comparisons of TURF 2H3 with homologous sequences from the 26S ribosomal gene and the tobacco chloroplast tRNA-Arg gene reveal three short 5 bp repeats in these regions of homology. Similar short repeats are found in the nucleotide sequence of the 18S and 5S ribosomal gene in Oenothera mitochondria and in flanking sequences of ribosomal genes and the *rbc*L gene of maize and barley chloroplasts (Brennicke et al., 1985; Takaiwa and Sugiura, 1982; Zurawski et al., 1984). Misalignment of sequences during replication through mispairing, formation of stem-loops, or slippage can result in short direct tandem repeats (Drake et al., 1983).

Three segments of TURF 2H3 have sequence homology with the maize 26S ribosomal gene. The first region of homology is interesting because the homologous sequences occur in the 5′ flanking region of three different genes. Twenty-nine of 30 nucleotides are conserved between sequences located 5′ of the 26S ribosomal gene, *atp* 6, and the open reading frames of TURF 2H3 (Figure 4). The location and conservation of these sequences suggests that they could play a role in the initiation and/or regulation of transcription.

Two long open reading frames, designated ORF 13 and ORF 25, are located in the transcribed region of TURF 2H3. ORF 13 could encode a polypeptide of 115 amino acids with an estimated molecular weight of 12,961. Because this open reading frame consists primarily of sequences derived from the 3′ flank and an interior region of the 26S ribosomal gene, it seems unusual that it would produce a functional protein product. Moreover, since the 5′ end of the transcript is not precisely mapped, it is uncertain whether transcription initiates before or after the first in-frame ATG codon shown in Figure 2. If ORF 13 is a functional reading frame, it would produce a protein product exclusive to the T cytoplasm because this region of TURF 2H3 is uniquely transcribed in *cms-T*.

In vitro protein synthesis studies with isolated mitochondria have revealed differences in the proteins produced by normal and T cytoplasms (Forde and Leaver, 1980). One-dimensional SDS gel electrophoresis has shown a 13,000 Mr protein product unique to T cytoplasm. In addition, a 21,000 Mr polypeptide distinguished in normal mitochondria is absent in T. The functions of these translational products have not been determined. The 13,000 Mr protein is apparently subject to nuclear regulation, since fertility restoration of the T cytoplasm with nuclear restorer genes *Rf1* and *Rf2* significantly suppresses synthesis of the 13,000 Mr product (Forde and Leaver, 1980). Leaver and coworkers have proposed that the 13,000 Mr polypeptide may play a part in the Texas type of cytoplasmic male sterility. It is interesting that the protein predicted by ORF 13 is similar in size to the 13,000 Mr polypeptide unique to *cms-T* discovered by Forde and Leaver (1980).

DNA and RNA hybridization studies suggest ORF 25 is an active plant mitochondrial gene. This view is supported by the fact that ORF 25 probes hybridize to mitochondrial transcripts in all four maize cytoplasms (normal, C, S, and T, Figure 6D) and that sequences homologous to ORF 25 are detectable in mtDNA digests of such diverse species as bean, pea, wheat, and rice. The latter result indicates that the reading frame is common to plant mitochondrial genomes. ORF 25 could code for a protein of 221 amino acids with a molecular weight of 24,675. Computer searches have failed to identify a similar protein among the data banks, so the function of this putative gene product is unknown. Hydropathy plots indicate that the predicted ORF 25 protein is hydrophilic; therefore, it is unlikely to be a membrane polypeptide. Interestingly, the 5′ end of the reading frame is still open at the point of recom-

bination with the interior segment of the 26S gene (position 1565, Figure 2). Consequently, the reading frame of the unrecombined form of this gene in normal maize cytoplasm may extend farther 5′, producing a polypeptide larger than ORF 25. Moreover, it is possible that ORF 25 may not be properly translated in TURF 2H3 if important regulatory sequences are interrupted by recombination in the 5′ end of the gene. Forde and Leaver (1980) have reported a polypeptide synthesized in normal cytoplasm (approximately 21,000 Mr) that is not present in *cms-T*.

ORF 25 is novel in that the last 14 amino acids are coded by DNA derived from the chloroplast genome. Although chloroplast-like sequences are prevalent in the mitochondrial genome, TURF 2H3 is distinct in that these incorporated chloroplast sequences are transcribed as part of a mitochondrial message. ORF 25 terminates with a TAG codon located in the 5′ aminoacyl stem of the chloroplast tRNA-Arg pseudogene (Figure 5). The pseudogene location is analogous to the organization of animal mitochondrial genomes in which genes are frequently terminated by functional tRNA genes (Anderson et al., 1981). In animal mitochondria, 3′-terminal tRNA genes are thought to aid in transcriptional processing (Attardi et al., 1982).

A complex transcriptional pattern is observed when TURF 2B is hybridized to Northern blots of maize mtRNA (Figure 6A). Part of the complex pattern results from cross hybridization of TURF 2B with transcripts produced by the 26S ribosomal gene. Similarly, complex mtRNA hybridization patterns are reported for maize genes coding for cytochrome oxidase subunit II (Fox and Leaver, 1981), apocytochrome b (Dawson et al., 1984), ATPase subunit 9 (Dewey et al., 1985a), and ATPase subunit 6 (Dewey et al., 1985b). Recombinations between transcribed regions of different mitochondrial genes may partially explain the complex transcriptional patterns of these and other maize mitochondrial genes.

Regeneration studies with tissue cultures of *cms-T* maize have discovered interesting changes in disease resistance and the cms phenotype. Reversion from male sterility to male fertility often occurs in plants regenerated from callus culture (Gengenbach et al., 1977; Brettell et al., 1979). Reversion is also invariably associated with a newly acquired resistance to Bipolaris maydis (Helminthosporium maydis), race T, and its fungal toxin (T toxin). Molecular changes are also associated with the reversion phenomenon. Significant suppression or total loss of the 13,000 Mr polypeptide unique to *cms-T* is reported in revertant plants (Dixon et al., 1982). At the DNA level, the disappearance of a 6.6 kb Xho I fragment in mtDNA digests is associated with reversion. In contrast, plants that maintain the male-sterile, susceptible phenotype during culture do not lose the 6.6 kb Xho I fragment. This specific alteration, presumably via rearrangements, deletions, or base substitutions leading to new restriction sites, is reported in 15 of 16 revertants examined, prompting speculation that this change may be associated with fertility restoration (Umbeck and Gengenbach, 1983). Wise et al. (1985) report that this 6.6 kb Xho I fragment is interior to a 9.0 kb Bam HI fragment in *cms-T* and also hybridizes to a 6.5 kb Bam HI fragment and a 4.5 kb Xho I fragment in mtDNA digests of both T and normal cytoplasms. We have observed the same hybridization pattern for TURF 2B (Figure 3, Figure 9), strongly suggesting that the 6.6 kb Xho I fragment located within the 9.0 kb TURF 2B molecule is the same fragment that disappears in revertant maize plants. The entire 3.5 kb TURF 2H3 fragment is internal to a 6.6 kb Xho I digestion fragment of *cms-T* mtDNA. Since the chimeric sequence, TURF 2H3, has been the site of numerous previous rearrangements, it is not surprising to find it associated with yet another change in the mtDNA. In any event, it is clear that the sequence has some unusual properties making it prone to change. At the moment, the evidence linking the loss of a 6.6 kb Xho I fragment with reversion to fertility and to the possibility that this region may be responsible for the T type of male sterility is circumstantial. Nevertheless, the chimeric and unstable nature of the sequence, the novel and abundant transcripts, and the open reading frames suggest a relationship between TURF 2H3 and the male sterility trait.

Evidence linking TURF 2H3 to the *cms-T* phenotype is also indicated by the effect of the nuclear restorer genes *Rf1* and *Rf2* on the TURF 2H3 transcripts. When *cms-T* plants are restored to fertility by the nuclear genes *Rf1* and *Rf2*, changes occur in the mitochondrial transcripts of TURF 2H3 (Figure 7). Nuclear genes affecting proper transcriptional processing of mitochondrial messages have been described in yeast (Pillar et al., 1983; Faye and Simon, 1984). *Rf1* and *Rf2*, however, have no apparent effect on transcripts of the mitochondrial genes coding for ATPase subunits 6 and 9. TURF 2H3 transcripts from *cms-T* that have been restored to fertility with *Rf1* and *Rf2* are distinguished from *cms-T* (sterile) by the presence of transcripts approximately 1600 and 600 nucleotides long, and the absence of a 1500 nucleotide RNA species. A 21 base long oligonucleotide, complementary to the message located near the 5′ end of ORF 13, hybridizes to the 1600 nucleotide RNA species in restored *cms-T*, but not to the 1500 nucleotide RNA species in sterile *cms-T*. Probes located farther 3′ to the 21 base long oligonucleotide hybridize to both RNAs. These results suggest that the size difference between the 1600 and 1500 nucleotide RNAs is caused by additional 5′ flanking nucleotides in the 1600 nucleotide species. In our view, the differences are most likely due to differential processing of the TURF 2H3 transcript. This proposal suggests that the gene product of either one or both of the nuclear restorer genes, *Rf1* and *Rf2*, are involved in RNA processing, perhaps as an RNA processing enzyme. Further studies will be required to determine the specific role of *Rf1* and *Rf2*, and the relationship between TURF 2H3 and the male sterility trait in *cms-T*.

Experimental Procedures

Isolation of Nucleic Acids

Mitochondrial RNA (mtRNA) and mtDNA were prepared from 6 to 7 day old, dark grown seedlings of Zea mays L., as previously described (Pring and Levings, 1978; Schuster et al., 1983). The following sterile (nonrestored) maize lines or crosses were used as sources of male-sterile cytoplasm: B73 × Mo17 (*cms-C*), B73 (*cms-Vg*), B73 (*cms-T*), and

B37 (*cms-T*). The *cms-Vg* cytoplasm is a member of the S group of male-sterile cytoplasms (Beckett, 1971). B37 (*cms-T*) *Rf1 Rf1 Rf2 Rf2* and B73 × Ky21 (*cms-T*) *Rf1 ___ Rf2 ___* were used as sources of the *cms-T* restored cytoplasm; they are male-fertile. The fertile hybrid, B73 × Mo17 (normal), was used as a source of male-fertile cytoplasm.

Construction and Screening of a Mitochondrial DNA Library

A Bam HI library of *cms-T* mtDNA was constructed and hybridized to end-labeled total mtRNA from *cms-T* and normal cytoplasms as previously described (Dewey et al., 1985a).

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. (1979). MtRNA was heat denatured and fractionated by electrophoresis in 1.2% agarose gels containing 6% formaldehyde and blotted to nitrocellulose as described by Thomas (1980). Double-stranded DNA was labeled with [α-^{32}P]dATP (NEN, 3200 Ci/mmol) by nick translation (Rigby et al., 1977). Single-stranded DNA clones in the bacteriophage M13 were labeled by the backpriming technique of Hu and Messing (1982).

Nucleic acid hybridizations were performed under conditions already described (Dewey et al., 1985a). The 18S (1986 nucleotides) and 26S (3546 nucleotides) ribosomal RNAs of maize mitochondria were used as markers for estimating RNA sizes. Hind III digests of bacteriophage lambda DNA were used as markers for estimating DNA sizes.

Oligonucleotide Synthesis and Hybridization

Oligonucleotide probes were prepared with the Applied Biosystems 380A DNA Synthesizer according to the manufacturer's instructions. Oligonucleotides were 5′ end-labeled with [γ-^{32}P]ATP (ICN, 7000 Ci/mmol) using T_4 polynucleotide kinase (Maxam and Gilbert, 1980). Northern blots were prehybridized in a solution containing 6× NET (0.75 M NaCl, 75 mM Tris–HCl, pH 7.8, 1 mM EDTA), 5× Denhardt's (0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone), 0.1% SDS, and 100 mg/ml salmon sperm DNA for 5 hr at 68°C. Labeled oligonucleotides were hybridized at 53°C in a solution containing 6× NET, 5× Denhardt's, and 0.1% SDS. Filters were washed in 6× SSC, 0.1% SDS at room temperature.

DNA Sequence Analysis

Cloning for sequence analysis was carried out using M13 bacteriophage vectors mp10 and mp11 (Messing, 1982). Ligation and transformation procedures were as outlined by New England Biolabs. DNA sequences were determined by the chain-termination method of Sanger et al. (1977) with a universal primer (PL Biochemicals). Sequencing gels were either 6% or 8% polyacrylamide and 0.4 mm thick. Sequencing strategies are shown in Figure 1.

Nucleotide and amino acid sequence analyses were performed by computer programs furnished by Bionet. Bionet accesses the National Institutes of Health (GenBank) and European Molecular Biology Laboratories DNA sequence libraries and the National Biomedical Research Foundation protein sequence database.

Acknowledgments

We are grateful to Carol Griffin, Jane Suddith, and Angela M. Grimes for expert technical assistance. This research was supported by grants from Agrigenetics Inc. and the National Science Foundation.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *"advertisement"* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 1, 1985; revised October 15, 1985

References

Anderson, S., Bankier, A. T., Barrell, B. G., De Bruijn, M. H. L., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, A. J. H., Staden, R., and Young, I. G. (1981). Sequence and organization of the human mitochondrial genome. Nature *290*, 457–465.

Attardi, G., Cantatore, P., Chomyn, A., Crews, S., Gelfand, R., Merkel, C., Montoya, J., and Ojala, D. (1982). A comprehensive view of mitochondrial gene expression in human cells. In Mitochondrial Genes, P. Slonimski, P. Borst, and G. Attardi, eds. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), pp. 51–71.

Beckett, J. B. (1971). Classification of male sterile cytoplasms in maize (*Zea mays* L). Crop Sci. *11*, 724–726.

Braun, C. J., and Levings, C. S., III (1985). Nucleotide sequence of the F_1-ATPase alpha subunit of maize mitochondria. Plant Physiol. *79*, 571–577.

Brennicke, A., Moller, S., and Blanz, P. A. (1985). The 18S and 5S ribosomal RNA genes in *Oenothera* mitochondria: sequence rearrangements in the 18S and 5S rRNA genes of higher plants. Mol. Gen. Genet. *198*, 404–410.

Brettell, R. I. S., Goddard, B. V. D., and Ingram, D. S. (1979). Selection of Tms-cytoplasm maize tissue cultures resistant to *Drechslera maydis* T-toxin. Maydica *24*, 203–213.

Dale, R. M. K., Mendu, N., Ginsburg, H., and Kridl, J. C. (1984). Sequence analysis of the maize mitochondrial 26 S rRNA gene and flanking regions. Plasmid *11*, 141–150.

Dawson, A. J., Jones, V. P., and Leaver, C. J. (1984). The apocytochome b gene in maize mitochondria does not contain introns and is preceded by a potential ribosome binding site. EMBO J. *3*, 2107–2113.

Dewey, R. E., Schuster, A. M., Levings, C. S., III, and Timothy, D. H. (1985a). Nucleotide sequence of F_O-ATPase proteolipid (subunit 9) gene of maize mitochondria. Proc. Natl. Acad. Sci. USA *82*, 1015–1019.

Dewey, R. E., Levings, C. S., III, and Timothy, D. H. (1985b). Nucleotide sequence of ATPase subunit 6 gene of maize mitochondria. Plant Physiol. *79*, 914–919.

Dixon, L. K., Leaver, C. J., Brettell, R. I. S., and Gengenbach, B. G. (1982). Mitochondrial sensitivity to *Drechslera maydis* T-toxin and the synthesis of a variant mitochondrial polypeptide in plants derived from maize tissue cultures with Texas male-sterile cytoplasm. Theor. Appl. Genet. *63*, 75–80.

Drake, J. W., Glickman, B. W., and Ripley, L. S. (1983). Updating the theory of mutation. Amer. Scientist *71*, 621–630.

Duvick, D. N. (1965). Cytoplasmic pollen sterility in corn. Adv. Genet. *13*, 1–56.

Faye, G., and Simon, M. (1984). Steps in processing of the mitochondrial cytochrome oxidase subunit I pre-mRNA affected by a nuclear mutation in yeast. Proc. Natl. Acad. Sci. USA *81*, 8–12.

Forde, B. G., and Leaver, C. J. (1980). Nuclear and cytoplasmic genes controlling synthesis of variant mitochondrial polypeptides in male-sterile maize. Proc. Natl. Acad. Sci. USA *77*, 418–422.

Forde, B. G., Oliver, R. J. C., and Leaver, C. J. (1978). Variation in mitochondrial translation products associated with male-sterile cytoplasms in maize. Proc. Natl. Acad. Sci. USA *75*, 3841–3845.

Fox, T. D., and Leaver, C. J. (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.

Gengenbach, B. G., Green, C. E., and Donovan, C. M. (1977). Inheritance of selected pathotoxin resistance in maize plants regenerated from cell cultures. Proc. Natl. Acad. Sci. USA *74*, 5113–5117.

Hooker, A. L., Smith, D. R., Lim, S. M., and Beckett, J. B. (1970). Reaction of corn seedlings with male-sterile cytoplasm to *Helminthosporium maydis*. Plant Dis. Rep. *54*, 708–712.

Hu, N., and Messing, J. (1982). The making of strand-specific M13 probes. Gene *17*, 271–277.

Iams, K. P., and Sinclair, J. H. (1982). Mapping the mitochondrial DNA of *Zea mays*: ribosomal gene localization. Proc. Natl. Acad. Sci. USA *79*, 5926–5929.

Kato, A., Takaiwa, F., Shinozaki, K., and Sugiura, M. (1985). Location and nucleotide sequence of the genes for tobacco chloroplast $tRNA^{Arg}$ (ACG) and $tRNA^{Leu}$ (UAG). Curr. Genet. *9*, 405–409.

Kemble, R. J., Mans, R. J., Gabay-Laughnan, S., and Laughnan, J. R. (1983). Sequences homologous to episomal mitochondrial DNAs in the maize nuclear genome. Nature *304*, 744–747.

Keus, R. J. A., Stam, N. J., Zwiers, T., de Heij, H. T., and Groot,

G. S. P. (1984). The nucleotide sequences of the genes coding for tRNA-Arg(UCU), tRNA-Arg(ACG) and tRNA-Asn(GUU) on *Spirodela oligorhiza* chloroplast DNA. Nucl. Acids Res. *12*, 5639–5646.

Levings, C. S., III (1983a). The plant mitochondrial genome and its mutants. Cell *32*, 659–661.

Levings, C. S., III (1983b). Cytoplasmic male sterility. In Genetic Engineering of Plants, T. Kosuge, C. P. Meredith, and A. Hollaender, eds. (New York: Plenum), pp. 81–92.

Levings, C. S., III, Shah, D. M., Hu, W. W. L., Pring, D. R., and Timothy, D. H. (1979). Molecular heterogeneity among mitochondrial DNAs from different maize cytoplasms. In Extrachromosomal DNA, ICN-UCLA Symposium on Molecular and Cellular Biology, Volume 25, D. J. Cummings, P. Borst, I. G. David, and S. M. Weissman, eds. (New York: Academic Press), pp. 63–73.

Lonsdale, D. M., Hodge, T. P., and Fauron, C. M. R. (1984). The physical map and organisation of the mitochondrial genome from the fertile cytoplasm of maize. Nucl. Acids Res. *12*, 9249–9261.

Maxam, A. M., and Gilbert, W. (1980). Sequencing end-labeled DNA with base-specific chemical cleavages. Meth. Enzymol. *65*, 499–560.

Messing, J. (1982). An integrative strategy of DNA sequencing and experiments beyond. In Genetic Engineering—Principles and Methods, J. K. Setlow and A. Hollaender, eds. (New York: Plenum), pp. 19–35.

Orozco, E. M., Jr., and Hallick, R. B. (1982). *Euglena gracilis* chloroplast transfer RNA transcription units. J. Biol. Chem. *257*, 3265–3275.

Palmer, J. D., and Shields, C. R. (1984). Tripartite structure of the *Brassica campestris* mitochondrial genome. Nature *307*, 437–440.

Pillar, T., Lang, B. F., Steinberger, I., Vogt, B., and Kaudewitz, F. (1983). Expression of the split gene *cob* in yeast mtDNA. Nuclear mutations specifically block the excision of different introns from its primary transcript. J. Biol. Chem. *258*, 7945–7959.

Pring, D. R., and Levings, C. S., III (1978). Heterogeneity of maize cytoplasmic genomes among male-sterile cytoplasms. Genetics *89*, 121–136.

Rigby, P. W. J., Dieckmann, M., Rhodes, C., and Berg, P. (1977). Labeling deoxyribonucleic acids to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. *113*, 237–251.

Sanger, F., Nicklen, S., and Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA *74*, 5463–5467.

Schardl, C. L., Lonsdale, D. M., Pring, D. R., and Rose, K. R. (1984). Linearization of maize mitochondrial chromosomes by recombination with linear episomes. Nature *310*, 292–296.

Schuster, A. M., Sisco, P. H., and Levings, C. S., III (1983). Two unique RNAS in *cms*-S and RU maize mitochondria. In Plant Molecular Biology, R. B. Goldberg, ed. (New York: Liss), pp. 437–444.

Schuster, W., and Brennicke, A. (1985). TGA-termination codon in the apocytochrome b gene from *Oenothera* mitochondria. Curr. Genet. *9*, 157–163.

Sederoff, R. R., Levings, C. S., III, Timothy, D. H., and Hu, W. W. L. (1981). Evolution of DNA sequence organization in mitochondrial genomes of *Zea*. Proc. Natl. Acad. Sci. USA *78*, 5953–5957.

Seldon, R. F., Steinmetz, A., McIntosh, L., Bogorad, L., Burkard, G., Mubumbila, M., Kuntz, M., Crouse, E. J., and Weil, J. H. (1983). Transfer RNA genes of *Zea mays* chloroplast DNA. Plant Mol. Biol. *2*, 141–153.

Stern, D. B., and Palmer, J. D. (1984). Extensive and widespread homologies between mitochondrial DNA and chloroplast DNA in plants. Proc. Natl. Acad. Sci. USA *81*, 1946–1950.

Stern, D. B., and Lonsdale, D. M. (1982). Mitochondrial and chloroplast genomes of maize have a 12-kilobase DNA sequence in common. Nature *299*, 698–702.

Takaiwa, F., and Sugiura, M. (1982). Nucleotide sequence of the 16S–23S spacer region in an rRNA gene cluster from tobacco chloroplast DNA. Nucl. Acids Res. *10*, 2665–2676.

Thomas, P. S. (1980). Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA *77*, 5201–5205.

Umbeck, P. F., and Gengenbach, B. G. (1983). Reversion of male-sterile T-cytoplasm maize to male fertility in tissue culture. Crop Sci. *23*, 584–588.

Vieira, J., and Messing, J. (1982). The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene *19*, 259–268.

Wahl, G. M., Stern, M., and Stark, G. R. (1979). Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization using dextran sulfate. Proc. Natl. Acad. Sci. USA *76*, 3683–3687.

Wise, R. P., Pring, D. R., and Gengenbach, B. G. (1985). Mitochondrial DNA rearrangements associated with reversion of T cytoplasm to male fertility and disease resistance. Maize Genet. Coop. Newsletter *59*, 50.

Zurawski, G., Clegg, M. T., and Brown, A. H. D. (1984). The nature of nucleotide sequence divergence between barley and maize chloroplast DNA. Genetics *106*, 735–749.