A Maturase-like Subunit of the Sequence-specific Endonuclease Endo.SceI from Yeast Mitochondria*

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Some yeast strains possess a sequence-specific endonuclease, Endo.SceI, which is a heterodimeric enzyme localized in mitochondria. The larger subunit (75 kDa) of Endo.SceI, encoded by a nuclear gene (ENS1), is transported from the cytosol into the mitochondria. In this study, we determined the partial amino acid sequence of the smaller subunit (50 kDa) of Endo.SceI. The determined sequence matched well the partial sequence deduced from a mitochondrial open reading frame (RF3). The RF3 locus is known to exhibit polymorphism since this reading frame in some yeast strains is supposed to encode a maturase-like protein, whereas in other strains, the frame is interrupted by GC clusters, which thus break the frame. Southern blot analysis of various yeast strains showed that the continuity of RF3 is correlated with the presence of Endo.SceI activity. These data indicate that the continuous RF3 sequence is a functional gene (ENS2) coding for the smaller subunit of Endo.SceI. The results of cytoduction, by which the continuous RF3 sequence was transferred into a yeast strain lacking mitochondrial DNA, confirmed this conclusion. This study suggests the involvement of Endo.SceI in genetic recombination of mitochondrial DNA.

There is a growing amount of evidence showing that sequence-specific endonucleases which cause double-strand breaks are involved in site-specific gene conversion (Szostak et al., 1983; Colleaux et al., 1986; Xiong and Eickbush, 1988; Wenzlau et al., 1989; Delahodde et al., 1989). In addition, recent data showed that a double-strand break also appears at the initiation sites for general recombination (Nicolas et al., 1989; Sun et al., 1989).

In Saccharomyces cerevisiae, three sequence-specific endonucleases have been identified as initiators of the site-specific gene conversion process: HO-endonuclease for mating type switching (Kosirken et al., 1983) and α- and α4-endonucleases for mitochondrial intron propagation (Jaquier and Dujon, 1988; Macreudie et al., 1985; Wenzlau et al., 1989; Delahodde et al., 1989). These endonucleases display several common enzymatic properties (Colleaux et al., 1986; Wenzlau et al., 1989; Delahodde et al., 1989). First, they show complex sequence specificities, unlike prokaryotic restriction endonucleases, because the sequences recognized and required for digestion are longer than 18 base pairs with no apparent dyad symmetry. Second, DNA is cleaved by these enzymes to create cohesive ends with 4-base 3'-overhangs.

Endo.SceI, purified from S. cerevisiae IAM4274 (Watabe et al., 1981, 1983), was the first example of a eukaryotic sequence-specific endonuclease which causes in vitro double-strand scission at well-defined sites on DNA. The cleavage mode of Endo.SceI is similar to those of the other sequence-specific endonucleases described above, and its cutting sites are expected to be located at every several thousand base pairs on the genome, suggesting that this enzyme is involved in general genetic recombination in yeasts (Shibata et al., 1984). Endo.SceI has been revealed to be a heterodimer of 75- and 50-kDa subunits, both of which are required for full enzymatic activity (Watabe et al., 1983; Nakagawa et al., 1988). In our previous study, the 75-kDa subunit was shown to be encoded by an essential nuclear gene, ENS1, which is a member of the 70-kDa heat shock protein family (Morishima et al., 1990). The sequence data suggested that the 75-kDa subunit is not a catalytic subunit and that the 50-kDa subunit thus bears the active site of the endonuclease. This conjecture has been confirmed by the results of recent biochemical analyses. The 75-kDa subunit has an N-terminal leader sequence which is a potential targeting signal for mitochondrial localization of the protein. As expected from this result, both the 75-kDa subunit and the Endo.SceI activity were detected almost exclusively in the mitochondria, indicating the presence of the 50-kDa subunit in the mitochondria (Morishima et al., 1990). To elucidate the molecular properties of the 50-kDa subunit, particularly its primary sequence and subcellular localization, we identified and analyzed the gene for the 50-kDa subunit.

EXPERIMENTAL PROCEDURES

Materials—Restriction endonucleases were purchased from Takara Biomedicals (Kyoto, Japan) and New England Biolabs, Inc. Escherichia coli DNA polymerase I (large fragment), T4 DNA ligase, exonuclease III, manganese nuclease, bacterial alkaline phosphatase, and polynucleotide kinase were obtained from Takara Biomedicals. [α-32P]dCTP (~110 TBq/mmol), [γ-32P]ATP (>185 TBq/mmol), and α-32P-4dTTP (>22 TBq/mmol) were from The Radiochemical Centre (Amersham, England). All other reagents were of analytical grade.

Yeast Strains and Microbial Techniques—The yeast strains used in this study are listed in Table I. Yeast media were essentially as described by Sherman et al. (1986).

Cytoductants were constructed by the method of Lancashire and Mattoon (1979) as follows. First, a ρ0 strain (N548-1) was isolated from a kar1 strain (deficient in nuclear fusion during mating), N548, by treatment of the cells with ethidium bromide (Sherman et al., 1986). The absence of mitochondrial DNA was checked by Southern blot analysis of total DNA using purified mitochondrial DNA from S. cerevisiae CG379, fragmented by EcoRI digestion and radiolabeled with [32P]dCTP (~110 TBq/mmol). The results of these analyses suggested that the strain was a cytoductant, as indicated by its growth on minimal medium lacking uracil and its sensitivity to 5-fluoroorotic acid (5-FOA) (Lancashire and Mattoon, 1979).

1 K. Kawaski, M. Takahashi, T. Ando, and T. Shibata, unpublished observations.

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Karl boring the mitochondria from IAM4274,NCYC74, MT371a-6B, and X1049-9C, respectively, were isolated. Selection of these strains with N548-1 by protoplast fusion (Spencer et al., 1983) using strain JM109. Minipreps of plasmid DNAs were obtained from the culture collection of the Institute for Fermentation, Osaka, Japan.

**Table 1 Yeast strains**

| Strain | Genotype and remarks |
|--------|---------------------|
| 1. IAM4274 | From the culture collection of the Institute of Applied Microbiology (University of Tokyo) |
| 2.NCYC74 | S. uvarum MATa/MATa |
| 3. IF02109 | From the culture collection of the Institute for Fermentation, Osaka, Japan |
| 4. MT371a-6B | MATa spo7 leu-2-3-leu-2-112 his4-j19 trpl 1 ura3 |
| 5. D160-4D | MATa arg6 ura3 his3 hsl1 trpl2 ade6 met1 |
| 6. S50 | MATa met2 leu-2-1 |
| 7. X1049-9C | MATa spo7 trpl 1 ura3 his3 arg8 |
| 8. N548 | MATa kar1 his4 |
| 9. CG378 | MATa ade6 can1-leu-2-3-leu-2-112 trpl-289 ura3-52 |
| 10. CG379 | MATa ade6 his4-2 leu-2-3-leu-2-112 trpl-289 ura3-52 |
| 11. N548-1 | p1 derivative Sub568 |
| 12. CG378-1 | p1 derivative of CG378 |
| 13. CD6-1 | MATa ade5 can1-leu-2-3-leu-2-112 trpl-289 ura3-52 | ENS2 (IAM4274) |
| 14. CD6-2 | MATa ade5 can1-leu-2-3-leu-2-112 trpl-289 ura3-52 | ENS2 (NCYC74) |
| 15. CD6-3 | MATa ade5 can1-leu-2-3-leu-2-112 trpl-289 ura3-52 | en2 (MT371a-6B) |
| 16. CD6-4 | MATa ade5 can1-leu-2-3-leu-2-112 trpl-289 ura3-52 | en2 (X1049-9C) |

Strain Genotype
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3. IF02109 From the culture collection of the Institute for Fermentation, Osaka, Japan
4. MT371a-6B MATa spo7 leu-2-3-leu-2-112 his4-j19 trpl 1 ura3
5. D160-4D MATa arg6 ura3 his3 hsl1 trpl2 ade6 met1
6. S50 MATa met2 leu-2-1 |
7. X1049-9C MATa spo7 trpl 1 ura3 his3 arg8
8. N548 MATa kar1 his4
9. CG378 MATa ade6 can1-leu-2-3-leu-2-112 trpl-289 ura3-52
10. CG379 MATa ade6 his4-2 leu-2-3-leu-2-112 trpl-289 ura3-52
11. N548-1 p1 derivative Sub568
12. CG378-1 p1 derivative of CG378
13. CD6-1 MATa ade5 can1-leu-2-3-leu-2-112 trpl-289 ura3-52 | ENS2 (IAM4274)
14. CD6-2 MATa ade5 can1-leu-2-3-leu-2-112 trpl-289 ura3-52 | ENS2 (NCYC74)
15. CD6-3 MATa ade5 can1-leu-2-3-leu-2-112 trpl-289 ura3-52 | en2 (MT371a-6B)
16. CD6-4 MATa ade5 can1-leu-2-3-leu-2-112 trpl-289 ura3-52 | en2 (X1049-9C)

The smaller subunit was isolated by gel filtration (final concentration: 3.5%). Mixtures of peptides were separated with a Model 130A separation system (Applied Biosystems, Inc.) equipped with an RP-300 microbore reverse-phase column (2.1 x 50 mm). The following buffer systems were used: buffer A (0.1% trifluoroacetic acid) and buffer B (70% (v/v) acetonitrile in 0.1% trifluoroacetic acid). A linear gradient was formed, over 45 min, between 0% (buffer A) and 60% acetonitrile in trifluoroacetic acid at a flow rate of 100 μl/min. Elution of peptides were monitored by their absorbance at 215 nm, and peptide-containing fractions were collected manually for amino acid sequence analysis.

**N-terminal Sequence Analysis**—Amino acid sequence analysis was performed with an Applied Biosystems 470A Protein Sequencer.

**Computer Analysis**—The partial amino acid sequence of the smaller subunit was used to search for homology using the LSRCNP program (William and Lipman, 1983) at the National Biomedical Research Foundation Protein Identification Resource (Release 23.0). The oligonucleotide probe was designed based on a partial amino acid sequence (that of peptide 11 shown in Table II). The sequence of the complete degenerate 20-mer comprised the first letter for the glutamic acid codon through the second letter for the asparagine codon as follows: 5'-GAGA(G/A)GAT(A/G)GAA(T/C)AA(T/C)AA(T/C)AA-3'. The oligonucleotide was synthesized with a Du Pont-New England Nuclear Coder 300 DNA synthesizer and then purified with a NenSorb 20 cartridge (DuPont New England Nuclear). The 5'-end of the oligonucleotide was radioactively labeled with [γ-32P]ATP using a Megalabel end-labeling kit (Takara Biomedicals).

**Southern Blot Analysis**—Southern blot analysis of mitochondrial DNA using an oligonucleotide probe was carried out as follows. Mitochondrial DNA was prepared from S. cerevisiae IAM4274 cells by the method of Hudsip et al. (1980). The DNA was digested with restriction enzymes, and then the digestion products were subjected to agarose gel electrophoresis for Southern blot analysis (Southern, 1975). DNAs were electroblotted onto a Millipore nylon membrane (Micron Separations) as described by the manufacturer. The blotted membrane was hybridized to the 5'-end-labeled oligonucleotide in 6 x SSPE (SSPE: 10 mM Na2HPO4, pH 7.0, 1 mM EDTA, 0.18 M NaCl), 10 x Denhardt's solution (0.2% Ficoll 400 (Pharmacia LKB Biotechnology Inc.), 0.2% polyvinylpyrrolidone, and 0.2% bovine serum albumin), and 1% SDS at 39°C for 15 h. After hybridization, the membrane was washed twice with 6 x SSPE and 0.1% SDS at room temperature, and finally with the same solution at 39°C for 1.5 min.

For examination of the continuity of the ENS2 frame, total DNAs were prepared from various yeast strains by the method described by Sherman et al. (1986). The DNAs were digested with EcoRi and Hpal II and then subjected to agarose gel electrophoresis with a Tris/borate/EDTA buffer system (Maniatis et al., 1982). The digests were blotted onto a BA85 nitrocellulose membrane (Schleicher & Schuell) and then probed with the 1.7-kb EcoRl fragment of mitochondrial DNA isolated by means of a Multiprime labeling kit. Hybridization was performed at 65°C overnight, and the membrane was washed with 0.5 x SSC (SSC: 0.15 M sodium chloride containing 15 mM sodium citrate) and 0.1% sodium lauroylsarcosine at 65°C.

**DNA Sequencing**—A 1.7-kb EcoRl fragment of mitochondrial DNA from S. cerevisiae IAM4274 was cloned into plasmid pUC118. A series of truncated clones was produced by Dral partial digestion or exonuclease III treatment (Henikoff, 1984) of the cloned fragment. Sequencing was performed by the chain termination method (Sanger et al., 1977; Messing et al., 1981) with a-32P-dCTP and a 7-deaza sequencing kit (Takara Biomedicals).

**Enzyme Assays**—For assaying the sequence-specific endonucleases from various yeast strains, cell-free extracts were prepared with a French press (Morishima et al., 1990). The extracts were loaded on a TOSOH high performance liquid chromatography column (TSK G3000SW, 7.5 x 800 mm). The column was eluted with 50 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.2 M ammonium sulfate, 1 mM phenylmethylsulfonyl fluoride, 10 mM β-mercaptoethanol, and 10% glycerol at 0°C. Fractions containing proteins with apparent molecular masses of 120-130 kDa were pooled and then dialyzed against 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 mM β-mercaptoethanol, and 50% glycerol prior to the assay. EnDoScl activity was measured using plasmid pS05 DNA as a substrate as previously described (Watabe et al., 1984).
RESULTS

Amino Acid Sequence of Smaller Subunit—The smaller subunit from purified Endo.SceI was prepared for amino acid sequence analysis (Watabe et al., 1984). The smaller subunit (50 kDa) was well separated from the larger subunit (75 kDa) by SDS-polyacrylamide gel electrophoresis. N-terminal sequence analysis of the smaller subunit blotted from an SDS-polyacrylamide gel onto a membrane, however, was unsuccessful, probably because the N terminus was blocked. To obtain proteolytic fragments for amino acid sequencing, the smaller subunit was subjected to in situ digestion on a nitrocellulose membrane (Aebersold et al., 1987) with a lysine-specific protease. After elution of the mixture of proteolytic fragments from the membrane, eight fragments were purified by high performance liquid chromatography (Fig. 1). These fragments were selected because they showed symmetrical elution profiles, indicating their purity as high enough for the sequence analysis. The primary sequences of these peptides were determined by automated Edman degradation.

Table II shows the partial amino acid sequences of the proteolytic fragments. These sequences were used to search for homology using the LSRCHP program (Wilbur and Lipman, 1983) at the National Biomedical Research Foundation Protein Identification Resource. All the sequences completely matched a probable maturase-like protein, which is encoded by a mitochondrial open reading frame (RF3). RF3 is a part of the nuclear and mitochondrial codons, based on the primary sequence of peptide 11 (Table II). The probe hybridized to a single DNA fragment obtained by EcoRI single or EcoRI/PstI double digestion of the purified mitochondrial DNA (1.7 and 1.2 kb, respectively) (Fig. 2). The sizes of the hybridized bands are consistent with the restriction map of mitochondrial RF3, which contains a unique PstI site (Séraphin et al., 1987). The results of Southern analysis indicate the presence of the gene for the smaller subunit of Endo.SceI (designated as ENS2) in mitochondrial DNA from strain IAM4274.

Nucleotide Sequence of Probable ENS2 Gene—RF3 in the mitochondrial genome has been shown to exhibit polymorphism among yeast strains because a Saccharomyces uvarum strain possesses a continuous RF3 sequence, whereas in some other yeast strains, the reading frame is interrupted by GC clusters, which thus break the frame (Séraphin et al., 1987). To confirm that S. cerevisiae IAM4274 possesses a functional RF3 sequence, we cloned and sequenced the 1.7-kb EcoRI fragment of mitochondrial DNA, which was found to hybridize to an oligonucleotide probe, as stated above. Fig. 3 shows the nucleotide sequence of the EcoRI fragment and the predicted amino acid sequence. The EcoRI fragment consisted of 1671 base pairs with two Ado deletions, compared to the equivalent region of S. uvarum NCYC74 (Séraphin et al., 1987), in Ado stretches within the 5' noncoding region. As expected, the fragment contained a continuous RF3 sequence, a probable ENS2 gene, with no GC clusters, which encodes a polypeptide of 476 amino acid residues with a molecular mass of 58 kDa. The coding sequence is almost identical to that of the continuous RF3 sequence reported by Séraphin et al. (1987) with seven nucleotide substitutions (indicated by un-

![Fig. 1. Reverse-phase high performance liquid chromatography of proteolytic digest of 50-kDa subunit. A proteolytic digest of the 50-kDa subunit was loaded onto an RP-300 reverse-phase column (2.1 × 30 mm) and eluted with a 0–60% gradient of acetonitrile in 0.1% trifluoroacetic acid: 0–5.2 min (0%) and 5.2–50.2 min (0–60%). The elution of peptides was monitored with regard to the absorbance at 215 nm. The flow rate was 100 μl/min. The fractions collected are indicated by the numbers.](image)

![Fig. 2. Southern blot analysis of mitochondrial DNA with oligonucleotide probe. Mitochondrial DNA from S. cerevisiae IAM4274 was digested with EcoRI (lane a) and EcoRI/PstI (lane b) and then subjected to a 1.2% agarose gel electrophoresis. The digests were blotted onto a nylon membrane and then hybridized with a 32P-labeled oligonucleotide mixture. Autoradiographs of the hybridization bands are presented. The lengths (in kilobase pairs) of the hybridization bands are indicated to the right.](image)
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**Fig. 3. Nucleotide sequence of ENS2 gene and its flanking sequence.** The deduced amino acid sequence and the nucleotide sequence of ENS2 are shown. The reading frame of ENS2 starts at position 159. A single-letter notation is used for amino acids. The termination codon is indicated by the dot. The boxed partial sequences determined by amino acid sequence analysis. Note that all the identified sequences are preceded by lysine residues. Underlining shows nucleotide or amino acid substitutions compared to the sequence of the corresponding region of *S. cerevisiae* IAM4274 (see the text). Asterisks show the positions of Ado deletions in comparison with the sequence in the *NCYC74* strain. Two conserved dodecamer sequences, which have been observed in several maturases (see "Discussion"), are indicated in the shaded boxes. In the 5'-upstream region of ENS2, the 3'-end of oli2 corresponding to the last 27 amino acid residues is also indicated.

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fractionated by high performance liquid chromatography (data not shown). Because there was clear correlation between the RF3 region in group A and the presence of Endo.SceI activity (we have confirmed the absence of Endo.SceI activity in another 15 strains of groups B and C), this result is consistent with our conclusion that the continuous RF3 is the functional gene (ENS2) encoding the smaller subunit of Endo.SceI.

In a similar way, several strains of *S. uvarum* were analyzed with regard to the gene organization of the RF3 region and the sequence-specific endonuclease activity because RF3 of *S. uvarum* is also polymorphic (data not shown). Among the *S. uvarum* strains tested, a group A strain (NCYC74) alone (Fig. 4, lane 2), like the group A strains of *S. cerevisiae*, was shown to exhibit sequence-specific endonuclease activity under these conditions (Fig. 5, lane 2). This sequence-specific activity was also detected in isolated mitochondria (data not shown). The level of endonuclease activity was apparently the same as that of Endo.SceI from *S. cerevisiae*. This result suggests that the continuous RF3 sequence in *S. uvarum* is also a functional gene encoding a subunit of the endonuclease. The profile of fragments produced by the endonuclease from *S. uvarum*, however, was not identical to those in the case of *S. cerevisiae* group A strains (lanes 1–3). Two bands, which did not clearly appear with the Endo.SceI from *S. cerevisiae*, were detected for the digests with the *S. uvarum* endonuclease (indicated by arrowheads in lane 2). In contrast, an intense band was visible in the *S. cerevisiae* digests (indicated by arrowhead in lane 1), whereas there was only a faint band at the corresponding position in lane 2. These results suggest therefore that the group A strain of *S. uvarum* possesses another sequence-specific endonuclease(s) (probably homologue(s) of Endo-SceI) whose sequence specificity is different from that of Endo.SceI. It remains to be determined whether or not the endonuclease activity detected here is due to a single enzyme species.

**Fig. 4.** Southern blot analysis of ENS2 regions in mitochondrial DNAs from various yeast strains. Total DNA preparations from several yeast strains were digested with EcoRI and HapII. The digests were run on a 2% agarose gel with a Tris/borate/EDTA buffer system. The 1.7-kb EcoRI fragment containing the ENS2 gene from *S. uvarum* NCYC74 was used as a hybridization probe. The sizes (in base pairs) of the hybridization bands are indicated to the left.

**Fig. 5.** Site-specific endonuclease activity in cell-free extracts from various yeast strains. Cell-free extracts from yeast cells were prepared with a French press. Fractions containing proteins with apparent molecular masses of 120–130 kDa were obtained from the extracts by high performance liquid chromatography and were used for the assay. Endonuclease activity was assayed using phage ϕ105 DNA. DNA (14 μM) was treated with cell-free extracts containing ~45 μg of proteins at 37 °C for 30 min in 100 μl of 50 mM Tris-HCl (pH 7.5) containing 10 mM MgCl₂ and 5 mM β-mercaptoethanol as described by Watabe et al. (1984). The DNA digests were deproteinized by phenol extraction; and subsequently, a one-sixth of each sample was run on a 0.8% agarose gel. The lanes correspond to the cell-free extracts prepared from the strains shown in Fig. 4 with the same numbers. *Lane M*, HindIII digests of λDNA; *lane S*, ϕ105 DNA reacted with purified Endo.SceI; *lane φ*, ϕ105 DNA. Arrowheads indicate the diagnostic bands for either Endo.SceI (lane 1) or *S. uvarum* endonuclease (lane 2).

each other (for unknown reasons, the substrate DNA could not be completely digested by Endo.SceI) (Watabe et al., 1984). These profiles were identical to the characteristic band pattern obtained with purified Endo.SceI (lane S) (Watabe et al., 1984). In addition, the specific endonuclease activity was detected almost exclusively in the mitochondria (data not shown), as reported previously for strain IAM4274 (Morishima et al., 1990). This result indicates the presence of Endo.SceI in mitochondria from the group A strains. For the other strains (groups B and C), sequence-specific endonuclease activity could not be detected in the fractions corresponding to apparent molecular masses of 120–130 kDa prepared from these strains (lanes 4–9). There was also no detectable Endo.SceI activity in the cell-free extracts which had not been

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**Cytduction Experiment Involving ENS2 and ρ₀ Strains—** To confirm that the Endo.SceI activity is dependent on ENS2 in the mitochondrial genome and independent of nuclear genes other than ENS1 (the gene for the larger subunit of Endo.SceI, which is essential for cell growth) (Morishima et al., 1990), we carried out mitochondrial transfer through cytduction (Lancashire and Mattoon, 1979; Spencer et al., 1989). By this technique, mitochondria were transferred from a group A strain (possessing Endo.SceI activity) to a ρ₀ strain (CG378-1), which lacks mitochondrial DNA and Endo.SceI activity. A haploid cytoductant (CD6-1) was constructed to contain the mitochondria from *S. cerevisiae* IAM4274. Fig. 6 shows the results of Endo.SceI assaying of the cytoductant and the original strain, CG378 (ρ₀, but having neither an RF3 sequence nor Endo.SceI activity, as shown in Fig. 4 (lane 9) and Fig. 5 (lane 9), respectively). Although these two strains were isonuclear with regard to each other, Endo.SceI activity was detected in CD6-1, but not in CG378 (Fig. 6, lanes 9, 13, and 1). In a similar way, another cytoductant, CD6-2, was constructed using CG378-1 and *S. uvarum* NCYC74. Consistent with the results of sequence-specific endonuclease assay- ing of NCYC74, Endo.SceI-like activity was detected in CD6-2 (lanes 14 and 2). Essentially the same results were obtained with the use of diploid strains harboring mitochondria of either IAM4247 or NCYC74 origin, excluding the possibility that the ploidy of cells affects the Endo.SceI activity (see "Experimental Procedures"). Negative control experiments showed that neither group B (CD6-3) (lane 15) nor group C (CD6-4) (lane 16) mitochondria yielded sequence-specific endonuclease activity in the CG378-1 strain. These results,
together with the results described above, demonstrated that the occurrence of Endo.SceI (or its homologue) activity is dependent on ENS2 in the mitochondrial genome.

DISCUSSION

We obtained consistent results from amino acid sequencing, Southern blot analysis, and cytoducttion experiments demonstrating that the smaller subunit of Endo.SceI is a maturase-like protein encoded by a mitochondrial open reading frame (RF3). The open reading frame contains two conserved dodecamer sequences which have been observed in several maturases (Michel et al., 1982, 1983; Shibata et al., 1990). These numbers over the lanes correspond to those used in Table I. The yeast strains used were CG578 (lane 9), CD6-1 (lane 13), CD6-2 (lane 14), CD6-3 (lane 15), CD6-4 (lane 16), IAM4274 (lane 1), and NCYC74 (lane 2). Numbers in parentheses indicate the origins of mitochondria (Mit.) in cytoductants. Lane M, HindIII digests of \( \lambda \)DNA.

In the case of HO-endonuclease, the second motif could not be readily found; instead, a sequence displaying weak homology to the second motif was found at ~100 residues on the N-terminal side of the first motif. Therefore, the structural domain of HO-endonuclease may be distinct from those of other endonucleases.

The molecular mass of the smaller subunit calculated from its deduced amino acid sequence (55 kDa) is slightly larger than the apparent molecular mass of the subunit determined by SDS-polyacrylamide gel electrophoresis (50 kDa) (Watabe et al., 1983). The difference between the calculated and measured molecular masses could be explained by the fact that the 50-kDa subunit shows anomalously high mobility on SDS-polyacrylamide gel, as do other mitochondrial proteins (Groot et al., 1978; Nobrega and Tzagoloff, 1980; Weiss-Brummer et al., 1982). The proteolytic fragments of the subunit that we identified by amino acid sequence analysis are dispersed within the region from Asp32 to Lys41 in the deduced sequence (Fig. 3). Therefore, the possibilities that processing of the de novo synthesized subunit occurs and/or that the translation of ENS2 mRNA is initiated at codon 12 for the second methionine cannot be excluded.

As previously suggested by us (Morishima et al., 1990), this study strongly supports the idea that the 50-kDa subunit bears the active site of the endonuclease because the subunit contains the conserved sequence found in maturase-related endonucleases. Supporting data have recently been obtained from biochemical analysis of Endo.SceI. An apparent difference between the 50-kDa subunit of Endo.SceI and other site-specific endonucleases from yeasts is that the subunit alone exhibits little endonuclease activity. The results of our previous biochemical and immunochemical experiments (Watabe et al., 1983; Nakagawa et al., 1988) showed that the smaller subunit (maturase-like protein) exhibits full enzymatic activity when the protein is associated with a larger subunit of 75 kDa, which has now been revealed to be a nuclear-encoded heat shock protein (Morishima et al., 1990). Therefore, the 75-kDa subunit is likely required to function as, for example, a modulator of the protein structure of the 50-kDa subunit for the expression of full enzymatic activity.

In mitochondria from \( \textit{S. uvarum} \) NCYC74, we detected a new sequence-specific endonuclease activity which is apparently different from the Endo.SceI activity in its sequence specificity (Fig. 5). Since both \( \textit{S. cerevisiae} \) and \( \textit{S. uvarum} \) exhibit mitochondrial sequence-specific endonuclease activity
which is dependent on or correlated at least with the continuity of the ENS2 reading frame, it is possible that the endonuclease encoded by the reading frame in *S. uvarum* has a different sequence specificity from that of Endo.SceI. On comparison of the deduced amino acid sequences of ENS2 in *S. cerevisiae* IAM4274 and RF3 in *S. uvarum*NCYC74 (Séraphin et al., 1985, 1987), two amino acid changes were observed (Gly\(^{317}\) and Asn\(^{466}\) in the former for Lys\(^{317}\) and Asp\(^{466}\) in the latter). The mutations at these sites, which are close to the conserved dodecamer sequences described above, could alter the sequence specificity of the endonuclease.

As to a genetic study on the 75-kDa subunit, this work reveals an apparent discrepancy as to the requirement of Endo.SceI for the growth of yeasts. A null mutation in ENS2 (groups B and C in Figs. 5 and 6) does not affect cell viability, whereas ENS1, which encodes the 75-kDa subunit, is essential for growth (Morishima et al., 1990). The tester strain we used in a previous genetic study (diploid constructed through the mating of CG376 and CG379) has null alleles of ENS2, as judged by Southern blot analysis (Fig. 5). This suggests that the Endo.SceI activity is not essential, but that the 75-kDa subunit (heat shock protein) plays another unidentified role, i.e., besides functioning as a subunit of Endo.SceI. Yeast mitochondrial DNA contains several maturases, among which was found an essential protein for the splicing of the introns in the cytochrome oxidase subunit I gene (I. Abeoosse and Slonimski, 1983). There are also two maturase-like sequences other than RF3 within the mitochondrial genome (named RF1 and RF2) (Coruzzi et al., 1981; Michel, 1984). It is therefore possible that the 75-kDa protein is associated with some of these proteins. To examine this possibility, we have initiated a survey for such protein(s).

Whether Endo.SceI has a latent maturase activity or not remains to be examined. Unlike maturases, however, the 50-kDa subunit of Endo.SceI is not encoded within an intron of another gene (this study and Sbraphin et al., 1987). A possible target RNA for Endo.SceI is not apparent. Considering that aI4-endonuclease, which cleaves an intron at the DNA level, has latent maturase activity for the intron at the RNA level (as described above), it is possible that the 75-kDa protein is associated with some of these proteins. To examine this possibility, we have initiated a survey for such protein(s).

Our present knowledge suggests that Endo.SceI plays a role in the genetic recombination of mitochondrial DNA from yeasts. As to its biochemical characteristics, the mode of cleavage of Endo.SceI is similar to that of HO-endonuclease and \(\alpha\)-endonuclease (Kostriken et al., 1963; Shibata et al., 1984; Colleaux et al., 1986). The latter two enzymes are involved in the gene conversion process of the mating type locus and rDNA locus, respectively. In addition, aI4-endonuclease, which is supposed to be involved in the propagation of an intron through a unidirectional gene conversion, also exhibited a similar mode of cleavage (Wenzlau et al., 1989; Delahodde et al., 1989). Therefore, Endo.SceI could cleave mitochondrial DNA at the site(s) where gene recombination occurs. This work provides further supporting data, on a molecular basis, indicating that Endo.SceI possesses a function related to that of other sequence-specific endonucleases.

There is a significant difference, however, between the enzymatic characteristics of Endo.SceI and other endonucleases. Unlike HO- and \(\alpha\)-endonucleases, which are initiators of site-specific gene conversion, Endo.SceI has multiple recognition sequences (Shibata et al., 1984) and actually exhibits multiple digestion of yeast mitochondrial DNA in vitro (as described above), suggesting that it is involved in general recombination. As a step to identify the target sequences for Endo.SceI in vivo, we are trying to identify the cleavage sites within the mitochondrial DNA for the endonuclease.

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