The region of yeast mitochondrial DNA between 10.7 and 17.9 map units has been characterized by restriction analysis and DNA sequencing. The DNA sequence was obtained from the partially overlapping genomes of the two ρ− mutants DS200/A1 and DS302. Two tRNA genes have been found in the sequence upstream of the oxil locus. The deduced secondary structures indicate that the genes code for the methionine (5′-CAU-3′) and asparagine (5′-GUU-3′) tRNAs of yeast mitochondria. The region between 10.7 and 17.9 map units contains two reading frames. One of these corresponds to the oxil gene previously shown to code for subunit 2 of cytochrome oxidase (Coruzzi, G., and Tzagoloff, A. (1979) J. Biol. Chem. 254, 9324-9330; Fox, T. D. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 6534-6538). The second reading frame can potentially code for a basic protein with 386 amino acid residues. It is not known at present if this putative gene is translated in vivo.

Northern blots of wild type mitochondrial RNA were hybridized to single-stranded probes from the oxil gene and flanking regions. The results of these analyses indicate that the primary transcript of the oxil region is a high molecular weight RNA (larger than 3 kilobase pair) which is processed in discrete steps to a mature 850-nucleotide messenger. The 5′ leader of the messenger has been established to be 54 nucleotides long and to have a sequence identical with that of the genomic DNA immediately upstream of the oxil gene.

The oxil locus of yeast mitochondrial DNA (mtDNA) has been shown to code for subunit 2 of cytochrome oxidase (1, 2). These earlier studies indicated the gene to be 756 nucleotides long, spanning the wild type mtDNA from 14.2 to 15.3 map units. The agreement of the known amino acid sequence of bovine subunit 2 (3) and the deduced sequence of the yeast protein suggested a co-linear gene lacking intervening sequences (1, 2). In the present study, we have extended the DNA sequence analysis to the regions of mtDNA flanking the oxil locus. The new data provide a continuous sequence of the genome from 10.7 to 17.9 map units. The region downstream from oxil has been found to contain the genes of the asparagine and methionine tRNAs. The DNA sequence of the region downstream from oxil reveals the presence of a new reading frame (referred to as RF1) potentially capable of coding for a protein with 386 amino acid residues. This reading frame occurs in the same DNA strand as the subunit 2 gene. Analyses of the transcripts originating from the oxil region have enabled us to characterize the subunit 1 messenger and to identify some of the precursors to the messenger.

MATERIALS AND METHODS

Yeast Strains—The genotype and sources of the strains of Saccharomyces cerevisiae are listed in Table I. The following protocol was used to isolate DS302. The respiratory-competent strain S. cerevisiae D273-10B/A1 was mutagenized with ethidium bromide under non-growing conditions (10) and the clone DS300 selected for the retention of markers between the cap and par loci (Table II). This mutant was subjected to two additional cycles of mutagenesis with ethidium bromide to obtain DS302. The marker retention of DS302 was confined to the oxil locus. The details of the genetic manipulations have been described previously (6).

Purification of Mitochondrial DNA and RNA—The ρ− mutants were grown at 30 °C in 2% glucose medium supplemented with 1% yeast extract and 1% peptone. Cells were harvested in stationary phase and mitochondria were prepared by the method of Faye et al. (11). mtDNA was extracted with 2% Sarkosyl and purified on CsCl density gradients (12). Mitochondrial RNA was prepared by the method of Bonitz et al. (13) from the wild type S. cerevisiae D273-10B/A1.

Restriction Endonuclease Analysis—Restriction enzymes were purchased from New England Biolabs. All digestions were performed at 37 °C in a buffer containing 10 mM Tris-Cl, pH 7.5, 6 mM MgCl2, 6 mM 2-mercaptoethanol. The digestion products were analyzed on 1 or 2% agarose gels using the Tris/borate buffer system of Peacock and Dingman (14). Ex174 RF DNA digested with Hae III (15) and λ-DNA digested with HindIII (16) were used as molecular weight standards.

Preparative Isolation of Restriction Fragments—mtDNA of DS200/A1 and DS302 (100-200 μg) were digested with HindIII and Hpa II, respectively. The restriction fragments were separated on an 0.8-cm-thick slab of 1% agarose. The gel was stained with ethidium bromide and sections containing the desired fragments were cut out. The DNA was eluted from the gel electrophoretically and further purified by extraction with phenol and several precipitations with alcohol in the presence of 2 M ammonium acetate.

DNA Sequencing—mtDNA digested with single or combinations of restriction endonucleases was 5′-end labeled with [γ-32P]ATP (2000–3000 Ci/mmol, New England Nuclear Corp.) in the presence of T4 polynucleotide kinase (17). The labeled fragments were separated into the single strands on 4, 6, or 8% polyacrylamide gels and sequenced by the method of Maxam and Gilbert (17).

Northern Blots—Yeast mtRNA was denatured in the presence of 10 mM methyl mercaptan hydroxide and 2 μg were loaded on 0.6-cm-wide gels of 1.2% agarose, 10 mM methyl mercaptan gels. After electrophoresis at 5 V/cm for 4–5 h, the gel was treated with 14.3 mM 2-mercaptoethanol, stained with ethidium bromide, and photographed. The RNA was transferred to DBM paper by the method of Alwine et al. (18). Single-stranded DNA fragments 5′-end labeled with 32P were hybridized to DBM strips overnight (19). The strips were exposed for 1–2 days to Kodak XR-1 film with an intensifying screen.
**Table I**

*Names and genotype of S. cerevisiae strains*

| Strain       | Genotype            | Reference |
|--------------|---------------------|-----------|
| D273-10B/A21 | amet⁺, HpaI, PstI    |           |
| D273-10B/A1  | amet⁺, HpaI, PstI    |           |
| KL14-4A     | a, adel1, p⁺, oxil1  |           |
| CB11        | a, adel1, p⁺         | R. B. Needleman |
| aM9-94-4B   | a, adel1, p⁺, oxil1  |           |
| aM9-3-6C    | a, adel1, p⁺, oxil2  |           |
| aM5-16-2D   | a, adel1, p⁺, oxil3  |           |
| aM7-40-5B   | a, adel1, p⁺, cryl   |           |
| aM17-162-4D | a, adel1, p⁺, cryl1  |           |
| aM338-45-6A | a, adel1, p⁺, phol2  |           |
| aM19-229-24C| a, adel1, p⁺, phol1  |           |
| aM7-37-7C   | a, adel1, p⁺, tRNAR  |           |
| aX14-25-15B | a, adel1, p⁺, tRNA   |           |
| DS200/A1    | amet⁺                |           |

_**TABLE II**

*Genotypes of **p** mutants*

- Minus and plus signs indicate the loss or retention of the various loci. In addition to the markers shown in the table, the **p** mutants were also deleted with respect to oxil, cotl, cobl, oti1, and phol2.

**RESULTS**

**Restriction Maps of the DS200/A1 and DS302 mtDNA Segments**—The two **p**mutants used in the present study were isolated from the wild type parental strain _S. cerevisiae_ D273-10B/A21. The restriction map of the mtDNA segment retained in the mutant DS200/A1 has been reported previously (1). The mitochondrial genome of this mutant includes the wild type sequence from 10.7-17 map units (Fig. 1). The second mutant DS302 was obtained after several cycles of mutagenesis of the wild type parent with ethidium bromide. After two additional washes with 50% ethanol, the material was dried, dissolved in 0.1 M formamide, heated at 80°C for 3 min and loaded onto a DNA sequencing gel (21).

**Transcripts from the oxil Region of Yeast mtDNA**

- The two **p** mutants used in the present study were isolated from the wild type parental strain _S. cerevisiae_ D273-10B/A21.
- The DNA sequences of the two segments. The DS302 segment lacked two _Hpa_II fragments (fragments 2 and 4 in Fig. 1) and had an extra 620-base pair _Hpa_II fragment (fragment 5) that was mapped at the extreme right hand side of the segment.

**Nucleotide Sequence of the DS200/A1 and DS302 mtDNA Segments**—Together the segments of DS200/A1 and DS302 cover the region of the wild type mtDNA from 10.7 to 17.9 units. The restriction fragments used for the sequence analysis are shown in Fig. 2. Most of the digestions were done directly on the mitochondrial DNA of each mutant. In some instances, however, _Hpa_II and _Hin_II restriction fragments were isolated on a preparative scale and subjected to secondary cleavages.

- Single-stranded restriction fragments were hybridized either to total mitochondrial DNA or to the 8-14 sRNA fraction obtained from the sucrose gradient. The hybridizations were done in 50 mM Tris/acetate, pH 8.4, 6 mM Mg acetate, 0.1 M NaCl, 10 µM concentration of the four deoxynucleoside triphosphates, 10 µg/ml of actinomycin D, and 0.2 µM of [α-32P]-ATP (200-300 or 3000 Ci/mmol, New England Nuclear Corp.). The mixture was divided into 4 equal parts (70 µl) and to each was added one of the four dideoxynucleoside triphosphates (120 µM final concentration) to inhibit the reverse transcriptase (20). The reaction was started by the addition of 6 units of reverse transcriptase (J. W. Beard, Life Science, St. Petersburg, FL) and allowed to proceed for 15 min at 42°C. The reactions were chased to completion by the addition of 15 µl of a solution 250 µM in each of the four deoxynucleoside triphosphates and further incubated for 15 min at 42°C. The reactions were stopped with an equal volume of 4 M ammonium acetate and the cDNA was precipitated with 3 volumes of ethanol. The precipitate was dissolved in 280 µl of a solution containing 50 mM Tris-acetate, pH 7.5, 1 M NaCl, and 1 mM EDTA. The RNA was size-fractionated by centrifugation at 22,000 rpm in a Spinco SW27 rotor for 27 h at 15°C. The gradient was divided into 17 fractions which were analyzed for the size distribution of the RNA on agarose gels.

- Single-stranded restriction fragments were hybridized either to total mitochondrial DNA or to the 8-14 sRNA fraction obtained from the sucrose gradient. The hybridizations were done in 50 µl at 68°C. Following incubation at 42°C for 90 min, the mixture was diluted with 1.0 volume of 4 M ammonium acetate and the RNA was precipitated with 3 volumes of ethanol. The precipitate was dissolved in 280 µl of a solution containing 50 mM Tris-acetate, pH 7.5, 6 mM Mg acetate, 60 mM NaCl, 10 mM dithiothreitol, 120 µM concentration of the four deoxynucleoside triphosphates, 10 µg/ml of actinomycin D, and 0.2 µM of [α-32P]-ATP (200-300 or 3000 Ci/mmol, New England Nuclear Corp.). The mixture was divided into 4 equal parts (70 µl) and to each was added one of the four dideoxynucleoside triphosphates (120 µM final concentration) to inhibit the reverse transcriptase (20). The reaction was started by the addition of 6 units of reverse transcriptase (J. W. Beard, Life Science, St. Petersburg, FL) and allowed to proceed for 15 min at 42°C. The reactions were chased to completion by the addition of 15 µl of a solution 250 µM in each of the four deoxynucleoside triphosphates and further incubated for 15 min at 42°C. The reactions were stopped with an equal volume of 4 M ammonium acetate and the cDNA was precipitated with 3 volumes of ethanol. The cDNA was reprecipitated from 2 M ammonium acetate. After two additional washes with 90% ethanol, the material was dried, dissolved in 0.1 M formamide, heated at 80°C for 3 min and loaded onto a DNA sequencing gel (21).
Transcripts from the ox1 Region of Yeast mtDNA
Transcripts from the ox1 Region of Yeast mtDNA

Fig. 3. DNA sequences of yeast mtDNA from 10.7 to 17.9 map units. The sequence shown is that of the sense strand. The sequences of the tRNA genes are in lower case. Only the ATG initiation and TAA termination codons of the ox1 gene have been included in the sequence (for the complete sequence, see Refs. 1 or 2). The amino acid sequence encoded in the unidentified reading frame (+998 to +2158) is based on the assignments of the universal code except for UGA which has been shown to code for tryptophan (2, 23) and the CUN family which codes for threonine in yeast mitochondria (24). The sequence enclosed by the brackets may have one or two extra nucleotides.

Prior to 5'-end labeling. All the sequence data were obtained on 5'-end-labeled single strands. With a few exceptions, most of the restriction sites were sequenced from neighboring sites (Fig. 2). The nucleotide sequence is presented in a linear form starting with the sequence (for the complete sequence, see Refs. 1 or 2). The amino acid sequence encoded in the unidentified reading frame (+998 to +2158) is based on the assignments of the universal code except for UGA which has been shown to code for tryptophan (2, 23) and the CUN family which codes for threonine in yeast mitochondria (24). The sequence enclosed by the brackets may have one or two extra nucleotides.

Aside from the ox1 gene (nucleotides +1 to +756), there are three other sequences of interest. This first is a 72-nucleotide-long sequence (−2182 to −2111) that can be folded to form a cloverleaf structure with a 5'-GUU-3' anticodon. We tentatively identify this to be the gene of the mitochondrial asparagine tRNA. The second tRNA gene is located at 12.4 map units (2, 23) and the CUN family which codes for threonine in yeast mitochondria (24). The sequence enclosed by the brackets may have one or two extra nucleotides.

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Since the tRNAs have not been sequenced, the modified bases are not known. Although we believe the sequence shown to be correct, there could be a single-nucleotide insertion or deletion which would cause a frame to terminate either at nucleotide +1905 or +1928. Assuming that RF1 is 1161 nucleotides long, it can generate a protein with a molecular weight of 48,000. The sequence has several unusual features. The G + C content is only 19%, a value significantly lower than found in other yeast mitochondrial genes (2, 30–32). While most of the codons in RF1 are similar to those present in the genes of apocytochrome b and of the cytochrome oxidase and ATPase subunits, there are some exceptions. Most notable is the occurrence of CGU codons for arginine and the UGG codon for tryptophan (Table III). Neither of these codons has been found in the identified yeast mitochondrial genes. It is also of interest that RF1 traverses at least part of a G + C cluster (+1862 to +1902) whose sequence is virtually identical with another cluster upstream of the asparagine tRNA gene (−2400 to −2360). Similar types of sequences are known to be scattered in many other regions of the genome, although none have been found within coding sequences (33, 34).

The reading frame reported here is highly reminiscent of the reading frames recently discovered in the intron regions of several mitochondrial genes. Table IV compares the amino acid compositions of the proteins encoded by these putative genes. In each case, the proteins are characterized by a high content of lysine, asparagine, tyrosine, leucine, and isoleucine, i.e., amino acids with an A and/or U in the first two positions of their codons.

**Transcription of the oxil Region—**A number of transcripts, the smallest of which is an 11 S RNA, have been reported to hybridize specifically to the oxil region of yeast mtDNA (38, 39). That the 11 S transcript is the subunit 2 messenger is supported by *in vitro* translation experiments. DeRonde et al. (40) have shown that an 11–13 S fraction of yeast mitochondrial RNA can direct the synthesis of subunit 2 in a wheat germ translation system supplemented with a yeast UGA suppressor. While the larger transcripts might also function as messengers, this has not been experimentally verified and the most reasonable assumption is that they are incompletely processed precursors of the 11 S RNA.

As a first approach to characterizing the oxil transcripts, we have prepared a series of single-stranded DNA fragments with defined sequences from within the oxil gene and its flanking regions. The probes were hybridized to total mitochondrial RNA separated on agarose gels and transferred to DBM paper. The restriction fragments used in these hybridizations are shown in Fig. 5. One of the fragments had a sequence upstream from the gene (probe C); others originated either from within the gene (probes A, B, K) or from RF1 (probes D–H). Together, the fragments spanned the region of DNA from 13.7 to 18.0 units.

A representative autoradiograph of a Northern blot challenged with the pure oxil probe C is shown in Fig. 6. The probe is seen to hybridize to a transcript with an estimated size of 850 nucleotides. This RNA species corresponds to the 11 S transcript previously identified as the mature subunit 2 messenger (39, 40). The Northern blot reveals the presence of at least 9 other large transcripts with the oxil sequence. Although we have assigned sizes to these less abundant transcripts, they are only approximate values based on migration relative to the 15 S and 21 S rRNAs.

The results of the hybridizations with the different probes are summarized in Table V. Probes A and B with different sequences of the oxil gene hybridized to 10 different transcripts with sizes ranging from approximately 4 to 0.85 kb. Probe K from the sense strand of the oxil did not detect these transcripts (Fig. 6). This negative result confirms the specificity of the hybridizations and suggests that either only one strand of the DNA in this region is transcribed or gives rise to stable RNAs. Probe C whose 5′ end lies at the first *Hinfl* site immediately upstream from the gene hybridizes to all the transcripts except for the 0.85-kb messenger.

Fig. 4. **Secondary structures of the tRNAs** (A) and tRNAs (B). The structures shown are based on the DNA sequences of the genes. Since the tRNAs have not been sequenced, the modified bases are not known.
Five of the probes used (D through H) contained RF1 sequences. Probe D was complementary to RF1. Probes E through H were complementary to the opposite strand, whose sequence does not contain any reading frame of significant length. Probe D hybridized only to some of the high molecular weight transcripts that were detected by the oxil probes. For example, transcripts 2, 6, 7, 9, and 10 failed to hybridize to probe D, indicating that their 3' termini cannot extend more than 300 nucleotides beyond the end of the oxil gene. It is of interest that probe D did not reveal any new abundant transcript. Unexpectedly, both probes E and F with sequences from the sense strand hybridized to a new transcript of 1.5 kb (Fig. 6). Since this transcript was not detected by probes G through J, at most it contains a sequence of 0.7 kb from 16.6 to 17.6 map units. The discrepancy between the length of sequence homology and the size of the transcript cannot be accounted for but could indicate that it is a spliced product.

The following conclusions can be drawn from the Northern blot hybridizations. 1) The primary transcript of the oxil gene is a high molecular weight RNA with a minimal size in excess of 3 kb. 2) The mature messenger of subunit 2 of cytochrome oxidase is most likely formed by stepwise cleavages of a primary transcript at both the 5' and 3' ends. The hybridizations to upstream and downstream probes further suggest that the same precursor may be processed by alternative pathways. At least 9 intermediates are sufficiently stable to be detected as discrete size RNA species. 3) Transcripts with the sequence of RF1 are found only in high molecular weight oxil precursor RNAs. Although a unique transcript from the RF1 region does exist, this RNA contains the sequence complementary to the reading frame.

**5' Terminus of the Subunit 2 Messenger—**Based on its estimated size, the subunit 2 messenger exceeds the known length of the gene by approximately 100 nucleotides. In order to further characterize its composition, parts of the messenger were sequenced by reverse transcription in the presence of dideoxynucleotides (20). Two different restriction fragments were used as primers for the reverse transcription. The first

**Fig. 6. Autoradiographs of Northern blots.** Total mitochondrial RNA was separated on a 1.2% agarose gel. The gel was stained with ethidium bromide and photographed (lanes 5 and 6). Following transfer of the RNA to DBM paper, the strips were hybridized with single-stranded fragments labeled at their 5' ends with 32P. The strip with probe B (lane 1), probe A (lane 2), probe D (lane 3), probe K (lane 4), and probe E (lane 7). The strip with probe B (lane 1) was overexposed in order to detect all oxil transcripts. The transcripts have been numbered in the left- and right-hand margins. The calibration scale used to calculate the sizes of the transcripts is shown next to the ethidium bromide-stained gels.

| TABLE IV |
| --- |
| **Amino acid compositions of proteins encoded in putative yeast mitochondrial genes** |
| All values are expressed as mole per cent. |

| RFI* | Subunit 1 introns* | Apocytochrome b introns | 21S rRNA intron* |
| --- | --- | --- | --- |
| Ala | 1.5 | 3.1 | 2.9 | 3.0 |
| Arg | 2.8 | 5.6 | 5.4 | 3.6 |
| Asn | 12.4 | 8.6 | 9.2 | 151 |
| Asp | 5.2 | 4.2 | 4.1 | 3.6 |
| Cys | 0.8 | 1.7 | 2.3 | 0.9 |
| Glu | 41 | 1.4 | 1.3 | 1.8 |
| Glu | 1.0 | 1.6 | 3.8 | 2.9 |
| Gly | 2.3 | 5.9 | 6.0 | 4.5 |
| His | 0.8 | 2.0 | 3.6 | 1.2 |
| Ile | 13.5 | 13.2 | 9.4 | 8.7 |
| Leu | 9.8 | 8.6 | 9.9 | 10.8 |
| Lys | 10.9 | 10.5 | 9.3 | 9.3 |
| Met | 1.0 | 0.9 | 2.2 | 0.9 |
| Phe | 4.4 | 3.3 | 4.3 | 3.0 |
| Pro | 4.1 | 3.7 | 3.1 | 1.8 |
| Ser | 5.4 | 5.1 | 7.5 | 7.2 |
| Thr | 6.7 | 4.8 | 4.8 | 6.0 |
| Trp | 2.1 | 0.9 | 0.6 | 1.5 |
| Tyr | 7.2 | 4.6 | 4.7 | 8.4 |
| Val | 3.6 | 5.0 | 5.5 | 4.5 |

*Refers to the unidentified reading frame found downstream from the oxil gene.  
*Taken from Bonitz et al. (13).  
*Calculated from the 21S rRNA intron sequence reported by Dujon (37).  
*First intron of the apocytochrome b gene in S. cerevisiae 8.4.13 (37).  
*Second intron of the apocytochrome b gene in S. cerevisiae 777-3A (38).
was a *Hinf*-*Rsa*I fragment complementary to the gene sequence from nucleotides +434 to +506. The sequence of the DNA copy synthesized from the 3' end of this primer was perfectly complementary to the previously reported sequence of the gene (Fig. 7 and Ref. 1). The second primer was a *Hinf*-*Rsa*I fragment with a 3' end 22 nucleotides upstream from the initiation codon and a 5' end at nucleotide 50 internal to the gene. The cDNA made from this primer is shown in Fig. 8. The cDNA extension is 32 nucleotides long and is complementary to the sequence upstream of the distal *Hinf* site up to nucleotide -54 (see Fig. 3). The 11 S transcript therefore has a 54-nucleotide leader whose sequence matches the corresponding DNA sequence. The leader combined with the 756-nucleotide-long gene sequence comes close to the measured size of the 11 S transcript. From S1 mapping, Fox and Boerner (39) have concluded that the 3' extension of the messenger must be less than 75 nucleotides in length.

Although the 5' and 3' ends of the messenger are generally consistent with the results of the Northern blot experiments, it is not completely clear why probe C which contains part (32 nucleotides) of the leader sequence did not hybridize to the 11 S transcript. A possible explanation is that the conditions used for the hybridizations were too stringent to favor formation of the hybrid, particularly since the complementary region in this probe was confined to 32 nucleotides consisting almost entirely of A + T.

![Fig. 7. Sequence of the cDNA copied from a region internal to the ox1II gene. The primer for the reverse transcriptase was a 73-nucleotide-long *Hinf*-*Rsa*I fragment (+433 to +506). The single-stranded fragment from the nonsense strand was hybridized to total mitochondrial RNA and reverse-transcribed in the presence of deoxynucleoside triphosphates as described under "Materials and Methods."](image)

![Fig. 8. The 5' leader of the ox1I messenger. The primer used for the reverse transcriptase was a 72-nucleotide-long *Hinf*-*Rsa*I fragment (-22 to +50). The single-stranded fragment complementary to the sense strand was hybridized to the 8-14 S RNA fraction enriched on a sucrose gradient and reverse-transcribed in the presence of deoxynucleoside triphosphates. The sequence read from the gel is complementary to the region upstream of the ox1I gene from nucleotides -23 to -54 (see sequence in Fig. 3).](image)

**DISCUSSION**

The two *p−* mutants, DS200/A1 and DS302, were selected for the retention of markers in the ox1I locus. The mitochondrial genomes of these mutants were established to span the region of the wild type map from 10.7 to 17.9 units. The nucleotide sequences of the mtDNA segments have enabled us to identify the genes of the asparagine and methionine tRNAs. Both genes are located upstream of the ox1I locus at 10.9 (asparagine) and 12.4 (methionine) map units and are transcribed from the same DNA strand as subunit 2 of cytochrome oxidase (1, 2).

In addition to the ox1I gene, the region sequenced contains a second reading frame (RF1) that has an ATG initiator at 15.5 units and is probably continuous for 1161 nucleotides. The protein encoded in this putative gene consists of 388 amino acid residues and has a molecular weight of 48,000. Even though this sequence is not part of an intron, it is in many respects similar to a number of reading frames recently found in the intervening regions of yeast mitochondrial genes (13, 35-37). RF1 has a G + C content of only 19% and is rich in codons for asparagine, lysine, and tyrosine. It also utilizes codons such as the CGU codon for arginine and the UGG codon for tryptophan which up to now have only been found in intron reading frames.

The transcripts copied from the ox1 region have been characterized by hybridization of single-stranded DNA probes
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to total mitochondrial RNA by the Northern blot technique (18). These studies indicate that the mature messenger of subunit 2 is formed by a stepwise cleavage of large precursor transcripts. The processing appears to involve the removal of both 5’ and 3’ sequences from large precursor transcripts. At least 9 intermediates with discrete sizes are detected by probes containing either structural gene or flanking sequences.

Several lines of evidence indicate the mature messenger of subunit 2 to be an 11 S RNA. It is the most abundant and smallest RNA species to hybridize to probes from the oxI gene (38, 39). More convincingly still, DeRonde et al. (40) have been able to synthesize subunit 2 in an in vitro wheat germ system programmed with an 11–13 S-enriched fraction from yeast mitochondrial RNA. In the present study, the 5’ terminus of the 11 S transcript has been determined from its cDNA sequence. The leader of the 11 S transcript starts 54 nucleotides upstream from the AUG initiator and has a 3’–5’ end. The processing appears to involve the removal of endonucleolytic cleavage or exonucleolytic trimming of one of the larger precursors.

Probes complementary to RF1 detect only high molecular weight RNAs which also hybridize to probes from the oxI gene. A single unique transcript with an estimated size of 1.5 kb, however, hybridizes specifically to probes with sequences from the sense strand of RF1. The function of this transcript is not clear to us at present.

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