The gene *atp6*, encoding subunit 6 of the mitochondrial F$_1$-ATPase complex, has been characterized from both the normal (fertile) and Ogura (male-sterile) radish cytoplasms in order to determine if previously identified *atp6* transcriptional differences could play a role in cytoplasmic male sterility. Normal radish *atp6* encodes a 282-amino acid polypeptide that exhibits approximately 80% sequence identity with other plant *atp6* polypeptides. A *tRNA*$_{Val}$ gene is located 150 base pairs 5' to *atp6*, and the two genes may be co-transcribed. As a result of extensive rearrangement, sequences that comprise the normal *atp6* locus are present in three widely separated regions of the Ogura mitochondrial genome. Both 5' and 3' rearrangement breakpoints have been identified and found to be associated with short repeated sequences. The normal and Ogura *atp6* loci share a common 987-base pair region containing most of the *atp6* coding region and 106 base pairs of the 3'-flanking region. A 105-codon open reading frame is transcribed as the first gene of an Ogura *atp6* bicistronic mRNA. This sequence is not present in normal radish mitochondrial DNA. There are many nucleotide differences in the Ogura *atp6* 5'-flanking and coding regions that can be expected to eliminate normal translation of *atp6*. At least two possibilities exist for translation of Ogura *atp6*, both of which would result in an NH$_2$-terminal amino acid sequence different from that of normal radish.

Cytoplasmic male sterility (CMS)$^1$ is a maternally inherited trait in which plants fail to produce functional pollen but maintain female fertility (1–3). The CMS trait is associated with mitochondrial dysfunction. Alterations in mitochondrial DNA (mtDNA) structure have been identified in every CMS which would result in an NH$_2$-terminal amino acid sequence different from that of normal radish.

The original observation of CMS in the crucifers (family Cruciferae) was by Ogura (14) who found male-sterile radish (*Raphanus sativus*) plants growing among an escaped radish cultivar in Japan. In Ogura radish, meiosis proceeds normally up to early microspore formation when a collapse of the tapetal tissue leads to microspore degeneration (15, 16). Three nuclear loci have been identified in European cultivars of radish that restore fertility to the Ogura cytoplasm (15, 16).

Ogura CMS, like all other examples of CMS, is found to be associated with mitochondrial alterations. We have recently shown that the Ogura mitochondrial genome is highly rearranged relative to that of normal (i.e. male-fertile) radish (13). In addition, altered transcript patterns were identified for three mitochondrial genes: *atp4*, *atp6*, and *coxI*. The transcript pattern for one of these, *atp4*, was found to be affected by nuclear restorer genes. In order to identify the alteration(s) responsible for Ogura CMS, we have further characterized the Ogura mitochondrial genome and report here a detailed analysis of the *atp6* locus from both normal and Ogura radish. An Ogura-specific *atp6* transcriptional unit containing both a disrupted *atp6* coding region and a novel 105-codon ORF has been generated as a result of multiple rearrangements. Numerous nucleotide differences at the 5' end of Ogura *atp6* eliminate the normal radish translation start site. The possible roles of these alterations in Ogura CMS are discussed.

**MATERIALS AND METHODS**

Plant Material—The *R. sativus* cultivar Scarlet Knight was the source of the normal (wild-type) cytoplasm. The mitochondrial genome of Scarlet Knight exhibits the same restriction enzyme profiles as those of three other commonly used male-fertile radish cultivars (17) and thus is used to represent what we term the "normal" cytoplasm of radish. The rapid-cycling radish line CrGC15 (R1rr; obtained from the Crucifer Genetics Cooperative) was the source of the Ogura cytoplasm. Sterile and fertile (nuclear restored) plants containing the Ogura cytoplasm were distinguished by their ability to produce functional pollen.

Isolation of Nucleic Acids—Mitochondrial DNA was isolated from leaves of 6-week-old plants by the DNase I procedure (18). Mitochondrial RNA (mtRNA) was isolated in the presence of aurintricarboxylic acid from mitochondria purified by differential centrifugation (19). Plassid DNA was isolated using the alkaline lysis procedure (20).

Sequence Determination and Analysis—Normal and Ogura *atp6* were isolated previously on 3.1- and 19.4-kb SalI clones in pUC8 and pTZ18, respectively (13, 21). Standard techniques were used for the preparation of recombinant plasmids in *Escherichia coli* (22). Exonuclease III deletion clones (23) were generated from plasmids containing either a 2.4-kb BamHI fragment in pTZ19R (Ogura *atp6*) or a 3.1-kb SalI fragment in pTZ18R (normal *atp6*) prior to sequencing by the dideoxy chain termination procedure of Sanger (24). The normal radish *atp6* 5'-flanking sequence was localized to a 0.49-kb XbaI/ClaI fragment from the Ogura 5.1-kb SalI fragment (13) by Southern analysis. This fragment was then cloned into Bluescript plasmids.

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1. Cytoplasmic male sterility (CMS) is a trait found in certain plants, where the female reproductive organs are functional but the male reproductive organs are not.
2. This research was supported by Grants R01 GM35087 (to J. D. P.) and F32 GM113323 (to C. A. M.) from the National Institutes of Health. 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.
3. To whom correspondence should be addressed.
4. The abbreviations used are: CMS, cytoplasmic male sterility; bp, base pair(s); kb, kilobase pair(s); PIPEs, piperezine-N,N'-bis[2-ethanesulfonic acid]; ORF, open reading frame.

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Characterization of Oqura Radish atp6

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SK* and SK- and sequenced. DNA sequences were analyzed on a Sun Microsystems, Inc. minicomputer using the Eugene program (Department of Cell Biology, Baylor College of Medicine, Houston, TX).

Southern Analysis of atp6 and Flanking Regions—In order to identify the position and orientation of specific rearranged fragments, exonuclease III-generated subclones containing various segments of the normal and Oqura atp6 loci were hybridized to Southern blots containing a series of single and double restriction digestes (PstI, SalI, BglII, and NruI) of Oqura and normal radish mtDNAs (see Ref. 13 for restriction fragment profiles and maps of the two genomes). Methods used for restriction endonuclease digestion, agarose gel electrophoresis of DNA fragments, bidirectional transfer from agarose gels to Zetabind (AMF Cuno) nylon membranes, and labeling of recombinant DNA fragments by nick translation were as described previously (22, 23).

Northern Hybridization and Nuclease Mapping—Conditions for the electrophoresis, transfer, and hybridization of RNA (7.5 μg/lane) to nick-translated probes were described previously (13). Nuclease-mapping experiments were conducted essentially as described (26, 27). RNA (10 μg) was ethanol precipitated together with a DNA probe that was labeled with either [α-32P]dATP by primer extension of single-stranded DNA using the Klenow fragment of DNA polymerase I (28) or [γ-32P]ATP with polynucleotide kinase (22). The precipitate was resuspended in 20 μl of hybridization buffer (40 mM PIPES, pH 6.4, 1 mM EDTA, 0.4 μM NaCl, and 50% formamide), denatured at 80 °C for 5 min, and allowed to anneal for 6-18 h at 46-56 °C (the GC content of the probe determined the annealing temperature). Samples were treated with 200 units of mung bean nuclease in 200 μl of a buffer containing 50 mM NaCl, 30 mM sodium acetate, pH 5.2, and 1 mM EDTA for 60 min at 37 °C. The samples were then phenol-extracted, ethanol-precipitated, resuspended in denaturing polycrylamide gels next to dideoxy sequencing ladders for precise size determinations.

RESULTS

Normal Radish atp6—The nucleotide sequence of a 3.1-kb SalI fragment from normal radish was determined by dyeoxy sequencing of deletion clones generated by exonuclease III (Fig. 1) and is shown in Fig. 2. This fragment contains a 262-codon open reading frame capable of encoding a 29,130-Da polypeptide that is identified as atp6 by DNA sequence homology. The deduced amino acid sequence exhibits 80-82% identity with atp6 polypeptides from maize (30), Oenothera (31), and tobacco (32). The AUG codon (nucleotides 1-3) assigned as the putative initiator methionine is the only in-frame methionine that precedes the highly conserved atp6 homologous sequence block (Figs. 2 and 3). Located 12 bp 5' to the putative initiator methionine is a 9-bp sequence that is 100% complementary to the 3' end of plant 18 S rRNAs and probably serves as a ribosome binding site (40). The NH2-terminal sequence of radish atp6 is more similar in length to that observed in fungal rather than plant mitochondrial atp6 genes (Fig. 3). Fungal atp6 genes encode short (5-10 amino acids) preresuences that are proteolytically cleaved to yield a mature protein with an NH2-terminal serine residue (39). In normal radish atp6, nine amino acids precede the highly conserved serine (Fig. 3), which has been shown to be the NH2-terminal residue of the mature Saccharomyces cerevisiae (38) and wheat polypeptides (39). Other plant atp6 preresuences could range in length from 4-144 amino acids (Fig. 3), but the actual translation initiation site is not certain.

Located on the same strand 150 bp 5' to normal atp6 is a gene for tRNA\textsuperscript{Met} (trnM). This radish sequence exhibits the typical cloverleaf structure and is 88 and 96% identical to bean tRNA\textsuperscript{Met} (41) and maize trnM (42), respectively. The 3'-terminal nucleotides CCA-OH characteristic of all mitochondrial tRNAs are not encoded in this gene, as has been observed in maize tRNA genes (42).

Nuclease mapping experiments using a uniformly labeled probe extending from within the normal atp6 coding sequence (nucleotides 55) to the SalI site at its 5' end produced protected fragments of 196, 207, and 1347 bp (Fig. 4). These protected fragments correspond to two major 5' transcript termini at nucleotides -152 and -141 (Fig. 2) and the fully protected probe. The transcript termini at -152 and -141 are 9 and 20 bases from the 3' end of the trnM gene, respectively, suggesting that the transcripts may represent processed forms of a larger transcript and that the two genes may be co-transcribed. This hypothesis is supported by the presence of a 2300-nucleotide atp6 transcript in Northern hybridization experiments (Fig. 5), which is also identified with the trnM-containing 0.49-kb XbaI/SalI fragment from Oqura radish (data not shown) and the presence of low levels of fully protected probe in nuclease mapping experiments. Analysis of the DNA sequences immediately 5' and 3' to trnM failed to identify sequences thought to be involved in transcript processing in plant mitochondria (43). Perhaps the tRNA cloverleaf structure itself acts as a processing signal, as has been postulated for the RNA processing events in animal mitochondria (44).

Located 97 bp 3' to normal radish atp6 is a 249-bp duplication of part of the radish coxl locus, containing 117 bp of 5'-flanking sequence and the first 44 codons of the coxl coding region. The coxl sequences are 99.6% identical. Oqura Radish atp6—Oqura radish atp6 was subcloned from a previously characterized 19.4-kb SalI clone (13) and sequenced as a 2.5-kb BamHI fragment (Fig. 1). This 2.5-kb Oqura fragment and the 3.1-kb atp6-containing fragment from normal radish share a common 987-bp region. Most of the atp6 coding region (754 bp) and part of the 3'-flanking sequence (106 bp) are highly conserved (99.7% identical), whereas a 118-bp segment containing the 5'-coding region and its flanking sequence exhibits only 75% sequence identity (Fig. 2). Upstream of nucleotide -91 and downstream of nucleotide 887, the sequences are unrelated with the exception of 66 bp (region A; positions -399 to -334 in normal radish and -764 to -699 in Oqura radish) common to both sequences.

There are 13 nucleotide differences between the Oqura and normal atp6 coding regions, all within the first 13 codons. The most striking changes are the A→C (nucleotide 1) and G→T (nucleotide 3) that change the putative initiator methionine to a leucine codon (Figs. 2 and 3).

\footnote{C. A. Makaroff, I. J. Apel, and J. D. Palmer, unpublished data.}
FIG. 2. Nucleotide and derived amino acid sequences of the atp6 loci from normal and Ogura radish. The Ogura sequence (top line) is aligned with that from normal radish (bottom line). Where the two are identical, only the normal sequence is shown. Numbering of nucleotides is from the predicted translational start of atp6 in normal radish. Amino acids are shown above (ORF-105) and below (atp6) the nucleotide sequence and are numbered relative to the predicted translation initiation codons. A trnM gene present upstream of atp6 in normal radish is boxed. Nucleotides complementary to the last 17 nucleotides of the 18 S rRNA, allowing for G-U base pairing, are indicated with solid circles under the corresponding sequence; where the two sequences are identical, the circles appear above the normal sequence. The 66-bp repeat, the T/A- and coxl-homologous sequence, and the cod-homologous sequence are overlined (solid line); the 44-bp and 35-bp direct repeats within ORF-105 are overlined with dashed lines. Major 5' transcript termini are represented by arrows (below the sequence for normal radish and above the sequence for Ogura radish).

Additional differences in the 91 bp immediately upstream of the coding region. Seven of these changes occur in the proposed ribosome binding site of normal atp6. Together these changes can be expected to eliminate normal translation of Ogura atp6. The leucine codon in Ogura radish that corresponds to the initiator methionine in normal radish is preceded by a 44-codon open reading frame; however, there are no in-frame methionine codons between the leucine codon and the first in-frame stop codon (Figs. 2 and 3). Located 23 amino acids into the coding region is a second methionine that is preceded by a sequence which shows limited sequence identity with the proposed plant mitochondrial ribosome binding site (40).

Ogura atp6 Is Co-transcribed with a Novel 105-Amino Acid ORF—Upstream of Ogura atp6 is a 105-codon open reading frame (ORF-105) that is capable of encoding a 12,052-Da polypeptide. This open reading frame may have been generated through a series of duplication and rearrangement events. Regions of 44 and 35 bp that are directly repeated a total two and three times, respectively, are found within the ORF-105 coding region. Northern analysis indicates that ORF-105 is co-transcribed with atp6 in Ogura mitochondria (Fig. 5). An ORF-105-homologous transcript is not detected in mtRNA from normal radish, consistent with the absence of this sequence from normal radish mtDNA (see below). Hybridization of the Ogura clones to multiple transcripts in both sterile and fertile Ogura mtRNAs (Fig. 5) is most likely due to the presence of repeated sequences on the Ogura clones. When normal radish or Brossica campestris atp6 clones are used to probe these RNA preparations, several low abundance transcripts are identified, but the 1400-nucleotide transcript is the only one of the abundant transcripts shown in Fig. 5 that is
Characterization of Ogura Radish atp6

Fig. 3. Alignment of the deduced N\textsubscript{H}\textsubscript{2}-terminal amino acid sequence of normal and Ogura radish atp6 with those from maize (30), tobacco (32), Oenothera (31), soybean (33), wheat (34), Aspergillus nidulans (35), Neurospora crassa (36), and S. cerevisiae (37). Identical amino acids are overlined and underlined, and in-frame methionines are shown in boldface. In-frame stop codons in the radish sequences are represented by an X. The position of the N\textsubscript{H}\textsubscript{2}-terminal serine residue of the mature proteins as determined for wheat (38) and S. cerevisiae (39) is indicated with an arrow. (*, a discrepancy exists between the deduced amino acid sequence of wheat atp6 and the N\textsubscript{H}\textsubscript{2}-terminal protein sequence data; see Footnote 4.)

...detected (data not shown; Ref. 13). The large, low abundance transcripts may be unprocessed forms of the atp6 transcript (see below).

Nuclease mapping experiments using a probe end-labeled at the XbaI site (nucleotide -437) and extending to the BanHI site (nucleotide -1090) resulted in protected fragments of 293 and 653 nucleotides (Fig. 4). These protected fragments correspond to a 5'-transcript terminus centered at -730 and the fully protected probe. This site (-730) is located within repeated region A (Fig. 2). This result, along with that from Northern analysis, indicates that the 5'-transcript terminus at -730 is most likely a processed form of a primary transcript that extends upstream of the sequenced region. Nuclease mapping experiments using probes labeled at a site within the atp6 coding region failed to identify any shorter Ogura atp6 transcripts (data not shown). This result is also consistent with Northern experiments that indicate that ORF-105 and atp6 are transcribed together as part of a polycistronic RNA in Ogura radish.

Although we do not yet know if ORF-105 is translated, its codon usage is similar to that of the normal and Ogura radish atp6, atp4, and cox1 genes. In addition, it is preceded by a 16-bp sequence that is 75% complementary to the 3' end of plant 18 S rRNAs (40). Because there is no similarity between...
characterization of Ogura Radish atp6

Fig. 4. Nuclease mapping of the 5' end of normal and Ogura radish atp6 transcripts. Top, autoradiographs of nuclease mapping of normal (1-3) and Ogura (4-6) atp6 using the probes A and B, respectively (shown below). Lane 1, probe A; lane 2, probe A plus 10 μg of normal radish mtRNA treated with mung bean nuclease; lane 3, probe A plus 20 μg of yeast tRNA treated with mung bean nuclease; lane 4, probe B; lane 5, probe B plus 10 μg of Ogura radish mtRNA treated with mung bean nuclease; lane 6, probe B plus 20 μg of yeast tRNA treated with nuclease; lane 7, 32P-labeled Bluescript KS+ digested with HindII. Protected fragments are marked with arrows. Sizes are shown in base pairs. Bottom, schematic illustration of probes used in nuclease mapping and the resulting protected fragments. Probe A (heavy line) was a 1347-bp fragment extending from -55 to the beginning of the clone, nucleotide -1292, and was uniformly labeled as the complement of the atp6 mRNA by primer extension. Protected fragments (thin lines) of 196, 207, and 1347 nucleotides were observed with probe A. Probe B (heavy line) was a 653-bp fragment extending from -457 to -1089 that was end-labeled with polynucleotide kinase. Protected fragments (thin lines) of 293 and 653 nucleotides were observed with probe B.

ORF-105 and any sequences in the data banks, the function of the putative polypeptide is unknown.

Genomic Organization of the atp6 Loci—To determine which normal radish sequences comprise the Ogura atp6 locus and where on the Ogura mitochondrial genome the normal atp6 flanking sequences reside, a series of Southern hybridizations was conducted. Deletion clones containing the atp6 coding region, as well as the normal and Ogura radish 5’ and 3’-flanking regions, were used to probe Southern blots containing single and double digests of normal and Ogura radish mtDNA. A summary of these results is shown in Fig. 6. The Ogura atp6 locus can be divided into three regions (A, B, and C), two of which are present in normal radish mtDNA (B and C). Region B, the atp6 coding region, is separated by 77 kb from region C, the Ogura atp6 3’-flanking sequence, in normal radish mtDNA. Most of region A, which contains the Ogura atp6 5’-flanking region and ORF-105 is not present in normal radish mtDNA. However, a probe from this region hybridizes weakly to many fragments in both DNAs, indicating that there are sequences present in this segment that are repeated at least four times in each genome (Fig. 6). Consistent with this result is the presence of a 162-bp repeated sequence, nucleotides -664 to -503 (Fig. 2), that has been identified upstream of normal radish atpA and cox1 and Ogura cox1 by sequence analysis. Thus, much of region A may be present in normal radish but in an extensively rearranged form. The three segments that comprise the normal radish atp6 locus are widely separated in the Ogura radish mitochondrial genome (Fig. 6). Part of the normal radish 3’ flank (region E),
which contains the CoxI-homologous sequence, is also repeated in the two genomes.

**Rearrangements Breakpoints**—The normal radish atp6 5'-flanking region (region D) was used in Southern hybridization experiments to identify an XbaI/ClaI 0.49-kb clone, which contains this region in Ogura radish mtDNA. This fragment was sequenced and aligned with the normal and Ogura atp6 5' flanks to identify the 5' rearrangement breakpoint (Fig. 7). The XbaI/ClaI 0.49-kb fragment shares a 66-bp sequence present in the 5'-flanking region of both normal and Ogura atp6. In addition, this fragment contains the trnFM gene that is present upstream of normal radish atp6 and co-transcribed with it. Because there is no sharp break in sequence identity, the exact site of rearrangement cannot be determined. The number of nucleotide differences (75% sequence identity) in a 118-bp segment (∼79 to +38) at the 5' end of atp6 is far greater than can be expected by nucleotide substitution alone, given our earlier measurement that the Ogura and normal radish mitochondrial genomes are on average 99.8% identical in nucleotide sequence (13). Indeed, the 848-bp segment following this divergent 118-bp stretch shows 99.8% sequence identity between the two radish genomes (Fig. 2). We postulate that the large number of nucleotide differences in this 118-bp region are the result of ancient duplication events, followed first by a lengthy period of nucleotide divergence and then by recent rearrangement events that led to the replacement of the normal radish −79 to +38 region with more recently divergent sequences. Where the sequences are identical, only that perfect alignment and sequence comparison, 450 bp of the Ogura sequence is shown; identical amino acids are represented by an asterisk. Where the sequences are identical, only that identical bases are shown as =, missing bases as −. To facilitate alignment and sequence comparison, 450 bp of the Ogura sequence (nucleotides −552 to −102) are not shown. The deduced sequence for the 105-amino acid ORF is that is co-transcribed with atp6.

**DISCUSSION**

**Rearrangements Associated with atp6**—Detailed analyses of atp6 from both normal and Ogura radish have revealed that the two loci are highly rearranged relative to one another. The sequences that comprise the normal atp6 locus, including a trnFM gene that is co-transcribed with atp6, are present in three widely separated regions of the Ogura mtDNA. Similarly, the 3' flank of Ogura atp6 is far removed from the atp6 gene in normal radish, whereas the 5' flank of Ogura atp6 is not present in normal radish mtDNA. Within this 5'-flanking region is a 105-amino acid ORF that is co-transcribed with atp6.

Although the Ogura atp6 5' flank is not present as an intact unit in the normal radish mitochondrial genome, a 161-bp sequence (Fig. 2, region B) from this region has been found in two locations in the normal genome and once in Ogura by DNA sequencing. Furthermore, hybridization data indicate that this sequence may be present in as many as four copies in each of the two genomes. Within the ORF-105 sequence regions are 44 and 35 bp that are directly repeated a total of two and three times, respectively. Thus, much of Ogurasppecific atp6 DNA consists of short repetitive sequences.

Analysis of the 5' and 3' recombination breakpoints failed to identify any specific sequence or structure common to them. However, that repeated sequences, albeit different sets of them, are present at rearrangement sites suggests that they may be involved in homologous recombination events. It is believed that plant mitochondrial genomes exist as mixtures of different sized circular molecules that interconvert via frequent homologous recombination across sets of direct repeats (17, 45). Thus, the rapid structural evolution that plant mitochondrial genomes undergo may also be the result of this recombination, albeit at a much lower frequency.

Despite the highly rearranged nature of the two atp6 loci, sequences common between them are highly conserved at the DNA sequence level (99.8% identity). This agrees with our earlier findings, based entirely on restriction site mapping, that the two genomes have undergone extensive rearrangement relative to one another, but that unarranged regions are highly conserved (99.8% identical) at the DNA sequence level (13).

**CMS and atp6 Alterations**—Our studies suggest two possible ways in which atp6 could be involved in Ogura CMS: 1) an altered, semifunctional atp6 polypeptide is synthesized in Ogura mitochondria; 2) synthesis of ORF-105 has a detrimen- tal effect on mitochondrial function. In Ogura radish, the region predicted to encode the 5' end of atp6 contains many nucleotide substitutions relative to normal radish. These changes result in the elimination of the proposed ribosome binding site and seven amino acid substitutions, the most critical involving a change from the putative initiator methionine to a leucine codon (Figs. 2 and 3). If our assignment of Met-1 as the start of the normal atp6 polypeptide is correct, we would predict that if Ogura atp6 is translated, translation should begin at a different start codon and produce a polypeptide with an altered NH₂ terminus.

Plant atp6 polypeptides appear to be similar to those of...
funghi in that they are synthesized with presequences. NH₂-
terminal sequencing of the S. cerevisiae and wheat⁴ mito-
chondrial atp6 polypeptides has identified the sequence S P L E D
as the mature NH₂ terminus. We predict that the normal
radial polypeptide should be similar to those of S. cerevisiae
(39) and wheat (38) and should also contain a serine at the
NH₂ terminus of the mature polypeptide. There are two
candidates for alternate start codons in Ogura atp6. The first
is an internal methionine (Met-23) and the second is an
Ogura-specific GTC codon (nucleotides 10-12). Translation
from Met-23 would result in an Ogura protein lacking, at
minimum, the first 13 amino acids of the mature polypeptide.
Although unusual start codons have yet to be documented in
plant mitochondria, they are known to function in animal
mitochondria (46) and in E. coli (47). Utilization of GTG as
the start codon would produce an Ogura atp6 polypeptide
with a shorter presequence and four amino acid differences. If
processed correctly, the mature protein would contain only
one amino acid substitution: Asp 13 → Ser. Although it has
been suggested that the atp6 presequences may be involved
in membrane localization (32), the role of these sequences as
well as the identity of residues required for correct processing
are unknown. Therefore, if Ogura atp6 is translated from the
GTC codon, it is not clear whether the mutant protein would
be localized and processed correctly. Frameshift and nonsense
mutations in the S. cerevisiae oli2 (atp6) gene result in respira-
tory deficient, mit⁻ mutants (48), which can be predicted to
be lethal mutations in plants. It is also possible that Ogura
atp6 is not translated, but this seems unlikely.

It should also be noted that although there are several
examples of chimeric genes and pseudogenes in plant mito-
chondria (6, 10, 33, 40, 49), most often it is a normal
copy of the gene elsewhere in the genome. The Ogura radish
genome contains a single copy of atp6. The other only example
of an altered single copy mitochondrial gene is cox1 in the
9E cytoplasm of sulfur (10). It has been postulated that this
alteration of cox1 may be involved in sulfur CMS (10).

Although subunit 6 of the ATPase is universally encoded
by mitochondria, the polypeptide is highly variable in both
length and primary sequence. Moreover, analysis of yeast
coli- mutants suggests that the protein can tolerate amino
acid substitutions at many locations without a gross loss of
function (48). Thus, an altered Ogura atp6 polypeptide may
retain sufficient activity for ATP synthesis during most stages
of growth and development but fail during microsporogenesis.
This idea is supported by cytological and chemical evidence
indicating that aborted pollen grains in male-sterile cyto-
plasmic plants appear, in general, to have starved to death (15, 50).

A second possible role of the Ogura atp6 locus in CMS
could involve ORF-105. In the male sterility-T-cytoplasm of
maize, the gene T-urf13, generated through a series of re-
arrangements, has been shown to encode a 13-kDa polypep-
dide found to be associated with T-toxin sensitivity and male-
sterility (6-8). This polypeptide is found associated with the
membrane fraction of male-sterile mitochondria and, by anal-
ysis to its action in E. coli cells, is believed to disrupt mito-
chondrial respiration through dissipation of the proton gra-
dient (7). The C-terminal half of ORF-105 is hydrophobic and
characteristic of membrane-spanning domains. It is possible
that ORF-105 is analogous to the 13-kDa polypeptide of maize

⁴ A discrepancy exists between the reported NH₂-terminal amino
acid sequence of the mature wheat polypeptide (38) and the amino
acid sequence deduced from the atp6 nucleotide sequence (34). The
DNA sequence predicts a threonine residue at the N terminus iden-
tified as serine by protein sequencing.

CM-T and also has a detrimental effect on mitochondrial
function in Ogura radish.

Nuclear restorer genes have no effect on Ogura atp6 tran-
script patterns (13). Therefore, if either the atp6 polypeptide
or ORF-105 are causally related to Ogura CMS, we would
expect the restorer genes to act by either 1) supplying a
functional ATPase subunit 6 polypeptide, 2) supplying an
altered form of a different ATPase subunit to compensate for
the atp6 defect, or 3) blocking the expression or function of
ORF-105. Although both atp6 and ORF-105 are good can-
didates for being involved with Ogura CMS, further studies,
including experiments designed to investigate the ATPase
subunit 6 and ORF-105 polypeptides, are necessary to deter-
mine what role, if any, they have in this cytoplasmically
inherited trait.

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