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Structure and Activity of the Mitochondrial Intron-Encoded Endonuclease, I-SceIV

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Starting with crude yeast mitochondria, the intron homing endonuclease, I-SceIV, was purified to near homogeneity. This highly purified enzyme differs from some other well-characterized yeast mitochondrial intron-encoded endonucleases in terms of its structure and DNA cleavage specificity. The enzyme is a heterodimer with a native molecular mass of 92 kDa. A small catalytic subunit (32 kDa) is probably encoded largely or entirely by intron 5α of the cytochrome oxidase subunit I gene. A larger polypeptide subunit (60 kDa) may be a nuclear factor necessary for intron mobility. I-SceIV exhibits a low DNA sequence specificity as it cleaves a variety of DNA substrates. Analysis of kinetic parameters shows that the purified enzyme has a very high affinity for DNA and exhibits low turnover which may have implications for subsequent steps in the intron homing process.

Key Words: **DNA endonuclease; yeast; mitochondria; intron.**

A number of group I introns are mobile genetic elements (1, 2). These "infectious introns" are found in many genomes including fungal mitochondria (3-7), chloroplasts (8-10), nuclei (11, 12), eubacteria (13), and bacteriophages (14-16). In all cases, an open reading frame in the intron encodes an endonuclease required for its mobility (2, 17, 18). The intron-encoded endonucleases initiate intron mobility, known as intron homing (19), by introducing a double-stranded break in the DNA of the recipient allele (originally lacking the intron). The break in the recipient DNA strand is thought to be subsequently repaired by using the intron-containing allele as a template. This model is consistent with the double-strand break-gap repair model of gene conversion (20). The majority of the group I intron-encoded endonucleases share two homologous copies (P1 and P2) of an amino acid consensus sequence (LAGLI-DADG) that flanks a central region of about 115 amino acids (21). Mutations in P1 were shown to block the endonuclease activity of the protein encoded by the aI4α intron of the cytochrome oxidase subunit 1 gene (22). This structural homology is also found in non-intron-encoded proteins of yeast, HO endonuclease and Endo.SceI, and in non-intron-encoded insertions in protein coding sequences in yeast, *Mycobacterium tuberculosis* and archaebacteria (inteins, reviewed in (23, 24)). Thus, representatives of the LAGLI-DADG family of proteins are found in all three biological kingdoms.

The mitochondrial genome of *Saccharomyces cerevisiae* harbors some of the most well-characterized homing group I introns. The ω intron, a 1.1 kb intron of the 21S rRNA gene encodes the ω-endonuclease, I-SceI (4, 5, 25, 26). This enzyme is the product of a free-standing intron open reading frame (26). Intron aI4α, the fourth intron (1.01 kb) of the cytochrome oxidase subunit 1 gene, encodes the aI4α-endonuclease, I-SceII (3, 6). In this case, an open reading frame comprised of exons 1 to 4 and intron aI4α of the cytochrome oxidase subunit 1 gene is translated to yield a fusion polypeptide. Proteolytic processing of the fusion polypeptide is thought to yield the active endonuclease (27-29). Recent work has shown that the I-SceIII endonuclease activity (encoded by the 1.5 kb aI3α intron of the cytochrome oxidase subunit 1 gene) correlates with a 35 kDa protein that is produced upon proteolytic processing of the amino terminus of a 44 kDa precursor protein (30).

These endonucleases make a staggered double-stranded DNA break within a four base sequence near the intron insertion site (hereafter referred to as the cut-site) leaving 3′-hydroxyl group overhangs. It is important to note that the DNA substrates for intron insertion (which harbor a cut-site) are unique for each particular enzyme. These enzymes recognize and cleave an ~18-bp non-palindromic sequence, situated asymmetrically about the site of intron insertion. I-SceI cleaves symmetrically about its intron insertion site (31), while I-SceII cleaves both strands on one side

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of its intron insertion site (3, 6). The group I intron-encoded endonucleases are thus distinguished from the bacterial type II restriction enzymes which have short palindromic DNA recognition/cut-sites.

I-SceI, which was overproduced in *E. coli* from a synthetic gene on a plasmid, exhibits a very high degree of sequence specificity. This enzyme does not cut DNAs other than those containing its natural cut-site sequence (32, 33). By contrast, I-SceII, overproduced in *E. coli* (34) or purified from yeast cells (29, 35) has a less stringent nucleotide sequence specificity and cleaves DNAs with individual or multiple nucleotide substitutions relative to the natural cut-site sequence. Of the non-intron-encoded yeast proteins, Endo.SceI, is an endonuclease with very low stringency sequence recognition (36). One subunit of this heterodimeric enzyme is encoded by a mitochondrial gene (ENS2) while the other subunit is encoded by a nuclear gene (ENS1) which is thought to be related to the HSP70 gene family (37, 38). By contrast, the yeast nuclear encoded HO endonuclease, required for yeast mating type switching, has a very high degree of nucleotide sequence specificity (39, 40).

Genetic studies have shown that the 1.4 kb intron aI5α of the cytochrome oxidase subunit 1 gene is also a mobile genetic element (7, 41). In analogy with the other group I introns mentioned above, the aI5α intron encodes an endonuclease activity, I-SceIV, that is required for aI5α intron mobility (7, 41). The endonuclease cleaves the recipient genome lacking the intron near the intron insertion site, making a four base staggered cut with 3′-OH overhanging ends (7, 41). It is interesting to note that aI5α intron conversion is not as efficient as that observed for the ω intron (7). Further, the nuclear genotype is known to influence aI5α intron mobility (41).

This work describes the purification and partial characterization of I-SceIV. The purified enzyme is comprised of two polypeptide subunits. One polypeptide is likely to be of mitochondrial origin (derived from the expression of intron aI5α) while the other may be encoded by a nuclear gene. Like some other intron-encoded endonucleases, our experiments show that this enzyme exhibits little nucleotide sequence specificity. The differences in the efficiency of intron homing that have been noted (7) do not appear to correlate with differences in the kinetic parameters of the intron-encoded endonucleases.

MATERIALS AND METHODS

Yeast strains and growth conditions. *Saccharomyces cerevisiae* strain ID41-6/161 (a ade2 lys1/$\rho^+\omega^-$) was the source of the enzyme used in these studies. Cultures used for enzyme preparation were grown to late logarithmic phase for 48 h at 30°C in medium composed of 1% yeast extract, 1% Bacto-peptone, and 2% galactose.

Bacterial plasmids. The plasmids used in this study have been described previously (7). The plasmid, pJVM134+, contains the aI5α homing site of the mitochondrial DNA of yeast strain GII-0 cloned in pBLSKS+ as a 2.1 kb BamHI to EcoRI fragment. Plasmid pSMB-R1 contains the aI5α homing site of the mitochondrial DNA of strain D273-10B cloned in pBS+ as a 1.35 kb EcoRI fragment. Plasmid pΔ5α,β,γ contains the aI5α homing site, cloned in pBS+ as a 730 bp HindIII to EcoRI fragment from a derivative of strain ID41-6/161, which had lost introns aI5α, aI5β and aI5γ by reversion of C2116 a point mutant in domain 3 of intron 5γ.

Preparation of yeast mitochondria. Yeast cells were collected by centrifugation at 1000*g* and washed with 2 mM EDTA. These and all subsequent steps were performed at 4 °C. Seventy three grams (wet weight) of washed yeast cells were resuspended in a buffer containing 50 mM Tris-HCl, pH 7.5, 0.6 M sorbitol, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 mM sodium bisulfite, 1 mM benzamidine, 1 mM benzamide, 1 μg/mL leupeptin, 1 μg/mL aprotinin, and 2 mM dithiothreitol at a ratio of 2 mL/g of cells. The yeast cells were mechanically broken with glass beads in a Bead Beater (Biospec Products) by a 15-s homogenization cycle repeated every minute for 8 min. This procedure results in breakage of 90% of the cells as determined by microscopic analysis. Crude mitochondria were isolated by differential centrifugation for 50 min at 14,000*g*. The crude mitochondria were resuspended in the same buffer at 0.2 mL/g wet weight of cells and stored frozen at −80°C.

Mitochondrial extract. The crude mitochondrial solution was adjusted to achieve final concentrations of 1 M KCl, 10 mM $MgCl_2$, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 mM sodium bisulfite, 1 mM benzamidine, 1 mM benzamide, 1 μg/mL leupeptin, 1 μg/mL aprotinin, 2 mM dithiothreitol and 0.5% Triton X-100. The solution was incubated at 4°C for 1 h and centrifuged at 14,000*g* for 1 h to obtain the mitochondrial extract. The mitochondrial extract was dialyzed in 50 mM Tris-HCl, pH 7.5, 5% glycerol, 50 mM KCl, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 mM sodium bisulfite, 1 mM benzamidine, 1 mM benzamide, 1 μg/mL leupeptin, 1 μg/mL aprotinin, 2 mM dithiothreitol (Buffer A) and stored at −20°C (Fraction I).

I-SceIV endonuclease assay. Plasmid substrates were linearized at a unique ScaI site in the vector. DNAs were 3′-end labeled with (^{35}S) α-dATP or (^{32}P) α-dATP as described previously (7) unless indicated otherwise. The standard reaction mixture contained 25 mM Tris-HCl, pH 9.5, 10 mM $MgCl_2$, 50 mM KCl, 2 mM dithiothreitol, 100 ng pJVM134+ DNA substrate (unless indicated otherwise) and the enzyme sample. Reactions were incubated for 1 hr at 30°C (5 min for kinetic experiments), and stopped upon the addition of proteinase K and SDS (50 μg/mL and 0.5% final concentrations, respectively) followed by incubation for 1 hr at 37 °C. One-tenth volume of a gel loading buffer composed of 50 mM Tris-HCl, pH 7.5, 100 mM EDTA, 2% SDS, 2% Ficoll and 1% bromophenol blue was added to the reaction mixture and the reaction products were analyzed on a 0.7% agarose gel. Reaction products were visualized by autoradiography or phosphorimagery of the dried gel. Extents of reaction were quantified by use of a Bio-Rad Model GS-363 Molecular Imager System.

Chromatographic methods. Fraction I was applied to an anion exchange column (Econo Q, Bio-Rad, 100 mL packed volume, 3 mg protein/mL) which was pre-equilibrated with Buffer A, at a flow rate of 1 mL/min. The column was washed with 200 mL of Buffer A at the same flow rate and eluted by the step-wise application of three 120 mL portions of Buffer A containing 0.25, 0.5, and 1 M KCl, respectively. Sixty fractions (6 mL each) were collected and assayed for endonuclease activity. The peak of I-SceIV activity eluted upon application of the 0.25 M KCl step. The active fractions (11-18) were pooled and dialyzed against Buffer A (Fraction II).

Fraction II was applied to a 5-mL cation exchange column (Econo S, Bio-Rad) at a flow rate of 0.2 mL/min, which was pre-equilibrated with Buffer A. The column was washed with 20 mL of Buffer A at the same flow rate. The column was eluted by the step-wise application of three 20 mL portions of Buffer A containing 0.25, 0.5, and 1 M KCl,

respectively. Sixty 1 mL fractions were collected and assayed for I-SceIV endonuclease activity. The enzyme eluted upon application of the 0.25 M KCl step. The active fractions (13-20) were pooled, concentrated to 2 mL and applied to a Sephacryl S-200 HR (Sigma) gel filtration column (1.5 cm × 100 cm) that was pre-equilibrated with Buffer A containing 0.5 M KCl. The column was eluted with 140 mL of the same buffer at 0.1 mL/min. The void volume of the column (Vo) was 68 mL and the total column volume (Vt) was 133 mL. The I-SceIV enzyme elution volume (Ve) was 81 mL and Kav = 0.2 (where Kav = Ve − Vo/Vt − Vo (42)). The active fractions (15-19) were pooled and dialyzed against Buffer A (Fraction IV).

Pulsed field gel electrophoresis. Genomic DNAs (Promega) were resolved by electrophoresis on a 1% agarose gel using a CHEF-DRII apparatus (Bio-Rad). The switch interval was varied with a time ramp of 10 s (initial) to 75 s (final). The gel was run at 10 V/cm for 24 hr in 0.5× TBE buffer at 15°C.

Other methods. Protein samples were TCA precipitated and analyzed on a 10-18% polyacrylamide gel (ISS) using a Tris-glycine-SDS buffer in a Hoefer SE 200 Series Model Mighty Small Mini-Gel Unit. Prestained SDS-PAGE standards (2 μL, broad range, Bio-Rad) were included on the gel. The gel was stained with Coomassie Brilliant Blue R-250 (43) and then silver stained (43). Protein concentrations were determined using the method of Schaffner and Weissman (44).

RESULTS

I-SceIV can be purified from yeast mitochondria. The dialyzed mitochondrial extract (Fraction I) was applied to a strong anion exchange column (Figs. 1A and 1B). I-SceIV endonuclease eluted at about 0.25 M KCl. Active fractions (11-18) were pooled and dialyzed against Buffer A (Fraction II). This procedure results in a 15-fold increase in the specific activity of I-SceIV, with nearly a 50% yield of the activity (Table 1). Fraction II was applied to a medium strength cation exchange column (Fig. 2). I-SceIV activity eluted after application of Buffer A with 0.25 M KCl. The active fractions (13-20) were pooled and dialyzed against Buffer A (Fraction III). I consistently obtain an apparent low yield at this step (~30%, Table 1) with an apparent 3-fold increase in specific activity. However, the low yield may be due to the presence of an inhibitor that is concentrated in Fraction III, because activity is always regained in the next (and last) step. Thus, Fraction III was applied to a Sephacryl S200-HR gel filtration column to increase the degree of purification and to estimate the native molecular mass of I-SceIV (Figs. 3A and 3B). Fractions 15-19 were pooled and dialyzed against Buffer A. The apparent step yield is consistently ~250%, suggesting that some inhibitory component is removed at this point. This step results in an 11-fold increase in specific activity and yields the near homogeneous enzyme preparation. The overall yield of the I-SceIV

TABLE 1

Summary of the Purification of Yeast Mitochondrial I-SceIV

Fraction	Protein (mg)	Activity[a] (units × 10^{-3})	Specific activity (units/mg × 10^{-3})	Yield (%)
I. Mitochondrial extract	300	479	2	100
II. Anion exchange	7.7	221	29	46
III. Cation exchange	0.9	70	78	15
IV. Gel filtration	0.2	172	860	36

[a] One unit of activity cleaves 50 ng of double-stranded pJVM134+ DNA substrate/h at 30°C.

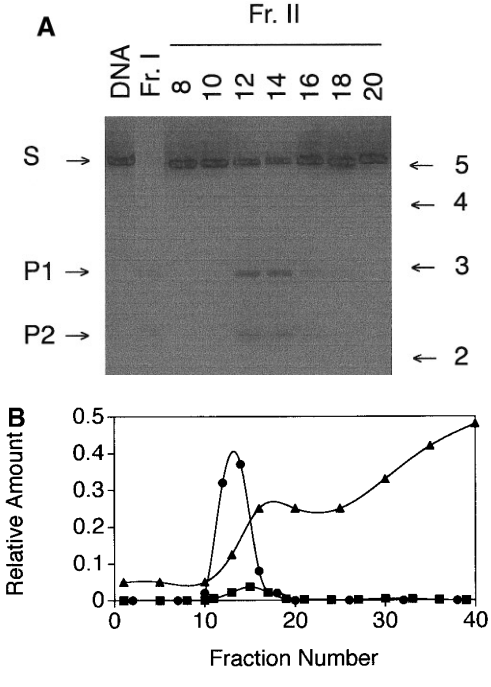

FIG. 1. (A) Anion exchange chromatography activity assay. Individual even numbered anion exchanged column fractions (8-20) were assayed for I-SceIV activity on pJVM134+ DNA as described in Materials and Methods. S, linear substrate DNA; P1, DNA cleavage product 1; P2, DNA cleavage product 2. The 1 kb DNA ladder (BRL) DNA size markers were included on the gel and are indicated on the right side of the figure as 2, 3, 4 and 5 (kb). (B) Anion exchange chromatography of the mitochondrial extract. Conditions for chromatography are described in Materials and Methods. Filled circles, I-SceIV activity (U/μL); filled squares, protein (mg/mL); filled triangles, KCl (M).

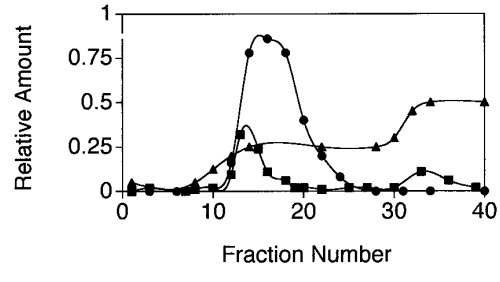

FIG. 2. Cation exchange chromatography. Conditions for chromatography are described in Materials and Methods. Filled circles, I-SceIV activity (U/μL); filled squares, protein (mg/mL); filled triangles, KCl (M).

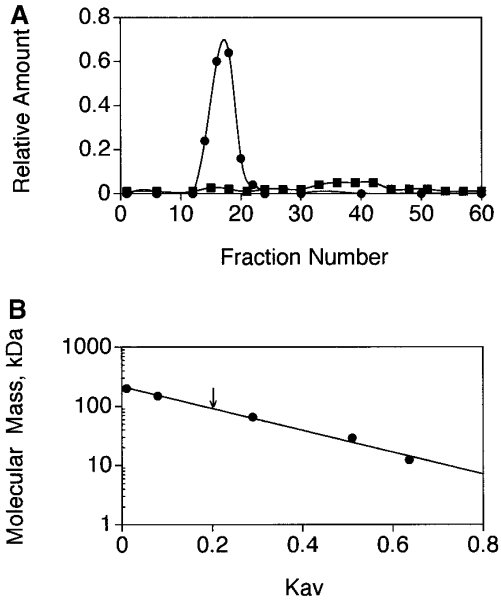

FIG. 3. (A) Gel filtration chromatography elution profile. A Sephacryl S200HR column was prepared and eluted as describe in Materials and Methods. Filled circles, I-SceIV activity (U/μL $\times$ 3); filled squares, protein (mg/mL). (B) Gel filtration chromatography reveals that I-SceIV has a native molecular mass of ~92 kDa. The native molecular mass was estimated as described in Materials and Methods. Filled circles, molecular mass standards. The standards used were β-amylase, 200,000 Da; alcohol dehydrogenase, 150,000 Da; albumin, 66,000 Da; carbonic anhydrase, 29,000 Da; and cytochrome c, 12,400 Da. The arrow indicates the Kav value for I-SceIV which correlates with an apparent molecular mass of 92,000 Da.

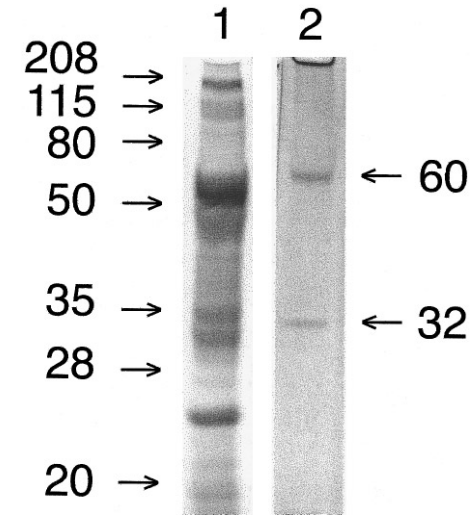

FIG. 5. SDS-PAGE analysis shows that the highly purified I-SceIV endonuclease is comprised of two polypeptides. Lane 1, Fraction III (100 U, 1.3 μg); lane 2, Fraction IV (100 U, 0.12 μg). Prestained SDS-PAGE standards were included on the gel and are indicated on the left side of the figure as 208, 115, 79.5, 49.5, 34.8, 28.3, and 20.4 (kDa).

activity is 36% and the specific activity is 860,000 U/mg. The gel filtration analysis also shows that the native molecular mass of I-SceIV is ~92 kDa (Fig. 3B) with a Stokes radius of 38 Å. The highly purified enzyme is optimally active in the presence of 5 mM $MgCl_2$, 100 mM KCl at pH 9.5 (Fig. 4).

Polypeptide subunit analysis shows that I-SceIV may be a heterodimer. The progress of the enzyme purification procedure was also monitored by SDS-polyacrylamide gel electrophoresis. Pooled column fractions were TCA precipitated and analyzed on a 10-18% gel as described in "Materials and Methods" (Fig. 5). Fractions I (mitochondrial extract) and II (anion exchange) have a complex array of polypeptide bands (data not shown). Lane 1 shows that a Fraction III preparation (cation exchange) contains several polypeptide bands. Lane 2 shows the two polypeptide bands that are consistently found the Fraction IV preparation (gel filtration). These polypeptides correlate with molecular masses of 60 and 32 kDa. This suggests that I-SceIV may be a heterodimer with an apparent native molecular mass of ~92 kDa.

DNA cleavage specificity and kinetics. Earlier, we showed that I-SceIV is active on three plasmid DNAs that differ from one another by one or more nucleotides at the I-SceIV cut-site/intron insertion region and in the amount of mitochondrial DNA that they contain (7). For this study, I measured the kinetic properties of the highly purified I-SceIV on the same

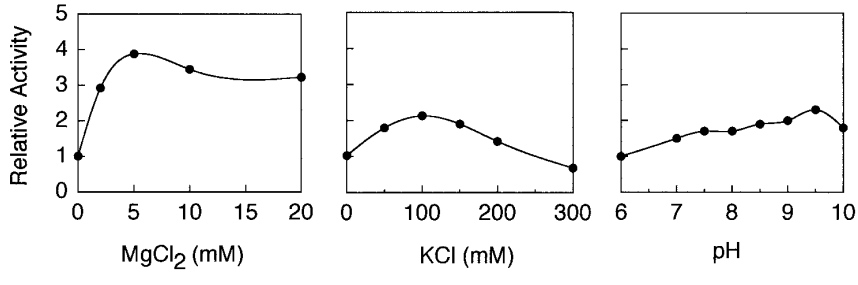

FIG. 4. Effect of divalent cation, monovalent cation and pH. The conditions for enzyme assay were as described in Materials and Methods except that $MgCl_2$, KCl and pH were varied as indicated. Filled circles, I-SceIV activity (U/μL).

TABLE 2

Nucleotide Sequences of Plasmid DNA Substrates[a]

Plasmid	Nucleotide sequence
pJVM134+	5′ . . AAAATCTTTTCTTGATTA ↓ GCCCTAATCTACGGT . . 3′
pSMB-RI	5′ . . AAAATCTTTTCTTGATTA ↓ GCCCT**G**ATCTACGGT . . 3′
pΔ5$\alpha\beta\gamma$	5′ . . AAAAT**T**TT**C**TC**A**TGATTA ↓ GC**T**CTA**CAT**TACGGT . . 3′

[a] The nucleotide sequence of one strand in each DNA plasmid is shown. I-SceIV cleaves each DNA strand after . . ATTA . . (↓) leaving a 4 base overhang and a 3′-OH. Nucleotide sequence differences relative to pJVM134+ are indicated in bold type.

three plasmid DNAs. The pJVM134+ DNA substrate contains the aI5α insertion site situated within 2.1 kb of flanking mitochondrial DNA. Plasmid pSMB-R1 contains a 1.35 kb mitochondrial DNA fragment that differs from the pJVM134+ DNA clone at the aI5α insertion site by one nucleotide. Plasmid pΔ5$\alpha\beta\gamma$ contains 730 bp of mitochondrial DNA and differs from the pJVM134+ DNA clone at the aI5α insertion site at seven nucleotides, and thus, differs from the pSMB-R1 clone at eight nucleotides. Table 2 shows the exact nucleotide sequence around the I-SceIV cut-site in each of these plasmid DNA substrates. The kinetic parameters for I-SceIV are nearly identical when measured with either the pJVM134+ or the pSMB-R1 cloned substrates (Table 3). The Km obtained with either DNA substrate is $\sim 1.5 \times 10^{-10}$ M which shows that the enzyme has a very high affinity for the DNA. The Km value for the pΔ5$\alpha\beta\gamma$ clone is about 5-fold higher suggesting that multiple mutations in the cut-site region decrease the DNA binding affinity somewhat but do not significantly interfere with enzyme/substrate recognition. The kinetic data also confirms that the homing endonuclease has a very low turnover rate as evidenced by the very small kcat values. The other well studied yeast mitochondrial intron-encoded endonucleases, I-SceI (32) and I-SceII (29, 45) also have a very high affinity for their DNA substrates as exhibited by the low Km and high specificity constant (kcat/Km).

I-SceIV also cuts yeast, bovine, pig, mouse (data not shown) and human genomic DNAs (Fig. 6). A CHEF gel analysis shows that I-SceIV alters the size distribution of human genomic DNA from a range of >50-350 kbp (lane 1) to about 50-150 kbp (lane 2). It is possible that the genomic DNAs contain a consensus I-SceIV cut-site that occurs frequently in the genome. However, the experiments we presented earlier (7) and those described above suggest that I-SceIV probably does not have a stringent DNA nucleotide sequence specificity *in vitro* as was found for I-SceII or Endo.SceI. Comparative DNA binding studies, cut-site mapping studies and cut-site nucleotide sequence analyses are underway and will provide further insight into the issues of enzyme turnover, DNA binding and cleavage site specificity.

DISCUSSION

Genetic and biochemical studies have shown that some yeast mitochondrial group I introns have long open reading frames which are expressed as site-specific endonucleases that promote intron homing. In the case of the expression of the cytochrome oxidase subunit I gene, it is thought that RNA splicing yields an in frame mRNA fusion consisting of upstream exon and the intron open reading frame sequences. It was proposed that translation produces a fusion polypeptide which is subsequently processed to yield the active endonuclease composed of intron-encoded amino acids (27-29). The yeast I-SceIII endonuclease is derived by proteolytic processing of a larger precursor protein (30). It is possible that this protein processing event is accomplished via an autocatalytic mechanism as has been shown for the inteins, which are non-intron encoded LAGLI-DADG proteins (23, 24).

Since the open reading frame of intron aI5α is in phase with the upstream exons it may also be ex-

TABLE 3

Kinetic Parameters for the Homing Endonuclease, I-SceIV

Plasmid	Km[a] ($M \times 10^{10}$)	Vmax ($M\ s^{-1} \times 10^{10}$)	kcat ($s^{-1} \times 10^{3}$)	1/kcat ($s \times 10^{-3}$)	kcat/Km ($M^{-1}\ s^{-1} \times 10^{-6}$)
pJVM134+	1.4	1.3	0.5	2.0	3.6
pSMB-R1	1.5	1.5	0.6	1.7	4.0
pΔ5$\alpha\beta\gamma$	7.7	0.8	0.3	3.4	3.9

[a] DNA concentrations are expressed as moles of cut-sites.

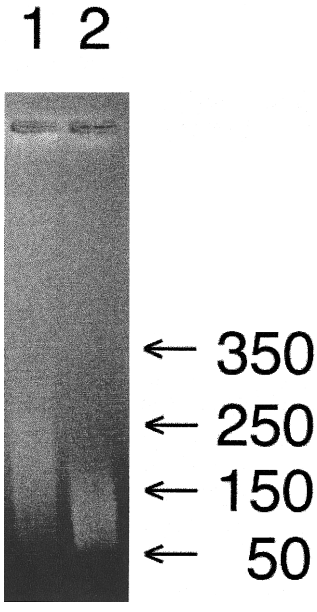

FIG. 6. CHEF gel analysis shows that I-SceIV cleaves genomic DNA. Lane 1, human genomic DNA (0.25 μg); lane 2, human genomic DNA (0.25 μg) treated with I-SceIV (20 U). Conditions for pulsed field gel analysis are described in "Materials and Methods".

pressed as a fusion protein, more than half of which is encoded by the upstream exon sequences. The intron open reading frame could potentially encode a 34 kDa polypeptide. The I-SceIV endonuclease activity is easy to detect in appropriate yeast strains and can be purified from yeast mitochondria with a good yield. Interestingly, the purified enzyme has a native molecular weight of about 92 kDa and appears to be a heterodimer—composed of a 60- and a 32-kDa polypeptide. Preliminary experiments suggest that the 32-kDa polypeptide possesses endonuclease activity and probably corresponds to the polypeptide encoded by the aI5α intron open reading frame. Amino acid sequence analysis of the 32-kDa polypeptide should allow confirmation of the identity of this polypeptide. Interestingly, amino acid sequence analysis of a cyanogen bromide digest of purified I-SceII endonuclease confirmed the identity of that protein with intron aI4α encoded amino acid sequences. However, it was not possible to obtain an amino terminal amino acid analysis as the amino terminus of the 31 kDa I-SceII monomer is blocked (Wernette and Butow, 1991, unpublished data). The identity and/or function of the 60 kDa polypeptide may also be clarified once amino acid sequencing studies are complete. Interestingly, the yeast enzyme Endo.SceI also has two subunits. Endonuclease activity is associated with the 50 kDa (small) subunit, while the 75 kDa (large) subunit is a member of the 70 kDa heat shock protein family (HSP70). Full endonucleolytic activity requires both subunits (36). By contrast, I-SceI, engineered in *E. coli*, is reported to be a monomeric protein of 27 kDa (32) and I-SceII, purified from yeast cells, is reported to be a homodimeric protein comprised of 31-kDa subunits (29). Thus, it appears that the yeast LAGLIDADG proteins comprise a structurally heterogeneous "family" of enzymes.

The low turnover number suggests that the enzyme may remain bound to the DNA product(s) after the DNA cleavage reaction. This tight binding might be evidence that the endonuclease is important in other steps of the intron homing process, either in positioning other necessary protein factors or facilitating DNA/DNA interactions. It was not surprising then to find that DNA affinity chromatography was not reliable as a component of the purification procedure. When Fraction IV was applied to a DNA affinity column, in the absence of magnesium (conditions that allow DNA binding but not the DNA cleavage reaction), the smaller I-SceIV polypeptide (~32 kDa) bound the DNA tightly and neither the polypeptide itself nor the enzyme activity were recovered. It seems likely that the mitochondrial endonuclease remained tightly bound to the DNA as suspected. By contrast, the larger I-SceIV polypeptide (~60 kDa) did not bind a DNA affinity column nor does it possess I-SceIV endonuclease activity (data not shown). This polypeptide may be a nuclear encoded factor necessary for intron mobility that was predicted by genetic studies (41).

I-SceIV has a broad DNA cleavage site specificity and exhibits endonuclease activity on a number of DNAs from different sources. The highly purified enzyme cleaves the substrate, pJVM134+, at the same position as the partially purified enzyme (data not shown). Sequence analysis and DNA mapping studies to define a minimal consensus sequence for I-SceIV are underway. Interestingly, the highly purified enzyme does not bind or cleave double-stranded synthetic oligonucleotides containing 26 basepairs (−13 to +13) surrounding the pJVM134+ DNA cut site (46). Thus, the minimal recognition site could be asymmetric or bipartite.

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