Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 6064–6068, June 1994
Biochemistry

Expression of a site-specific endonuclease stimulates homologous recombination in mammalian cells

(I-*Sce* I/extrachromosomal recombination/mouse cells)

PHILIPPE ROUET, FATIMA SMIH, AND MARIA JASIN*

Cell Biology and Genetics Program, Sloan–Kettering Institute and Cornell University Graduate School of Medical Sciences, 1275 York Avenue, New York, NY 10021

Communicated by Jerard Hurwitz, February 22, 1994 (received for review January 17, 1994)

ABSTRACT **Double-strand breaks introduced into DNA *in vivo* have been shown to enhance homologous recombination in a variety of chromosomal and extrachromosomal loci in *Saccharomyces cerevisiae*. To introduce double-strand breaks in DNA at defined locations in mammalian cells, we have constructed a mammalian expression vector for a modified form of I-*Sce* I, a yeast mitochondrial intron-encoded endonuclease with an 18-bp recognition sequence. Expression of the modified I-*Sce* I endonuclease in COS1 cells results in cleavage of model recombination substrates and enhanced extrachromosomal recombination, as assayed by chloramphenicol acetyltransferase activity and Southern blot analysis. Constitutive expression of the endonuclease in mouse 3T3 cells is not lethal, possibly due to either the lack of I-*Sce* I sites in the genome or sufficient repair of them. Expression of an endonuclease with such a long recognition sequence will provide a powerful approach to studying a number of molecular processes in mammalian cells, including homologous recombination.**

The repair of DNA double-strand breaks (DSBs) is important for the maintenance of genomic integrity in all organisms. In *Saccharomyces cerevisiae*, results from a number of studies have demonstrated that DSBs are primarily repaired by homologous recombination. Two distinct pathways have been implicated in the repair—DSB repair (1) and single-strand annealing (2–5). DSB repair is conservative, whereas single-strand annealing proceeds by a nonconservative mechanism in which some genetic information is lost.

Experiments in mammalian cells with transfected DNA have shown that both of these homologous recombination pathways are operational (6–12). In addition, and in contrast to *S. cerevisiae*, healing of broken DNA ends also occurs efficiently by nonhomologous end-joining (13). However, studies on the repair of DSBs in mammalian chromosomal DNA have been limited, due to the lack of expression systems for rare-cutting, site-specific endonucleases.

I-*Sce* I, which was discovered during analysis of the ω genetic system in *S. cerevisiae*, is one such endonuclease (reviewed in ref. 14). At the molecular level, ω^+ strains have an intron in the mitochondrial large rRNA gene. The intron efficiently moves (homes) to the intronless rRNA gene of ω^- strains, creating a new ω^+ allele (15, 16). Further analysis demonstrated that an endonuclease (I-*Sce* I) is encoded within the ω^+ intron and that a DSB appears transiently at the site of intron insertion in ω^- mitochondrial DNA (17). The DSB is repaired from the homologous ω^+ DNA by a gene conversion mechanism, details of which are compatible with the DSB repair model. Subsequent to the analysis of the ω system, many other examples of intron homing and homing endonucleases have been identified in a variety of organisms (14, 18), some of which are encoded in protein introns (19).

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

The length of the recognition site for I-*Sce* I and related endonucleases is longer than that of bacterial restriction enzymes. Mutagenesis of the site of intron insertion has shown that I-*Sce* I recognizes an 18-bp nonpalindromic sequence (20). The enzyme has been purified (21), and its DNA binding and cleavage activities have been characterized (22). The presumed infrequency of recognition sites in even very complex genomes makes I-*Sce* I an ideal endonuclease to study the repair of DSBs at defined sites.

With this goal in mind, we have constructed an I-*Sce* I expression vector for use in mammalian cells. We have begun to examine the effects of *in vivo*-generated DSBs on homologous recombination by using an extrachromosomal assay that measures reporter gene activity. We also address the question as to whether expression of I-*Sce* I has any deleterious consequences for murine cells.

MATERIAL AND METHODS

DNA Constructs. All I-*Sce* I plasmids were prepared in *Escherichia coli* JM101. The universal code equivalent of I-*Sce* I (23) was derived from pSCM525 (B. Dujon, Pasteur Institute). The 714-bp *Nde* I/*Sal* I fragment from pSCM525 was cloned into the *Nde* I and *Sal* I sites of pUC19, generating pUC19/I-Sce I. The unique *Nde* I site overlaps the ATG for I-*Sce* I. The influenza hemagglutinin (HA) epitope tag (24) was inserted by using the oligonucleotides 5′-TATGTACCCATACGATGTTCCTGACTATGCGGG and 5′-TACCCGCATAGTCAGGAACATCGTATGGGTACA. Annealing these generates a double-stranded oligonucleotide with *Nde* I overhangs, which was ligated into the *Nde* I site of pUC19/I-Sce I. In the correct orientation, the *Nde* I site is reconstructed on the 5′ side of the HA–I-*Sce* I fusion, generating pUC19/HA-I-Sce I. A nuclear localization signal (25) was inserted in a similar manner in the *Nde* I site of pUC19/HA-I-Sce I, generating pUC19/nls-HA-I-Sce I, with the oligonucleotides 5′-TATGGGATCATCATCAGACGACGAAGCAACAGCAGACGCACAACACGCAGCACCACCAAAAAAAAAAACGAAAAGTAGAAGACCCACGATT and 5′-TAAATCGTGGGTCTTCTACTTTTCGTTTTTTTTTTGGTGGTGCTGCGTGTTGTGCGTCTGCTGTTGCTTCGTCGTCTGATGATGATCCCA. A consensus eukaryotic translation initiation site (26) was added by inserting the annealed oligonucleotides 5′-TATGGATATCCCCGGGGGATCCGCCGCCAC and 5′-TAGTGGCGGCGGATCCCCCGGGGATATCCA into the *Nde* I site of pUC19/nls-HA-I-Sce I. A *Bam*HI site (underlined) is included in this insertion. The eukaryotic expression vector pCMV-I-Sce I was obtained by ligation of the *Bam*HI/

Abbreviations: DSB, double-strand DNA break; CAT, chloramphenicol acetyltransferase; nls, nuclear localization signal; HA, influenza hemagglutinin; SV40, simian virus 40; RSV, Rous sarcoma virus.

*To whom reprint requests should be addressed.

Sal I fragment of pUC19/Kozak-nls-HA-I-Sce I into the *Bgl* II/*Sal* I sites of plasmid pCMV5 (27).

All recombination substrates were prepared in *E. coli* XL1-Blue. Plasmid 4 has been described previously (28). Plasmids 5 and 6 were obtained by addition of a synthetic I-*Sce* I site (20) into the unique *Xba* I site of plasmid 4, which is situated between the chloramphenicol acetyltransferase (CAT) gene repeats. The synthetic site was obtained by annealing the oligonucleotides 5'-CTAGATAGGGATAA/CAGGGTAATA and 5'-CTAGTATTACCCTG/TTATCCCTAT. (The site of I-*Sce* I cleavage is indicated by the slash.) The CAT repeats are separated by 17 bp in plasmid 4 and 39 bp in plasmids 5 and 6. Plasmids 5 and 6 have the I-*Sce* I site in opposite orientations, as distinguished by the reconstruction of the *Xba* I site on opposite sides of the I-*Sce* I site. The integrity of the site in these plasmids was verified by *in vitro* cleavage with purified I-*Sce* I (Meganuclease; Boehringer Mannheim). Plasmids Δ5' and Δ3' were constructed by deletion of the *Mlu* I/*Xba* I 5' CAT fragment of plasmid 5 and the 3' CAT *Xba* I/*Hpa* I fragment of plasmid 6, respectively, using Klenow fill-in of the DNA ends followed by blunt-end ligation. Plasmids Δ5' and Δ3' each contain an I-*Sce* I site at the 5' and 3' ends, respectively, of the one remaining CAT repeat.

Cell Culture, Enzyme Assays, and Southern Analysis. COS1 and 3T3 cells were cultured on 10-cm-diameter plates and transfected by the calcium phosphate protocol as described (29) without reducing the percentage of CO_2 in the incubator. For COS1 cell transfections, which were performed in triplicate, 35 μg of DNA was used: 15 μg of the CAT plasmid(s), 15 μg of pCMV-I-Sce I (or pCMV5, as a control), and 5 μg of plasmid pgkH2lacZ (P.R., unpublished results), which was used to monitor transfection efficiency. Cells were harvested 60 h after transfection. CAT and β-galactosidase assays were performed as described (30) using 20% of the cell extract from each plate. For Southern analysis, cells were washed three times with PBS to eliminate DNA adhering to the outside of cells. Extrachromosomal DNA was extracted using a very fast miniprep protocol (31) and resuspended in 50 μl of TE (10 mM Tris·HCl, pH 7.5/1 mM EDTA). Southern analysis was performed on 15 μl of DNA using standard procedures (32).

For the stable transfections, 3T3 cells were cotransfected as above with 3 μg of plasmid pKJ1, which contains the *pgkneo* gene (33) and 17 μg of plasmid pCMV-I-Sce I (clones 7E and 7F) or pCMV5 (clone 6B). Selection was started 24 h after transfection with G418 (Geneticin; GIBCO) at 0.8 mg/ml. Colonies were picked about 2 weeks later. Transient CAT transfections of the isolated cell lines were done with 10 μg each of Δ5' and Δ3', or 20 μg of plasmids 4 and 5, plus 5 μg of *pgklacZ*. Data are presented as CAT cpm, since a higher endogenous β-galactosidase activity was detected for 3T3 cells. For Southern analysis, 62.5 μg each of Δ5' and Δ3' and 25 μg of *pgklacZ* were electroporated with a Bio-Rad gene pulser (960 μF, 250 V) into 3T3 or 7E cells from seven subconfluent plates. One-seventh of the cells were processed immediately after electroporation for DNA extraction. The rest of the cells were split onto six plates and were processed at various times. The Southern blot was done with 20% of the DNA.

RESULTS

Design of a Vector for Expression of I-*Sce* I in Mammalian Cells. Since I-*Sce* I is encoded by the yeast mitochondrial genome, its genetic code differs from the universal code. However, a universal code equivalent has previously been constructed for expression of the protein in *E. coli* (23). To direct expression of I-*Sce* I in mammalian cells, a DNA fragment containing the universal code equivalent for I-*Sce* I was subcloned into a mammalian expression vector. Initial attempts to detect endonucleolytic cleavage *in vivo* proved unsuccessful with this construct. Therefore, three modifications were undertaken (Fig. 1). Oligonucleotides encoding a simian virus 40 (SV40) nuclear localization signal (nls) and an HA epitope tag were fused in-frame to the 5' end of the I-*Sce* I coding region. A consensus eukaryotic translation initiation site (26) was also added. The modified I-*Sce* I gene was inserted into the pCMV5 expression vector (27), which has the strong human cytomegalovirus promoter/enhancer, a human growth hormone polyadenylylation site, and an SV40 origin for replication of the plasmid in COS1 cells. The complete vector is called pCMV-I-Sce I.

Expression of I-*Sce* I in COS1 cells transiently transfected with pCMV-I-Sce I was monitored by Northern blot analysis. A 1-kb I-*Sce* I-specific RNA accumulated rapidly, reaching very high levels 60 h posttransfection (data not shown). Although we have not as yet detected the protein via the epitope tag, functional studies have demonstrated the expression of the protein (see below).

Design of Recombination Substrates. In a wide variety of mammalian cells, extrachromosomal homologous recombination is stimulated when the transfected DNA has a DSB at the homology region. To detect enhanced recombination through the introduction of DSBs by I-*Sce* I *in vivo*, we have utilized plasmid substrates that are unable to express CAT except by a homologous recombination event (28). CAT plasmid substrates are designed to measure both intramolecular and intermolecular recombination in transient transfections of mammalian cells (Fig. 2).

Plasmids 4 and 5 are intramolecular substrates in which the CAT coding region contains an internal 403-bp duplication, rendering the gene nonfunctional. Homologous recombination within the repeats leads to a functional CAT gene expressed from the Rous sarcoma virus (RSV) promoter. In both plasmids, an *Xba* I site is present between the duplicated sequences. Plasmid 5 also has an I-*Sce* I site between the repeats. The intermolecular substrates, plasmids Δ5' and Δ3', have deletions at either the 5' or 3' ends of the CAT gene, respectively, which are delimited by an I-*Sce* I site. Recombination between plasmids Δ5' and Δ3' also leads to a functional CAT gene. None of the substrates contain a mammalian replication origin.

I-*Sce* I Is Active in Mammalian Cells and Stimulates Extrachromosomal Recombination. To monitor extrachromosomal recombination, plasmid substrates were transfected into COS1 cells, and CAT assays were performed 60 h posttransfection. In control transfections with uncut plasmids 4 and 5, we detected a low level of CAT activity that was similar for both plasmids (data not shown). As expected from our previous work (28), the amount of activity increased 10-fold if the DNA was cleaved between the repeats prior to transfection. The activity from the cleaved substrates was ≈10% that of the wild-type RSVCAT gene, indicating that ≈10% of the cut plasmid DNA recombined (data not shown).

To assess whether the I-*Sce* I endonuclease was functional *in vivo* and whether *in vivo* DSBs would enhance extrachromosomal recombination, plasmid substrates were cotransfected with pCMV-I-Sce I (Fig. 3*A*). Plasmid 5, which contains the I-*Sce* I site, produced a 3- to 4-fold higher level of CAT activity than did plasmid 4, which has no I-*Sce* I site. The level of activity is ≈40% that obtained if the plasmid DNA was cleaved between the repeats prior to transfection. Our interpretation is that a significant amount of plasmid 5 is

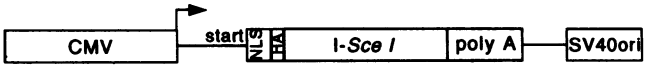

FIG. 1. pCMV-I-Sce I expression vector. A modified I-*Sce* I protein of 276 amino acids is encoded by this vector. CMV, cytomegalovirus promoter/enhancer; ori, origin for replication.

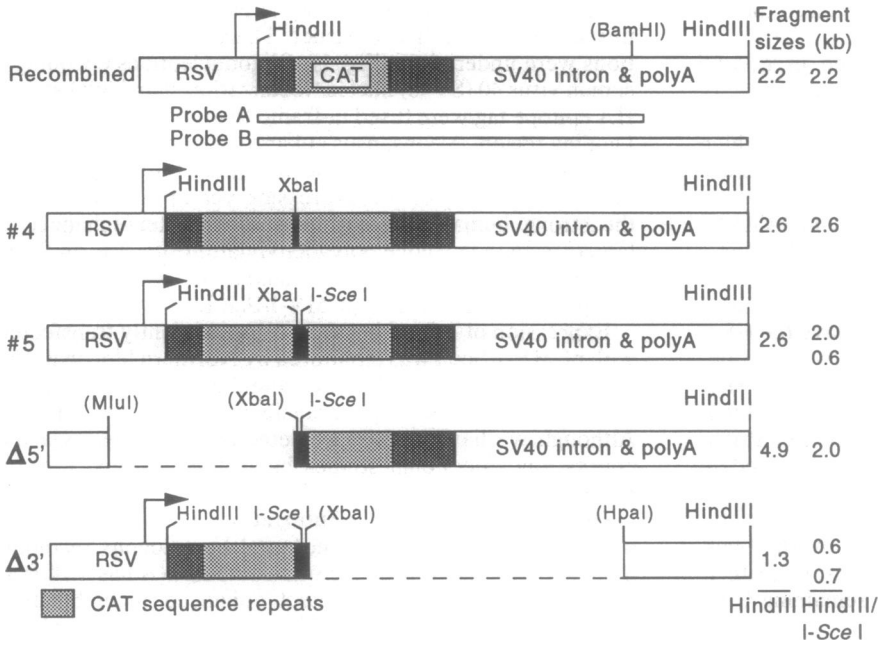

FIG. 2. CAT gene recombination substrates. An I-*Sce* I site is present in plasmids 5, Δ5′, and Δ3′, but is absent from plasmid 4. The CAT sequences are shaded; the repeated sequence has a lighter shading. SV40 sequences at the 3′ end of the CAT genes provide an intron and poly(A) site for correct processing of the CAT mRNA. The deletions in Δ5′ and Δ3′ are indicated by the dashed lines. Restriction fragment sizes and probes relevant to the Southern analysis shown in Figs. 4 and 5 are indicated. Probe A hybridizes only to the 0.6-kb I-*Sce* I/*Hin*dIII fragment of Δ3′, whereas probe B hybridizes to both the 0.6- and 0.7-kb fragments.

being cleaved *in vivo* by I-*Sce* I, stimulating recombination within the CAT repeats.

Expression of I-*Sce* I also enhanced intermolecular recombination (Fig. 3*B*). The substrates Δ5′ and Δ3′ by themselves showed very low CAT activity. When Δ5′ and Δ3′ were transfected together with pCMV5, a somewhat higher level of activity was observed, indicating a small amount of intermolecular recombination. This was increased almost 10-fold by I-*Sce* I expression, reaching almost 40% the level of activity obtained with the pre-cleaved substrates. This suggests that both Δ5′ and Δ3′ were efficiently cleaved *in vivo* by I-*Sce* I, stimulating recombination.

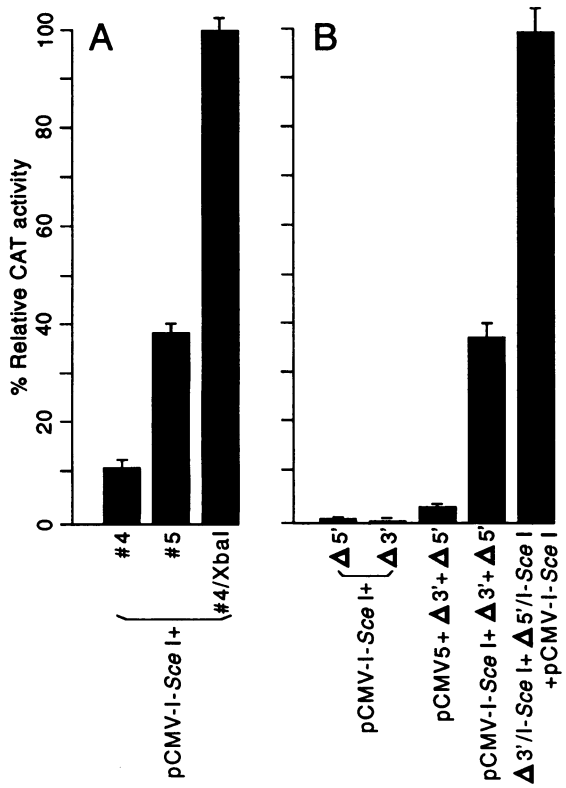

FIG. 3. CAT activity from COS 1 cells transiently transfected with the intramolecular (*A*) and intermolecular (*B*) recombination substrates and either pCMV-I-Sce I or pCMV5, as indicated. DNA is transfected uncut except as indicated; 4/XbaI indicates that plasmid 4 was cleaved with *Xba* I and Δ3′/I-*Sce* I and Δ5′/I-*Sce* I indicate that Δ3′ and Δ5′, respectively, were cleaved with I-*Sce* I prior to transfection. CAT activity is normalized to β-galactosidase activity from a cotransfected *lacZ* gene.

To monitor I-*Sce* I endonuclease cleavage *in vivo* and to verify that the enhanced CAT activity was due to recombination, the CAT plasmids were isolated from COS1 cells, cleaved with *Hin*dIII, and analyzed by Southern blotting. Fig. 4 shows results from transfections with the intermolecular substrates. These transfections were performed in parallel to those used for the CAT assays shown in Fig. 3*B*. When the substrates were cleaved with I-*Sce* I prior to transfection, bands of 2 kb and 0.6 kb were detected, from the combined I-*Sce* I/*Hin*dIII digestion of Δ5′ and Δ3′, respectively (lane 1). When they were transfected uncut, they showed the *Hin*dIII cleavage products of 4.9 kb and 1.3 kb, respectively (lane 3). Cotransfection of pCMV-I-Sce I with the uncut substrates produced both the *Hin*dIII and the I-*Sce* I/*Hin*dIII cleavage products, verifying that I-*Sce* I was functional *in vivo*. Uncut Δ3′ showed the *Hin*dIII band of 1.3 kb and the I-*Sce* I/*Hin*dIII band of 0.6 kb (lane 4), whereas Δ5′ showed the *Hin*dIII band of 4.9 kb and the I-*Sce* I/*Hin*dIII band of 2 kb (lane 5). When Δ3′ and Δ5′ were cotransfected along with pCMV-I-Sce I, all four bands were observed (lane 2). In this experiment, more than half of plasmid Δ5′ was cleaved by I-*Sce* I. Somewhat less Δ3′ was cleaved, although it is more difficult to quantitate the smaller cleavage product. A substantial level of *in vivo* cleavage was also detected with the intramolecular substrate (data not shown).

In addition to the cleavage products, the recombination product of 2.2 kb was also detected by Southern analysis (Fig. 4). This band was seen if the substrates Δ5′ and Δ3′ were cleaved *in vitro* by I-*Sce* I prior to transfection (lane 1) or if they were cleaved *in vivo* by expressed I-*Sce* I (lane 2). The recombined product was not detected if they were transfected uncut with the control pCMV5 vector (lane 3).

A similar amount of the recombined product was apparent in two experiments for both the *in vitro*-cleaved and the *in vivo*-cleaved DNA. Considering that the *in vivo*-cleaved DNA produced less CAT activity, ≈60% less recombined DNA might have been expected with *in vivo* cleavage. It is possible that when the substrates are introduced uncut with

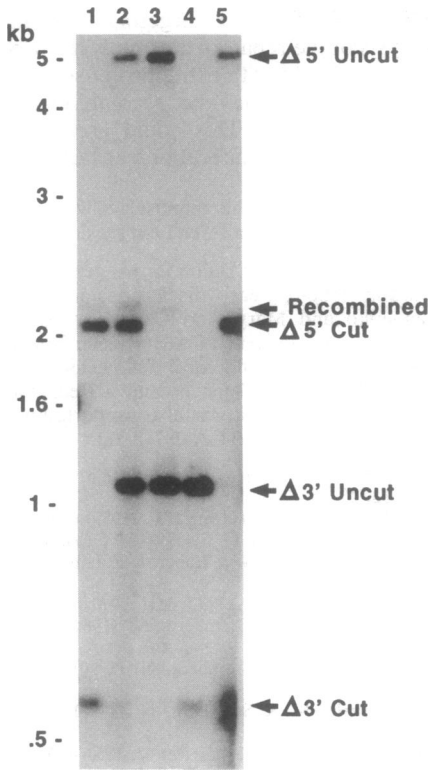

FIG. 4. Southern blot analysis of recombination substrates isolated from transiently transfected COS1 cells and cleaved with *Hin*dIII. Transfected DNA: lane 1, I-*Sce* I-cleaved Δ5′, I-*Sce* I-cleaved Δ3′, and pCMV-I-Sce I; lane 2, Δ5′, Δ3′, and pCMV-I-Sce I; lane 3, Δ5′, Δ3′, and pCMV5; lane 4, Δ3′ and pCMV-I-Sce I; lane 5, Δ5′ and pCMV-I-Sce I. The probe is probe A, as shown in Fig. 2.

pCMV-I-Sce I, recombination will be stimulated later in the transfection, as I-*Sce* I accumulates, than if the substrates were introduced cleaved. In that case, the CAT activity would experience a lag.

In addition to *Hin*dIII, Southern analysis of the isolated DNA was performed using a second restriction endonuclease digestion (*Kpn* I/*Sca* I; data not shown). Similar results were obtained that further substantiated *in vivo* cleavage by I-*Sce* I and the appearance of the recombined product.

Constitutive Expression of I-*Sce* I Is not Toxic to Murine Cells. Constitutive expression of I-*Sce* I could potentially be toxic to cells through the introduction of lesions in genomic DNA. Such deleterious effects may not be readily apparent in transient transfections. To determine if constitutive expression of I-*Sce* I is toxic to murine cells, stable transformations were performed. The expression plasmid pCMV-I-Sce I was transfected into 3T3 cells along with a selectable *neo* gene, under conditions that result in a high cotransfection efficiency, and G418-resistant colonies were selected. No difference in the number of G418-resistant colonies was evident in the cotransfections with pCMV-I-Sce I versus those without it. Northern blot analysis was performed on six randomly isolated clones, and five of these showed significant levels of I-*Sce* I mRNA (data not shown).

To verify that I-*Sce* I is produced in these cell lines and that it is functional, cells were electroporated with uncut Δ5′ and Δ3′ to monitor cleavage at their I-*Sce* I sites. DNA was isolated immediately after electroporation and at time points up to 8 h later. Southern blot analysis on *Hin*dIII-cleaved DNA is shown in Fig. 5. For the parental 3T3 cells, the DNA appeared unchanged 8 h after electroporation. However, the pCMV-I-Sce I-transfected clone 7E had a significant fraction of the plasmids cleaved at their I-*Sce* I sites, as first detected

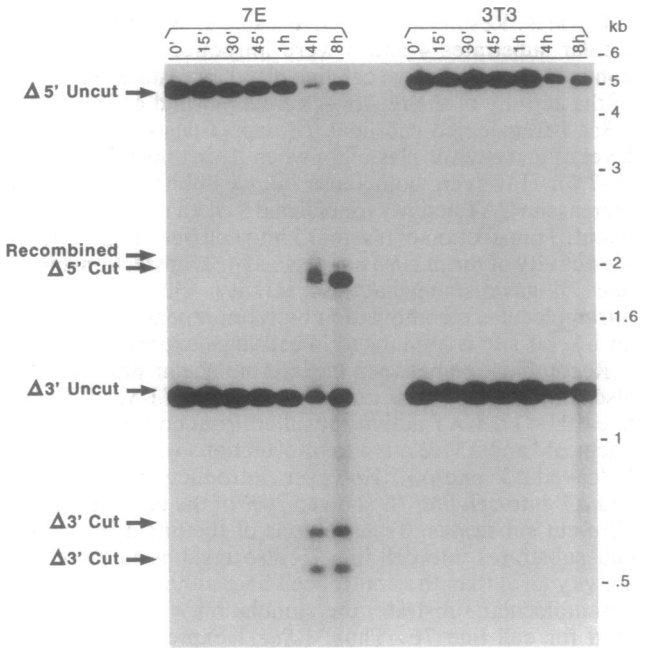

FIG. 5. Southern blot analysis of plasmids Δ5′ and Δ3′ isolated from electroporated 3T3 cell lines at the indicated times (from 15 min to 8 h) and cleaved with *Hin*dIII. Cell line 7E was derived from transfection of 3T3 cells with pCMV-I-Sce I, and it expresses I-*Sce* I. The probe is probe B, as shown in Fig. 2.

4 h after transfection. For Δ5′, the 4.9-kb plasmid was cut to produce a 2.0-kb band, and for Δ3′, the 1.3-kb *Hin*dIII band was cut to produce 0.6- and 0.7-kb I-*Sce* I/*Hin*dIII bands. Clearly, I-*Sce* I is constitutively produced in this cell line, and it can efficiently cleave introduced DNA. In addition to the cleavage products, the recombined product of Δ5′ and Δ3′ was also seen.

To quantitate the level of recombination in the cell lines producing I-*Sce* I, CAT activity was measured after transient transfections of both the intramolecular and intermolecular

Table 1. CAT activities in cell lines transiently transfected with intramolecular and intermolecular substrates

Cell line	Plasmid DNA*	CAT activity, cpm	% cpm†
	Intramolecular substrates		
6B	4	12,824 ± 3,824	9.6
	5	14,548 ± 2,366	10.9
	4/*Xba* I	133,546 ± 7,117	(100)
7E	4	3,644 ± 822	4.9
	5	29,782 ± 6,903	40.7
	4/*Xba* I	73,182 ± 15,574	(100)
7F	4	8,030 ± 1,318	6.5
	5	33,934 ± 16,563	27.4
	4/*Xba* I	123,792 ± 7,325	(100)
	Intermolecular substrates		
6B	Δ5′ + Δ3′	61 ± 32	0.3
	Δ5′/I-*Sce* I + Δ3′I-*Sce* I	22,731 ± 4,490	(100)
7E	Δ5′ + Δ3′	7,601	20.9
	Δ5′I-*Sce* I + Δ3′/I-*Sce* I	35,928 ± 4,204	(100)
7F	Δ5′ + Δ3′	1,539 ± 131	3.1
	Δ5′/I-*Sce* I + Δ3′/I-*Sce* I	50,813 ± 4,893	(100)

The results for the intramolecular substrates are from triplicate plates. The results for the intermolecular substrates are from duplicate plates, except for cell line 7E transfected with Δ5′ plus Δ3′. The values given are the mean ±SD.

**In vitro* cleavage by *Xba* I or I-*Sce* I prior to transfection is indicated by a slash and the endonuclease after the plasmid.

†Relative to *in vitro*-cut DNA.

substrates. Results are shown in Table 1. When the intramolecular substrates 4 and 5 were introduced uncut into the control I-*Sce* I-negative cell line 6B, they resulted in 10–11% of the activity of the *in vitro*-cleaved plasmid 4. The pCMV-I-Sce I-transfected cell lines 7E and 7F also showed a low level of activity for plasmid 4 when it was introduced uncut (5–7%). However, both these lines exhibited a substantial increase in CAT activity for plasmid 5 when it was introduced uncut. Transfection of plasmid 5 into cell line 7E gave 41% of the activity of the *in vitro* cut plasmid 4. Transfection into cell line 7F gave somewhat less activity, ≈27%. Therefore, intramolecular recombination between repeats separated by an I-*Sce* I site is stimulated in cell lines expressing I-*Sce* I.

Recombination between the intermolecular substrates was also stimulated by constitutive expression of I-*Sce* I. For cell line 6B, <1% CAT activity resulted from cotransfections of uncut Δ5′ and Δ3′ relative to transfections with *in vitro* I-*Sce* I-cleaved Δ5′ and Δ3′. However, introduction of uncut Δ5′ and Δ3′ into cell line 7E showed 20% of the activity of the *in vitro*-cut substrates. Transfections of the uncut intermolecular substrates into cell line 7F also resulted in more CAT activity (3%) than the control cell line, although, as with the intramolecular substrate, the stimulation was less than that seen for cell line 7E. Thus, I-*Sce* I expression is able to stimulate homologous recombination in these cell lines by cleaving recombination substrates at its recognition site.

DISCUSSION

We have expressed a modified form of I-*Sce* I, a mitochondrial intron-encoded endonuclease from *S. cerevisiae*, in mammalian cells. DNA containing the I-*Sce* I site is cleaved efficiently after transfection into cells transiently or stably expressing I-*Sce* I. As a result, extrachromosomal homologous recombination is stimulated *in vivo*. There are indications that the DNA is exonucleolytically processed prior to recombination, since Southern blots show a diffuse band after cleavage (Fig. 5). It will be interesting to determine if DNA ends in mammalian cells are processed in a similar fashion to what is seen in yeast and in *Xenopus* oocytes. In these cases, the 5′ end is exonucleolytically processed, generating 3′ tails (4, 5, 34).

The modifications we made to the I-*Sce* I open reading frame do not appear to substantially affect endonuclease activity, although the nls may enhance the rate of nuclear transport. However, it is not clear that its addition is essential for activity in the mammalian nucleus, since it has been shown that unmodified I-*Sce* I is able to enter the nucleus of yeast when expressed from the nuclear genome (35).

The question of whether constitutive expression of the endonuclease would be toxic to murine cells was addressed by performing stable transformations. The results convincingly demonstrate that no significant toxicity exists for murine cells. We have recently obtained evidence that I-*Sce* I sites integrated into chromosomal DNA are cleaved after transfection of pCMV-I-Sce I (unpublished results). Therefore, the lack of toxicity is likely due to either the lack of recognition sites in the genome or to adequate repair of them. Considering that I-*Sce* I has an 18-bp recognition site, a cleavage site is expected on average once every 7×10^{10} bp (4^{18}), which is more than 20 times the size of the mouse genome. However, base pair substitutions within the recognition site are tolerated by the purified enzyme, although they reduce the cleavage efficiency by various degrees (20).

Expression of site-specific endonucleases in mammalian cells will provide a powerful tool in genome studies. One application would be the creation of nested sets of chromosome deletions, in a modification of the telomere-associated chromosome fragmentation approach (36, 37). In addition, the introduction of I-*Sce* I recognition sites into the genome, either randomly or by gene targeting, will allow a more precise analysis of the repair of chromosomal DSBs than has so far been possible. As with extrachromosomal DNA, DSBs in chromosomal DNA may be recombinogenic so as to stimulate gene targeting. This could greatly facilitate the creation of subtle genetic alterations at targeted loci.

Note Added in Proof: While this manuscript was in preparation, a paper was published describing I-*Sce* I expression in plant cells (38).

We are indebted to Bernard Dujon for his generosity in providing pSCM525, to Jim Haber for early discussions on the project, and to Peter Romanienko and Mary Ann Osley for comments on the manuscript. P.R. is a recipient of a European Molecular Biology Organization long-term postdoctoral fellowship. M.J. is a Pew Scholar for the Biomedical Sciences and a Beckman Foundation Young Investigator. This work was also supported by the National Institutes of Health (Grant P30-CA-08748-26).

1. Szostak, J. W., Orr-Weaver, T. L., Rothstein, R. J. & Stahl, F. W. (1983) *Cell* **33**, 25–35.
2. Rudin, N., Sugarman, E. & Haber, J. E. (1989) *Genetics* **122**, 519–534.
3. Ozenburger, B. A. & Roeder, G. S. (1991) *Mol. Cell. Biol.* **11**, 1222–1231.
4. Fishman-Lobell, J., Rudin, N. & Haber, J. E. (1992) *Mol. Cell. Biol.* **12**, 1292–1303.
5. Sugawara, N. & Haber, J. E. (1992) *Mol. Cell. Biol.* **12**, 563–575.
6. Lin, F.-L., Sperle, K. & Sternberg, N. (1984) *Mol. Cell. Biol.* **4**, 1020–1034.
7. Lin, F.-L., Sperle, K. & Sternberg, N. (1990) *Mol. Cell. Biol.* **10**, 103–112.
8. Lin, F.-L., Sperle, K. & Sternberg, N. (1990) *Mol. Cell. Biol.* **10**, 113–119.
9. Jasin, M. & Berg, P. (1988) *Genes Dev.* **2**, 1353–1363.
10. Jasin, M., deVilliers, J., Weber, F. & Schaffner, W. (1985) *Cell* **43**, 695–703.
11. Subramani, S. & Seaton, B. L. (1988) in *Genetic Recombination*, eds. Kucherlapati, R. & Smith, G. R. (Am. Soc. Microbiol., Washington, DC), pp. 549–573.
12. Valancius, V. & Smithies, O. (1991) *Mol. Cell. Biol.* **11**, 4389–4397.
13. Roth, D. B. & Wilson, J. H. (1988) in *Genetic Recombination*, eds. Kucherlapati, R. & Smith, G. R. (Am. Soc. Microbiol., Washington, DC), pp. 621–653.
14. Dujon, B. (1989) *Gene* **82**, 91–114.
15. Bos, J. L., Heyting, C., Borst, P., Arnberg, A. C. & Van Bruggen, E. F. J. (1978) *Nature (London)* **275**, 336–338.
16. Faye, G., Dennebouy, N., Kujawa, C. & Jacq, C. (1979) *Mol. Gen. Genet.* **168**, 101–109.
17. Zinn, A. R. & Butow, R. A. (1985) *Cell* **40**, 887–895.
18. Dujon, B., Belfort, M., Butow, R. A., Jacq, C., Lemieux, C., Perlman, P. S. & Vogt, V. M. (1989) *Gene* **82**, 115–118.
19. Shub, D. A. & Goodrich-Blair, H. (1992) *Cell* **71**, 183–186.
20. Colleaux, L., d'Auriol, L., Gailbert, F. & Dujon, B. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6022–6026.
21. Monteilhet, C., Perrin, A., Thierry, A., Colleaux, L. & Dujon, B. (1990) *Nucleic Acids Res.* **18**, 1407–1413.
22. Perrin, A., Buckle, M. & Dujon, B. (1993) *EMBO J.* **12**, 2939–2947.
23. Colleaux, L., d'Auriol, L., Betermier, M., Cottarel, G., Jacquier, A., Galibert, F. & Dujon, B. (1986) *Cell* **44**, 521–533.
24. Field, J., Nikawa, J.-I., Broek, D., MacDonald, B., Rodgers, L., Wilson, I. A., Lerner, R. A. & Wigler, M. (1988) *Mol. Cell. Biol.* **8**, 2159–2165.
25. Rihs, H.-P., Jans, D. A., Fan, H. & Peters, R. (1991) *EMBO J.* **10**, 633–639.
26. Kozak, M. (1989) *J. Cell Biol.* **108**, 229–241.
27. Andersson, S., Davis, D. N., Dahlback, H., Jornvall, H. & Russell, D. W. (1989) *J. Biol. Chem.* **264**, 8222–8229.
28. Jasin, M. & Liang, F. (1991) *Nucleic Acids Res.* **19**, 7171–7175.
29. Chen, C. & Okayama, H. (1987) *Mol. Cell. Biol.* **7**, 2745–2752.
30. Rouet, P., Raguenez, G. & Salier, J. P. (1992) *BioTechniques* **13**, 700–701.
31. Serghini, M. A., Ritzenthaler, C. & Pinck, L. (1989) *Nucleic Acids Res.* **17**, 3604.
32. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.*
33. McBurney, M. W., Sutherland, L. C., Adra, C. N., Leclair, B., Rudnicki, M. A. & Jardine, K. (1991) *Nucleic Acids Res.* **19**, 5755–5761.
34. Maryon, E. & Carroll, D. (1989) *Mol. Cell. Biol.* **9**, 4862–4871.
35. Plessis, A., Perrin, A., Haber, J. E. & Dujon, B. (1992) *Genetics* **130**, 451–460.
36. Farr, C. J. Stevanovic, M., Thomson. E.J., Goodfellow,. P. N. & Cooke. H. J. (1992) *Nature Genet. (London)* **2**, 275–282.
37. Itzhaki, J. E., Barnett, M. A., MacCarthy, A. B., Buckle, V. J., Brown, W. R. A. & Porter, A. C. G. (1992) *Nature Genet. (London)* **2**, 283–287.
38. Puchta, H., Dujon, B. & Hohn, B. (1993) *Nucleic Acids Res.* **21**, 5034–5040.